Ash Yellows in Velvet Ash in Zion National Park, Utah: High Incidence but Low Impact

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ABSTRACT

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Possible causes of slow growth and dieback of velvet ash (*Fraxinus velutina*) in Zion National Park, Utah, were investigated. Mycoplasmalike organisms (MLOs) were detected by means of the DAPI (4′,6-diamidino-2-phenylindole·2HCl) fluorescence test in 5, 7, and 45% of velvet ash sampled in three localities but were not detected in singleleaf ash (*F. anomala*). In Zion Canyon, MLOs were detected in 51% of velvet ash ≥6 cm dbh and in 35% of saplings. Branch dieback occurred in 74% of trees ≥6 cm dbh in which MLOs were detected and in 56% of noninfected trees. Low titer of MLOs in velvet ash was indicated by sparse fluorescent particles in DAPI-treated root phloem observed by fluorescence microscopy and by 39% incidence of negative DAPI test results in trees retested 1–2 yr after MLOs were first detected in them. Witches'-brooms and dwarfed, simple-leaved shoots near ground level, diagnostic for ash yellows in eastern ash species, were uncommon. Tolerance of velvet ash for MLO infection was indicated by scarcity of diagnostic symptoms, similar vigor of MLO-infected and noninfected saplings observed for 3 yr, and continued vigorous growth of MLO-infected velvet ash, but not white ash, seedlings after graft-inoculation with a New York strain of ash yellows MLO. Many velvet ash in Zion Canyon sustained severe foliar damage by unidentified loopers (Lepidoptera: Geometridae), ash plant bugs (*Tropidosteptes pacificus*), and lace bugs (*Leptophya* sp.). Insect injury, rather than MLO infection, was a likely cause of declining vigor and dieback. Mature trees on some sites had also been damaged by water deficiency.

Ash yellows (AshY), a disease of *Fraxinus* species, is caused by noncultivable mycoplasmalike organisms (MLOs) that are genetically distinguishable from MLOs that infect most other plants (1,4,7,9,13). White ash (*F. americana* L.) affected by AshY sustains growth loss, rootlet necrosis, and dieback (5,6,8,12,17,19,20). AshY MLOs in green ash (*F. pennsylvanica* Marsh.) suppress growth (17) but have not been linked to dieback (10).

MLOs were detected in velvet ash (*F. velutina* Torr.) in Zion National Park, Utah, in 1988 (19). 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 (12). DNA hybridization data and immunofluorescence tests indicated that the MLOs in velvet ash were closely related to AshY MLOs of eastern North America (7). The research described here began in 1990 to learn the distribution and incidence of declining velvet ash and of AshY within Zion Park, to evaluate the relationship between MLO infection and health of this species, and to learn whether or not singleleaf ash (*F. anomala* Torr. ex S. Wats.) in Zion Park is also affected by MLOs.

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MATERIALS AND METHODS

Research sites and observations. Zion National Park, located in southwestern Utah, contains deep canyons carved in sandstone by the Virgin River and its tributaries (Fig. 1). Velvet ash occurs primarily along water courses in North Creek, Parunuweap, and Zion canyons at elevations below 1,500 m. It is most abundant in Zion Canyon, which is the largest and deepest in the park. Singleleaf ash, the only other *Fraxinus* species present, grows on slopes, alluvial terraces, and other relatively xeric sites at elevations from 1,250 to 1,900 m. Field research was conducted primarily in Zion Canyon. Surveys for occurrence of MLOs and symptoms of decline in velvet ash were conducted in North Creek and Parunuweap canyons.

During 1990–1991, velvet ash on 17 sites in Zion Canyon were observed and tested for MLO infection. On each site,

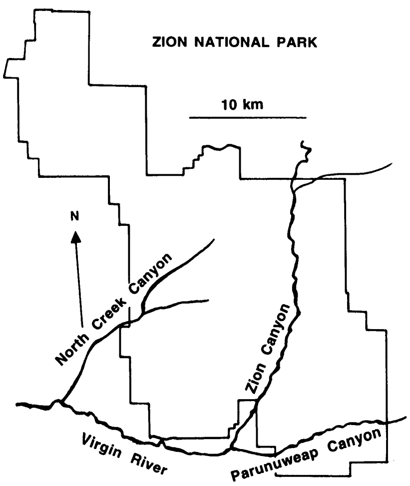

Fig. 1. Map of Zion National Park, Utah.

at least five trees ≥6 cm dbh, representing the range of health and debilitation of ash, were tagged and/or their locations plotted on maps. Each tree was tallied in one of five 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, and 5 = dead to near ground level. The character "thin canopy" refers to abnormal transparency to skylight as the result of missing or undersized foliage or stunted shoots. The selected trees, a total of 243, were also examined for evidence of injury by insects and for symptoms associated with AshY as previously observed in white ash: witches'-brooms, sprouts on the butt or bole, simple leaves on sprouts, deliquescent branching, and chlorosis (12,19). Observations were repeated in 1992. Weather records from the Zion Park meteorological station were consulted for evidence of unusual water shortage. Soil and other site conditions (e.g., evidence of prior disturbance by flash floods or construction) were recorded. One root sample for diagnostic testing was taken from each tree in 1990 and/or 1991 and again in 1992 as described below.

On four sites in Zion Canyon, a total of 139 velvet ash saplings <6 cm dbh were evaluated and sampled during 1990–1992 in the manner described for trees. Saplings were scarce or absent on most sites where larger trees were studied. They were considered separately because they provided an opportunity to assess possible MLO influence on young trees that had not been subject to long-term effects of adverse sites, weather, or insects. Velvet ash in the reproduction class (up to 1.4 m tall) were not considered, because they were not numerous.

In North Creek Canyon, a total of 79 velvet ash on two sites 7.5 km apart were rated for vigor as described above and sampled for MLO detection in 1991 and again in 1992. In Parunuweap Canyon, 70 velvet ash along a 5-km segment of the river were sampled for MLO detection in 1992. The sampling in both canyons included saplings and larger trees.

A total of 53 singleleaf ash on seven sites in Zion Canyon were observed and sampled for possible MLO detection as described below. Sampling was deliberately biased toward trees showing dieback or unusually slow growth, because these symptoms are associated with MLO infection in many tree species.

Mycoplasmal infection in relation to decline of velvet ash in Zion Canyon. The DAPI (4′,6-diamidino-2-phenylindole·2HCl) fluorescence test (14,18) was used for MLO detection. One rootlet, 1–4 mm in diameter, growing from the butt or a major root was exposed with hand tools, severed, and washed. Segments 1–4 × 8–10 mm were cut, placed immediately in a vial containing 2.5% aqueous glutaraldehyde, and stored in this fixative at 4 C for up to 3 mo. Longitudinal sections of one segment per tree were cut with a freezing microtome, treated with DAPI, and examined with a microscope equipped for epifluorescence (18). Sections from a second segment were examined if those from the first were unsatisfactory for interpretation. This intensity of sampling was known to be sufficient for 95% accuracy in detecting MLOs in white ash (18).

Relationships between tree health scores and diagnostic data were evaluated using contingency analyses of two types: tests for homogeneity of data and tests of the degree to which the distribution of health scores in MLO-infected trees conformed to the corresponding distribution in noninfected trees (21). These tests were performed separately for trees ≥6 cm dbh and for saplings <6 cm dbh.

Consistency of MLO detection by the DAPI test. MLO populations in roots of velvet ash, as detected by the DAPI test, were usually sparse in comparison to those previously observed in white ash. Therefore, we studied the incidence of false-negative results by retesting velvet ash in which MLOs were previously detected. In mid-May 1992, 85 trees in Zion Canyon that had been diagnosed as MLO-infected in 1990 or 1991 were resampled and retested with DAPI. The proportion of this group that was scored negative in 1992 provided an estimate of the frequency of false-negative results to be expected in any sampling of velvet ash in Zion Canyon in late spring or summer. False-positive results, believed to be rare, were not evaluated.

Reciprocal transmission of MLOs between velvet and white ash. Susceptibility of velvet ash to MLOs from white ash was tested in two experiments in a screened greenhouse. In experiment 1, four 2-yr-old velvet ash seedlings and eight 2-yr-old white ash seedlings (susceptible controls) were grafted with bark patches from diseased white ash growing at Ithaca, New York. Four control plants of each species were left untreated. In experiment 2, seven 2-yr-old velvet ash seedlings were cleft-grafted with leafy scions from diseased white ash and seven seedlings were left untreated. All grafts were wrapped with paraffin film, and scions in experiment 2 were covered for 1 mo with clear polyethylene bags sealed around the stems. Plants in both experiments were observed during three successive growth periods. DAPI tests were performed at the end of the second and third periods.

Two experiments were performed to assess the susceptibility of white ash to MLOs from velvet ash. Bark patches from roots of MLO-infected velvet ash saplings in Zion Canyon were grafted into stems of 15 (experiment 1) and 10 (experiment 2) potted white ash, 2–5 yr old, growing in a screened greenhouse. Grafts on equal numbers of control seedlings were prepared by removing and replacing patches of bark similar to the inoculum patches. Grafts were wrapped with paraffin film. The plants were observed during two successive growth periods and were tested with DAPI at the end of the second period.

RESULTS

Distribution and incidence of declining velvet ash in Zion Park. Velvet ash with branch dieback were most prominent in Zion Canyon. They occurred on diverse sites including dry terraces of deep sand or gravel, moist sand near the riverbed, and clayey soils. A few sites were irrigated, and some had been disturbed by construction or compaction. On one site, velvet ash had died after irrigation ceased. Records from the Zion Park meteorological station revealed no unusual precipitation deficiency during the 1980s, when much of the dieback apparently developed.

In North Creek Canyon, most of the velvet ash observed were saplings, of which approximately half were tallied as vigorous and half as growing slowly. Dieback was uncommon in the saplings but affected nearly all larger velvet ash. In Parunuweap Canyon, dieback of velvet ash was confined to trees larger than saplings.

Symptoms of decline, mycoplasmal infection, and injury by insects. Slow growth, dieback, and deliquescent branching in slowly growing trees were the most prominent symptoms. Trees of all sizes greater than approximately 6 cm dbh were affected. Foliar color was generally normal. Except for an occasional irrigated tree, the only vigorous specimens were saplings.

Other symptoms indicative of AshY were uncommon. These symptoms included witches'-brooms near or at ground level, shoots with simple leaves, and precocious axillary shoots (Fig. 2) within brooms or growing from the root collar, and light green or diffuse interveinal chlorosis of foliage on basal shoots. Witches'-brooms were seen only on trees with basal wounds made by beavers or construction equipment.

Damage by leaf-feeding insects was prominent in each year of the study. Defoliation by unidentified loopers (Lepidoptera: Geometridae) ranged from none to complete, even among trees in the same stand. Trees severely defoliated in successive years displayed slow twig growth and branch dieback. Many trees were also injured by ash plant bugs (*Tropidosteptes pacificus* (Van Duzee)) or lace bugs (*Leptophya* sp.). The former insect caused stunting and necrosis of developing leaves and shoots and stippling on expanded leaves. The latter insect caused stippling and general yellowing of mature foliage in summer.

Distribution and incidence of MLO infection of velvet ash in Zion Park. MLOs were detected in velvet ash in all three canyon habitats of this species in the park. If a tree was scored positive in the DAPI test in any year, it was tallied as diseased. In Zion Canyon, MLOs were detected at every site of sampling, in 51% of 243 trees ≥6 cm dbh, and in 34% of 139 saplings, for an overall infection rate of 45%. In North Creek Canyon, MLOs were detected at both sites sampled in 1991 but in only four of 79 trees and saplings. These 79 were retested in 1992, with no change in frequency of MLO detection. In Parunuweap Canyon, MLOs were detected in five scattered velvet ash (7%) among the 70 that were sampled.

Singleleaf ash. MLOs were not detected in singleleaf ash. Fluorescence of plant DNA occurred as expected under UV after treatment with DAPI. Therefore, test conditions were presumed to be suitable for detection of MLOs, had they been present.

Reliability of MLO detection by the DAPI test. To assess and improve the reliability of disease incidence data, we resampled, in May 1992, 278 velvet ash for which previous DAPI test results were available. Among 85 ash previously diagnosed as infected, 39% were scored negative for MLOs when retested. The 95% confidence interval for this proportion was calculated (21) as 28.4–49.2%. For the purpose of calculating diagnostic accuracy, the probability of a false-negative result in any single DAPI test was assumed to be 39%. Diagnostic results for trees scored negative in two annual DAPI tests were calculated to be 85% correct, based on the reasoning that the probability of a false-negative result for a given tree in 2 yr is the product of the separate probabilities (0.39^2, or 0.15).

MLO infection in relation to decline of velvet ash in Zion Canyon. Vigor scores and DAPI data were obtained for 209 trees ≥6 cm dbh and 109 saplings. The frequency of MLO infection varied among vigor classes (Table 1). Contingency analysis for homogeneity of data from trees ≥6 cm dbh revealed interaction of dieback and MLO infection (χ^2 with 1 df, adjusted for continuity, = 6.333, $P < 0.025$). Thus, the difference in incidence of dieback between infected and noninfected trees was greater than would likely be encountered on the basis of chance. The frequency distribution of vigor classes of MLO-infected trees was then compared to the corresponding distribution in noninfected trees (Fig. 3). Chi-square with 4 df was 18.28, $P < 0.005$. Thus, the difference in vigor class distribution between MLO-infected and noninfected trees could not be explained by chance. In the sapling group (Table 1), contingency analysis revealed no interaction of dieback and MLO infection (χ^2 with 1 df, adjusted for continuity, = 0.327, $P > 0.5$).

Reciprocal transmission of MLOs between velvet and white ash. New York strains of AshY MLOs were transmitted by bark patches and cleft grafts from white ash into velvet ash seedlings. MLOs were detected in three of four and six of seven inoculated plants in experiments 1 and 2, respectively. All controls were negative in DAPI tests. MLO-infected and noninfected velvet ash both grew vigorously throughout three cycles of growth and dormancy. Most MLO-infected plants produced thinner apical twigs, however, and some produced more axillary shoots than did healthy plants (Fig. 4). All eight white ash seedlings that were inoculated as susceptible standards became infected. These plants remained normal in appearance during the growth period in which grafting occurred, but they grew feebly thereafter, and five died. Control plants of white ash grew vigorously (Fig. 4C).

Ten weeks after the onset of growth in the second growth period, all plants

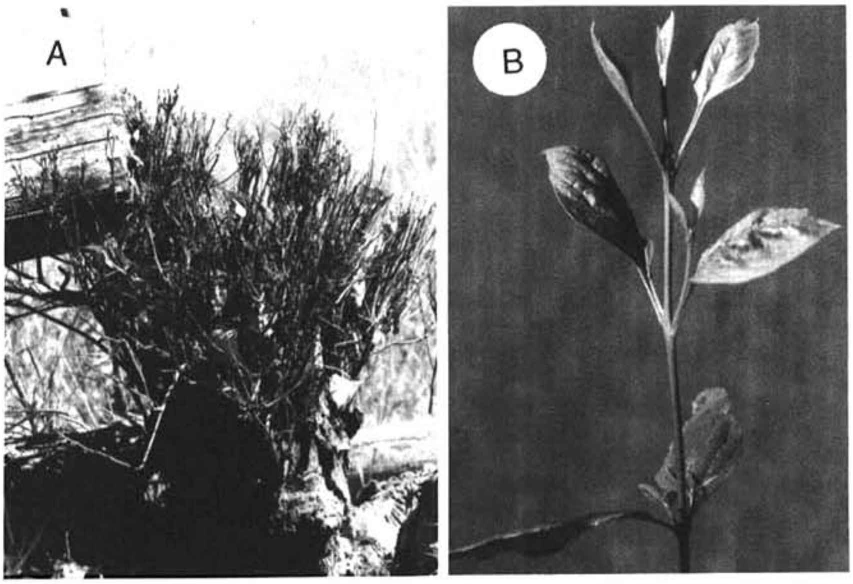

Fig. 2. (A) Witches'-broom on an MLO-infected stump of a velvet ash felled by a beaver. **(B)** Velvet ash shoot from a cluster growing at the base of a dying, MLO-infected tree, showing simple leaves and precocious axillary shoots. These symptoms were seen in the field only on MLO-infected trees.

Table 1. DAPI test scores in relation to vigor classes of velvet ash in Zion Canyon[a]

	Trees ≥6 cm dbh		Saplings	
Vigor class[b]	Number	DAPI-positive (%)	Number	DAPI-positive (%)
1 Normal and vigorous	7	57	10	60
2 Thin canopy or slow growth	62	44	71	41
3 Thin canopy, dieback of twigs or scattered branches	93	67	23	52
4 Dieback general or involving large limbs	32	59	5	40
5 Dead to near ground level	15	60	0	0
Totals	209	58	109	45

[a]Combined data of 1990–1992.

[b]Vigor scores were available for 209 of the 243 trees and 109 of the 139 saplings that were tested with DAPI. For trees scored more than once, the most recent score was tallied.

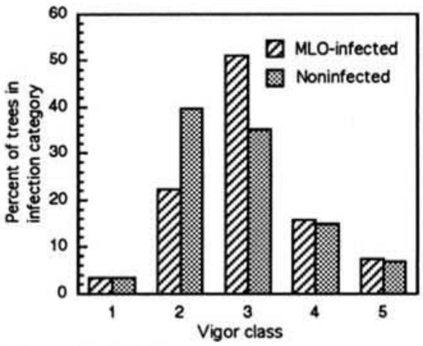

Fig. 3. Distribution of vigor classes among 121 MLO-infected and 88 noninfected velvet ash ≥6 cm dbh in Zion Canyon. Classes: 1 = normal in appearance and vigor, 2 = growing slowly and/or having a thin canopy, 3 = growing slowly and having a thin canopy and dieback of twigs and/or scattered branches, 4 = dieback of many branches or large limbs, 5 = dead to near ground level.

were removed from pots briefly, with undisturbed soil adhering to the roots, for inspection of roots that had grown against the walls of the pots. Dead roots were prominent in all MLO-infected white ash but were not apparent in white ash control plants or in either MLO-infected or healthy velvet ash.

MLOs were transmitted from roots of velvet ash in Zion Canyon to the stem of only one white ash seedling out of 25 inoculated. This seedling remained normal in foliar appearance during the growth period in which grafting occurred, but it developed rootlet necrosis and, after a dormant period, produced stunted shoots that developed chlorosis, then wilted and died.

DISCUSSION

Distribution and incidence of ash yellows within Zion Park. MLOs occur in velvet ash in all three canyon habitats of this species within the park. The incidence of infection as detected by DAPI tests is much higher (45%) in Zion Canyon than in North Creek or Parunuweap canyons (5–7%). The high incidence of AshY in Zion Canyon may reflect a more suitable habitat for insect vectors than occurs in the other two canyons. Zion Canyon is a more mesic habitat with more diverse vegetation than the parts of North Creek and Parunuweap canyons where velvet ash were sampled. The presence of MLO-infected saplings in all three canyons indicates that young velvet ash are at risk of infection, that overland spread of the MLOs by airborne vectors has probably occurred, and that incidence of AshY may have been increasing in Zion Park in recent years. MLO infection of Modesto ash, a variety of velvet ash, occurs in Las Vegas, Nevada (19), and Tempe, Arizona (2).

Several possible reasons exist for the apparent absence of MLO infection in singleleaf ash. This species could be an unsatisfactory food source for the vectors of AshY MLOs or could escape frequent infection because it occupies a habitat more xeric and less suitable for vector activity than that of velvet ash, or it could be resistant to AshY MLOs.

Consistency of MLO detection by the DAPI test. The 39% incidence of negative DAPI test results for velvet ash previously scored as MLO-infected was nearly fivefold greater than that detected in concurrent work with white ash in New York State (Sinclair and Griffiths, *unpublished*). False-negative DAPI results could lead to erroneously low estimates of incidence of MLO infection in velvet ash populations in which the true incidence of MLO infection is relatively high (e.g., in Zion Canyon) but would not have much effect on the validity of data from populations in which the true incidence of MLO infection is low (e.g., North Creek Canyon). Reliable estimates of disease incidence are possible in each circumstance if trees are sampled repeatedly.

Susceptibility of velvet and white ash to each other's MLOs. Differences between velvet ash and white ash seedlings in symptoms induced by a New York strain of AshY MLO provided direct evidence that velvet ash is more tolerant than white ash of MLO infection. Infected velvet ash continued to grow vigorously and had healthy-appearing roots. White ash seedlings infected with MLOs from the same source as those in velvet ash developed rootlet necrosis and produced stunted shoots that tended to wilt, a syndrome noted previously (5). Symptoms in the single white ash seedling that became infected with MLOs from velvet ash were similar to those induced in white ash seedlings by MLOs from white ash.

Bark patches (11) were used as scion material in most grafting experiments because growth stages of donor and recipient plants were not suitable for the use of budwood or leafy scions. Roots of velvet ash were used as sources of bark patches because the titer of MLOs in roots was thought likely to be higher than that in stems, based on work with white ash (3,15,18). Scarcity of MLOs in the scion material and formation of wound parenchyma at graft unions are possible reasons for the low frequency of MLO transmissions by bark-patch grafts from velvet ash. Previous work with white ash revealed that although such grafts usually make union, conductive phloem connections often fail to form because extensive wound parenchyma develops at the edges of patches. Without these connections, MLOs remain sequestered and degenerate in old phloem in the bark patches (Sinclair, *unpublished*).

Relationship of MLO infection to health of velvet ash in Zion Canyon. The role of MLOs in decline of velvet ash in Zion Canyon is apparently small. Dieback was more common in MLO-infected than in noninfected trees, but MLO-infected saplings observed for 3 yr were indistinguishable from noninfected saplings. Furthermore, no significant difference in average annual radial growth or growth pattern during the 1980s was detected between infected and noninfected trees (17).

Field observations and inoculation results both indicated that velvet ash is tolerant of infection by AshY MLOs. Possibly these MLOs diminish the ability of velvet ash to tolerate other stresses such as defoliation or water shortage. The greater frequency of dieback in MLO-infected than in noninfected trees

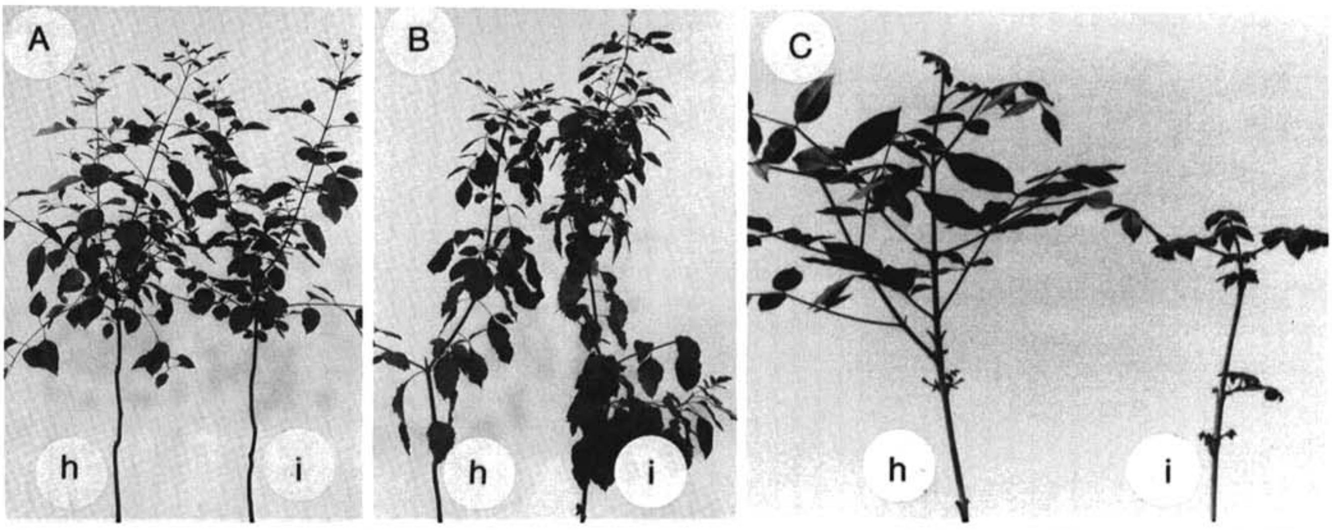

Fig. 4. Two-year-old velvet ash and white ash seedlings, healthy (h) or infected with a New York strain of AshY MLOs (i). **(A)** An infected velvet ash, growing vigorously, is indistinguishable from a healthy control. **(B)** An infected velvet ash, but not its healthy counterpart, has thin twigs and numerous axillary shoots, but has maintained apical growth and dominance. **(C)** New growth of a diseased white ash is stunted in comparison to that of a healthy control.

≥6 cm dbh is consistent with this possibility. Han et al (8) presented evidence that MLO infection suppressed the recovery of white ash from drought-induced growth depression. The MLO tolerance of velvet ash contrasts with the susceptibility of white ash, in which MLOs often contribute to slow growth and decline (8,12,17,19).

AshY MLOs could be widespread and innocuous in healthy-appearing as well as debilitated velvet ash. To date, detection of MLOs in velvet ash has been attempted only where declining trees were studied: Zion Park, Las Vegas, and the vicinity of Tempe (2,19). The finding of a velvet ash population with many slowly growing and declining trees and a high incidence of AshY MLO infection but without strong relationship between tree health and MLO infection was similar to the finding for green ash in midwestern states (10).

Insects and water shortage as possible stress inducers in velvet ash. Severe defoliation of velvet ash by insects was observed in the northern half of Zion Canyon in all 3 yr of the study (16). Radial growth of velvet ash there averaged less than 1 mm per year throughout the 1980s (17). Previous episodes of defoliation may have caused much of the observed slow growth and dieback.

Water shortage associated with changed site conditions but not with precipitation deficiency apparently contributed to decline of some velvet ash. This species colonizes only moist sites. Declining or dead specimens were found in a number of locations that were formerly irrigated or had become isolated from the river and are now dry.

Based on current knowledge, AshY in velvet ash in Zion Park seems inconsequential. On the other hand, the decline of mature trees is conspicuous. 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 National Park Service policy of allowing natural processes to proceed.

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