

Ash Yellows and Defoliating Insects Related to Decline of Velvet Ash in Zion National Park

By Wayne A. Sinclair, Helen M. Griffiths, Michael Treshow, and Robert E. Davis

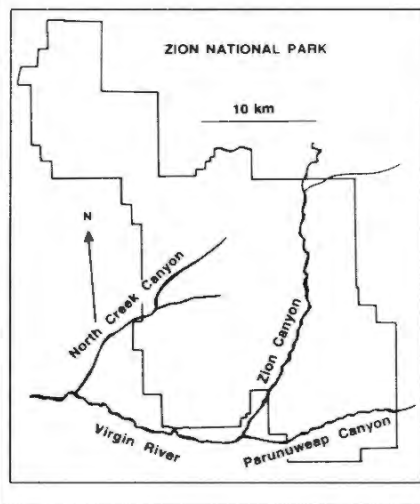
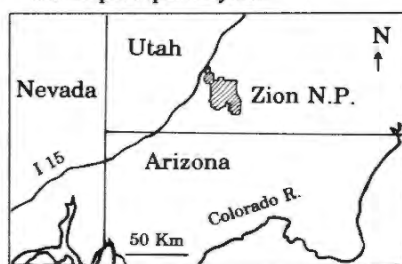
Zion National Park, on the southwestern flank of the Markagunt Plateau in southwestern Utah (Fig. 1), encompasses habitats ranging from arid to wet (Hamilton 1984). Velvet ash (*Fraxinus velutina*) colonizes moist sites in canyons there. In the late 1980s, a syndrome of slow growth and branch dieback was noticed affecting many velvet ash in Zion Canyon. The symptoms resembled those of a disease, ash yellows, that affects ash species in eastern and midwestern states (Matteoni and Sinclair 1985). Ash yellows is caused by noncultivable mycoplasma-like organisms, or MLOs.

MLOs are prokaryotic obligate parasites of plants and of the insects that serve as their vectors. MLOs belong to the class Mollicutes and cause several hundred plant diseases (Lee and Davis 1992). Within plants, MLOs colonize plants systemically by way of phloem sieve tubes and are confined to this cell type. Because MLOs can not yet be isolated and cultured apart from plant or insect hosts, they have not been named or classified at generic and species levels. The term *mycoplasma-like organism* connotes resemblance to mycoplasmas, a number of which are significant pathogens of birds and mammals (Maniloff et al 1992).

In northeastern states, ash yellows causes rootlet necrosis, growth loss, and dieback of white ash (*F. americana*) (Dyer and Sinclair 1991; Matteoni and Sinclair 1985; Sinclair et al 1990, 1993b; Smallidge et al 1991). Growth suppression also occurs in MLO-infected green ash (*F. pennsylvanica*) (Sinclair et al 1993b), but dieback in this species is not closely linked to yellows disease (Luley et al 1992). Witches'-brooms (Fig. 2a) are diagnostic for ash yellows, but only a minority of trees with the disease produce them.

MLOs were detected in velvet ash in Zion NP in 1988 (Sinclair et al 1990). Slow growth, dieback, and occasional witches'-brooms were noted. It seemed likely that velvet ash was displaying an MLO-induced syndrome similar to that described for white ash. The research summarized here (Sinclair et al 1993a, 1993b; 1994) began in 1990 to learn the distribution and incidence of declining velvet ash and of ash yellows within Zion NP, to evaluate the relationship between MLO infection and health of this species, and to learn whether or not singleleaf ash (*F. anomala*) in Zion NP is also affected by MLOs.

Figure 1. Location of Zion National Park and its principal canyons.



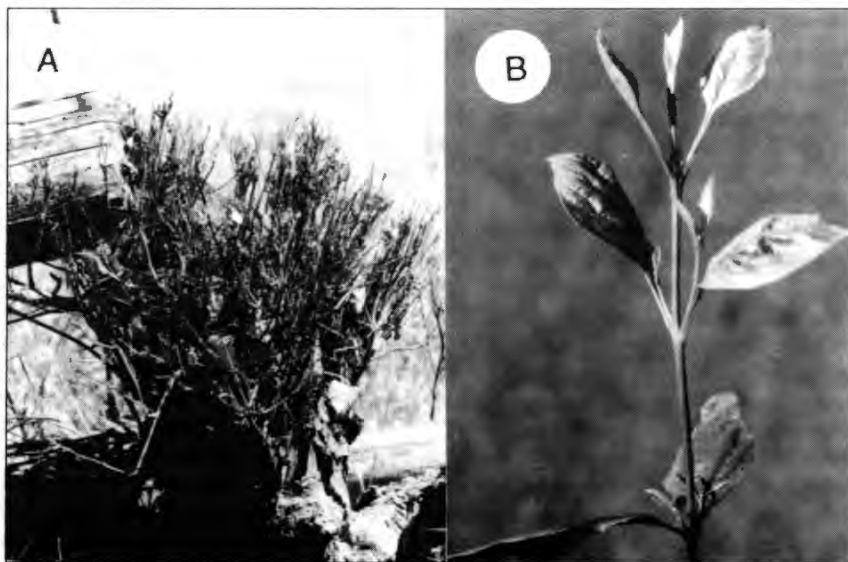
Methods

Velvet ash in the three largest canyons of the park (Fig. 1) were surveyed for health status and incidence of MLO infection. In Zion Canyon, ash on 17 plots were scored for degree of vigor and severity of dieback. The trees were also examined for evidence of injury by insects and for symptoms associated with ash yellows: witches'-brooms, sprouts on the butt or bole, simple leaves on sprouts, deliquescent branching, and chlorosis (Matteoni and Sinclair 1985). The condition of tree species associated with velvet ash was noted. Soil and other site conditions were recorded. Root samples for diagnostic testing by means of the DAPI (4',6-diamidino-2-phenylindole.2HCl) fluorescence method (Sinclair et al 1989) were taken from 382 velvet ash trees and saplings and from 53 singleleaf ash. This method permits detection of microorganisms in phloem sieve tubes based on fluorescence of their DNA when sections treated with DAPI are examined microscopically with UV illumination.

Relationships between vigor scores and diagnostic data were evaluated by means of contingency analyses that tested whether frequencies of symptoms observed in MLO-infected trees could differ from the corresponding frequencies in noninfected trees due to chance. These tests were performed

Figure 2. A. Witches'-broom on an MLO-infected stump of a velvet ash felled by a beaver.

B. Velvet ash shoot from a cluster growing at the base of a dying, MLO-infected tree, showing simple leaves and precocious axillary shoots. These symptoms were seen only on MLO-infected trees.



separately for trees 6 cm dbh and for saplings < 6 cm dbh. Growth of velvet ash as related to MLO infection was assessed by measuring widths of growth rings on increment cores and comparing average annual growth rates of MLO-infected and noninfected trees. The susceptibility of velvet ash to MLOs from white ash, and of white ash to MLOs from velvet ash was assessed by grafting potted trees of each species with bark patches or shoots from diseased trees of the other species. Grafted trees were then tested for infection and observed for symptoms.

MLOs in velvet ash in Zion Canyon were identified as ash yellows MLOs by three procedures: DNA hybridization tests utilizing cloned ash-yellows-specific probes derived from a New York strain of ash yellows MLO (Davis et al 1992), amplification of MLO DNA by a polymerase chain reaction (PCR) utilizing ash-yellows-specific primers derived from one of the DNA probes, and immunofluorescence microscopy utilizing an ash-yellows-specific monoclonal antibody (Griffiths et al 1994).

Results and Discussion

Slow growth, branch dieback, and deliquescent branching were the most prominent symptoms of distress in velvet ash. Trees of all sizes greater than approximately 6 cm dbh were affected, and they occurred on diverse sites. Foliar color was generally normal. Except for an occasional irrigated specimen, vigorous trees were confined to the sapling category. Water shortage associated with changed site conditions but not with precipitation deficiency apparently contributed to decline of some trees, because declining or dead specimens were found in a number of dry locations that were formerly irrigated or became isolated from the river. Records from the Zion NP meteorological station revealed no unusual precipitation deficiency during the 1980s, when much of the dieback apparently developed.

Symptoms diagnostic for ash yellows were uncommon. These symptoms included witches'-brooms near or at ground level (Fig. 2a) and shoots with simple leaves and precocious secondary shoots in leaf axils (Fig. 2b) within brooms or growing from the root collar.

Damage by defoliating insects was prominent on velvet ash, box elder (*Acer negundo*) and Fremont cottonwood (*Populus fremontii*). Severity of defoliation ranged from none to complete, even among individuals in the same stand. The insects responsible were unidentified loopers (*Lepidoptera: Geometridae*). Slow twig growth and dieback of twigs and branches occurred on ash and box elder on which severe defoliation was previously observed. Many velvet ash also sustained severe foliar injury by ash

plant bugs (*Tropidosteptes pacificus*) or lace bugs (*Leptophya* sp.). The former insect caused stunting and sometimes death of developing leaves and shoots and stippling on expanded leaves. The latter insect caused stippling and general yellowing of mature foliage in summer.

MLO infection was detected in velvet ash all three canyons surveyed. In Zion Canyon, 50 percent of 243 trees 6 cm dbh and 35 percent of 139 saplings tested were found to be infected. In North Creek and Parunuweap canyons, MLOs were detected in only 5 percent and 7 percent, respectively, of the 70 and 79 velvet ash tested. The high incidence of MLO infection in velvet ash in Zion Canyon was thought to reflect a more suitable habitat for vector insects (presumed to be leafhoppers) than occurs in the other two canyons. MLO infection was not detected in singleleaf ash. DNA hybridization and immunofluorescence tests both indicated close relatedness of MLOs in velvet ash in Zion NP to those in other ash species in eastern states (Griffiths et al 1994).

Velvet ash saplings infected with MLOs were found in all three canyons, indicating that young plants are at risk of infection, that overland spread of the MLOs by airborne vectors has probably occurred, and that incidence of infection may have been increasing in Zion NP in recent years. Possibly ash yellows is widespread in the Southwest, because MLO-infected Modesto ash, a variety of velvet ash, have been found in Las Vegas, NV (Sinclair et al 1990) and Tempe, AZ (Bricker and Stutz 1992).

The frequency of MLO infection varied among vigor categories of trees larger than saplings in Zion Canyon (Fig. 3). Dieback was observed in 74 percent of trees in which

MLOs were detected, versus 56 percent of trees tallied as not infected. This difference was greater than could be accounted for on the basis of chance. Moreover, the frequencies of various vigor scores of MLO-infected ash did not fit the ratio that would be predicted from the distribution of vigor scores of noninfected trees. These results were in accord with the interpretation that MLOs play a role in the decline of velvet ash in Zion Canyon. In velvet ash saplings, however, no significant association of MLO infection with dieback was detected.

Annual radial growth of velvet ash in Zion Canyon was found to have declined steadily during the 1980s, but trees in which MLOs were detected in 1990-1992 displayed the same growth trend and grew at nearly the same average rate as those in which MLOs were not detected (Fig. 4). In the northern half of the canyon, where defoliation was most

Figure 4. Annual mean radial growth of MLO-infected and noninfected velvet ash in 1970-1989. Data represent measurements on two increment cores from each of 38 infected and 19 noninfected trees in Zion Canyon.

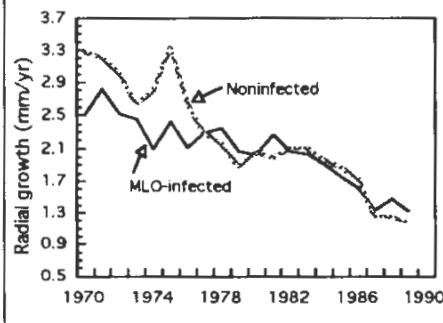
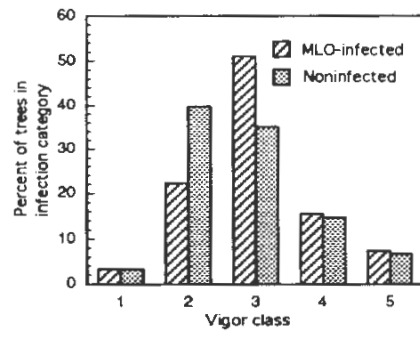


Figure 3. Distribution of vigor classes among MLO-infected and noninfected velvet ash 6 cm dbh in Zion Canyon. Classes: 1 = normal in appearance and vigor, 2 = growing slowly and/or having a thin canopy, 3 = growing slowly and having a thin canopy and dieback of twigs and/or scattered branches, 4 = dieback of many branches or large limbs, 5 = dead to near ground level.



conspicuous in each year of the study, radial growth averaged less than 1 mm per year throughout the 1980s (Sinclair et al 1993b). Previous episodes of defoliation may have caused the observed slow growth and dieback. No differences in growth or form were detected between MLO-infected and noninfected saplings that were observed for 3 years.

A New York strain of ash yellows MLO was transmitted by grafts from white ash into velvet ash and white ash seedlings. The latter species provided susceptible standards for comparison with velvet ash. MLO-infected velvet ash continued vigorous growth, while MLO-infected white ash developed rootlet necrosis and grew feebly. MLOs were transmitted from velvet ash growing in Zion Canyon to only one white ash seedling out of 25 grafted. This seedling developed rootlet necrosis and died.

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The findings of only a weak association of dieback with MLO infection, no difference in growth rate of MLO-infected and noninfected trees in Zion Canyon, and vigorous growth of young velvet ash inoculated with an eastern strain of ash yellows MLOs all indicated that velvet ash is tolerant of infection by these organisms. Perhaps MLOs affect the health of velvet ash only to the extent that infected trees may be more sensitive to, or may recover from, other stresses (e.g., defoliation, water shortage) less fully or rapidly than noninfected trees, as Han et al (1991) suggested for white ash. Or perhaps AshY MLOs are widespread and innocuous in healthy-appearing as well as debilitated velvet ash but have been detected only where declining trees were studied.

The role of MLOs in decline of velvet ash in Zion Canyon is apparently small. On the other hand, the decline of mature individuals of this species is conspicuous. This decline may have been caused primarily by defoliation by insects, with water shortage playing a role for some trees. The question for resource managers is whether measures to arrest or reverse the decline of velvet ash in Zion Canyon should be attempted. Feasible options for remedial action are limited by the policy of allowing natural processes to proceed.

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