ASH YELLOWS IN ZION NATIONAL PARK: IMPACT, IDENTITY OF PATHOGEN, MODE OF SPREAD, AND PROSPECTS FOR MANAGEMENT

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PROBLEM DESCRIPTION, PROJECT OBJECTIVES, AND SUMMARY OF PREVIOUS PROGRESS

♦ THE PROBLEM

Velvet ash (*Fraxinus velutina*) in Zion Canyon have declined in vigor, and some are dying. This species is aesthetically and ecologically important in the canyon, because it is one of only three tree species that commonly grow to large size on the canyon floor. Ash yellows (AshY), a disease caused by unnamed mycoplasmalike organisms (MLOs), is common in velvet ash in Zion Canyon and was suspected to contribute to the decline of this species. This project deals with the ecology and epidemiology of ash yellows and possibilities for managing velvet ash in Zion National Park.

♦ RESEARCH OBJECTIVES

1. Determine the extent of damage to the ash species in Zion Park, and map the distribution of ash yellows in the park.

2. Learn whether or not the MLOs in ash in Zion Park are closely related to those associated with ash yellows in Nevada and New York.

3. Determine the means and rate of spread of ash yellows and the rate at which the epidemic is progressing within the currently affected area.

4. Assess the possibilities for managing the disease.

SUMMARY OF FIRST 2 YEAR'S PROGRESS (MAY 1990-APRIL 1992)

For Objective 1. A survey along highways in and near Zion Park revealed that velvet ash is concentrated in Zion and North Creek canyons in the upper Virgin River watershed. Many trees of this species in Zion Canyon display dieback. Severe,

repeated defoliation by insects (mainly loopers), affecting not only ash but also boxelder and Fremont cottonwood, was judged to be the principal cause of dieback. Foliar damage to velvet ash by plant bugs (*Tropidosteptes pacificus*) and a lacebug (*Leptyphyia* sp.) was also prominent. Mature trees of all three species on deep sandy soils were apparently also stressed by water shortage.

The role of MLOs in decline of velvet ash was studied by means of diagnostic observations and radial growth measurements. Of 328 velvet ash in all vigor classes, sampled on 19 sites in Zion Canyon and tested for MLO infection by means of the DAPI fluorescence test, 32% were found to be infected. Incidence of infection was similar (25-35%) in all health classes of ash larger than 6 cm dbh. Incidence of infection in saplings (< 6 cm dbh) was greater in those with dieback than in those without dieback (44% vs. 26%). Data from increment cores indicated slower growth of velvet ash infected by MLOs than of noninfected trees in 1980-1989, although the difference lacked significance at the 95% confidence level. The preliminary conclusion was that MLOs play a secondary role in ash decline in Zion Canyon, probably by adding an increment of stress to that induced by insects.

Four percent of 71 trees sampled in North Creek Canyon were found to be infected with MLOs. The remoteness of the sites where AshY was detected in North Creek Canyon indicated that overland spread of the causal agent, presumably by insect vectors, has occurred.

MLOs were not detected in singleleaf ash (*F. anomala*).

For Objective 2. Preliminary evidence was obtained that the MLOs in velvet ash in Zion Canyon are related to MLOs associated with ash yellows in the East. DNA from one MLO-infected velvet ash (out of 16 from which DNA extracts were obtained) hybridized with each of four AshY-specific DNA probes derived from a New York strain of AshY MLO. Low MLO titer in velvet ash, as indicated by observations of DAPI-treated phloem, is the presumed reason for failure to detect AshY MLOs in most dot hybridizations with either AshY-specific probes or others that detect MLOs nonspecifically.

MLOs were transmitted from velvet ash to a white ash seedling by grafting in one out of 15 attempts. The diseased seedling ceased growth, wilted, and died -- a response previously observed in some white ash seedlings inoculated with New York strains of AshY MLOs.

For Objective 3. Insects, probably leafhoppers, are presumed to transmit AshY MLOs. Leafhoppers of approximately 30 taxa were caught in small numbers on yellow sticky traps, removed from the traps, and sorted as the first phase of work to detect and identify insect vectors of AshY.

Possible alternative plant hosts of AshY MLOs were sought on 10 sites in Zion Canyon. Plants that were stunted or had witches'-brooms or other symptoms resembling those commonly induced by MLOs were selected for diagnostic testing. MLOs were detected in only two plants, one of hairy golden aster (*Chrysopsis villosa*) and one of rabbitbrush (*Chrysothamnus nauseosus*), out of 30 tested. The relationship of the MLOs in hairy golden aster and rabbitbrush to those in ash was not determined. The lack of common detection of MLOs in plants other than velvet ash permits the tentative interpretation that the MLOs causing AshY in Zion Canyon do not have alternative host plants in the canyon.

For Objective 4. Experiments began for evaluation of kinetin solutions for chemotherapy of MLO-infected ash and other plants. Preliminary data gave no promise of beneficial effect.

ACTIVITIES DURING THE PERIOD OF REPORT

Project personnel visited Zion Park in May and August to continue disease surveys, resample trees on observation plots, perform diagnostic observations, collect plant specimens for diagnostic procedures that would be performed in the laboratory, collect more increment cores for study of ash growth as related to disease, and collect more insects for study of their possible role as vectors of ash yellows. Growth measurements on increment cores were performed in the laboratory of co-investigator Treshow at Salt Lake City. Other procedures were performed in Sinclair's laboratory at Ithaca.

SURVEY FOR ASH DECLINE AND ASHY IN ZION NATIONAL PARK

ASHY IN PARUNUWEAP AND NORTH CREEK CANYONS.

In August 1992, the survey was extended to Parunuweap Canyon (east fork of Virgin River), in which a velvet ash population was brought to our attention by NPS personnel. It was of interest to learn whether or not MLOs occur in velvet ash there, and if so, whether the frequency of infection is similar to that detected in Zion Canyon or North Creek Canyon. Roots of 70 velvet ash along a 5-km stretch of the River, beginning at the west boundary of the park, were sampled for MLO detection by means of the DAPI procedure. Symptoms of decline (dieback of branches) were infrequent in saplings but were observed in many larger trees among those sampled. As of this writing, MLOs have been detected in three of 43 specimens processed. Thus, the proportion infected is much lower than that in Zion Canyon, as is also true for trees sampled in North Creek Canyon.

Trees sampled for diagnosis in 1991 in North Creek Canyon were resampled in 1992. Half the samples have been processed; no change in frequency of MLO detection from 1991 to 1992 is indicated.

Occurrence of AshY in all three canyons indicates overland spread of the causal MLOs by wind-borne vectors, since Parunuweap Canyon and the part of North Creek Canyon within the park are undeveloped and the velvet ash population is discontinuous. Presence of AshY in all three canyons is consistent with the hypothesis that AshY MLOs are indigenous to the Zion Park area.

INCREASE OF ASHY IN ZION CANYON

Information about the rate at which new MLO infections are occurring in Zion Canyon is obtained by annually retesting saplings and larger trees that were first sampled and scored negative in DAPI tests of 1990-1991. In May, 1992, we resampled all available numbered trees and saplings within Zion Canyon that had been tested previously. MLOs were detected in 48% of 184 trees $\geq$ 6 cm dbh and 39% of 93 saplings. The over-all proportion of trees and saplings in which MLOs were detected at least once in 1990-1992 was 49.7% of 368 plants. Thus, the proportion of trees in which MLOs were detected increased approximately 12% between 1990 and 1992. This increase probably resulted in part from disease increase, but it may also reflect increasingly accurate diagnosis because of repeated sampling (see next paragraph).

SEASONAL AND ANNUAL CONSISTENCY OF MLO DETECTION BY THE DAPI TEST

Because detection of MLOs in velvet ash is often difficult due to low titer, and because the proportion of sampled trees in which MLOs were detected in May 1991 (28%) was lower than that in 1990 (38%), we speculated that seasonal or annual population fluctuations or discontinuous MLO distribution within trees may affect the results of DAPI tests. Accordingly, in late May 1992 we resampled 86 velvet ash that had previously been scored positive in DAPI tests. The time of sampling was chosen to allow seasonal buildup of MLO populations from assumed winter lows. The proportion of these trees scored negative for MLOs in 1992 could be used to derive the frequency of false negative diagnoses that might occur in any year. This proportion was 39%, which corresponded to 12.2% of the overall sample of 277 trees tested in 1992. This incidence of false negative diagnoses is more than twice the rate detected in comparable work with white ash in the East. Valid conservative estimates of disease increase are still possible, however, because only positive test data are utilized in the calculations.

RADIAL GROWTH OF VELVET ASH IN RELATION TO MLO INFECTION

Increment cores previously collected from 106 trees for which DAPI scores were also available were reexamined. Trees that averaged $<$ 1 mm radial growth per year during the 1980s were deleted from the sample, because these trees were considered to be growing too slowly for possible growth differences between MLO-infected and noninfected trees to be detectable. Annual radial growth in 1970-1989 was analyzed for 38 infected and 19 noninfected trees that grew at least 1.0 cm in 1980-89. No significant difference between these groups in annual mean growth was detected. Both groups displayed steadily declining growth rates (Figure 1). The lack of relationship between MLO infection and growth

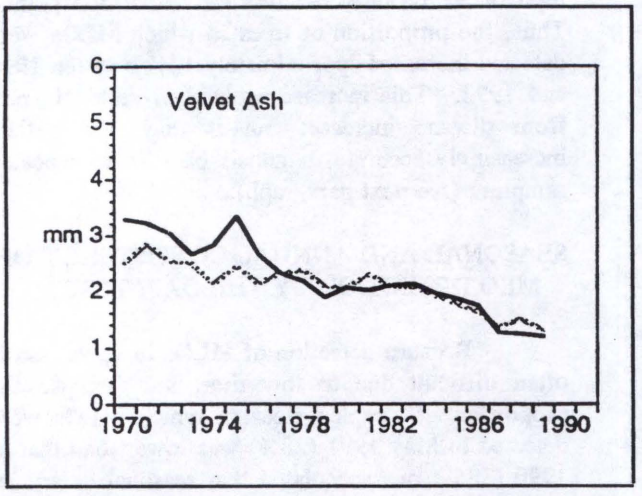

Figure 1. Annual mean radial growth of MLO-infected (solid lines) vs noninfected (hatched lines) velvet ash. Data represent measurements on two increment cores from each of 38 infected and 19 noninfected trees.

rate contradicted a preliminary finding of 23% slowergrowth of MLO-infected than noninfected trees during the 1980s (Sinclair et al. 1992b). Changing diagnoses caused the discrepancy. Initial diagnoses were made in 1990-91. When the "noninfected" trees were retested with DAPI in 1992, several were reclassified as infected. Thereafter, no growth impact of MLO infection was detected. Among the several explanations available for lack of detectable growth impact of MLOs in velvet ash, we favor the following. Velvet ash in Zion National Park may be tolerant of AshY MLOs, or MLOs may be present in many sampled trees in which they were not detected by the DAPI test. Either situation would result in the measurement similar growth rates in MLO-infected and "noninfected" groups.

ASSESSMENT OF SUSCEPTIBILITY OF SINGLELEAF ASH TO ASHY MLOS

Bark patches from roots of two diseased velvet ash saplings were grafted into the stems of 10 singleleaf ash in May, 1991, and this process was repeated with eight more trees in 1992. Several of the patches made union in each year. DAPI tests on root samples collected from the grafted trees in August 1992 revealed no evidence of MLO infection. This result could indicate either MLO resistance or lack of transmission of MLOs, because bark-patch grafts are relatively inefficient for AshY MLO transmission. Bark patches were used because budwood at an appropriate growth stage was not available.

IDENTIFICATION OF ASHY MLOS IN ZION PARK

Marginal effectiveness of the original approach to identification (dot hybridizations of DNA from velvet ash with AshY-specific DNA probes) has been indicated. AshY MLOs related to the New York strain AshY1 were detected in one out of 16 infected trees tested. Two new approaches are more promising. One involves diagnostic immunofluorescence microscopy utilizing an AshY-specific monoclonal antibody developed by collaborators J. R. Guo and T. A. Chen of Rutgers University (Guo & Chen 1993). Their antibody was prepared to MLO strain AshY2, which originated near Ithaca, NY. Its specificity for AshY MLOs (including those causing lilac witches'-broom (Hibben et al. 1991) has been verified in tests with approximately 18 MLO strains. We performed immunofluorescence tests on phloem from five velvet ash in Zion Canyon in which MLOs had been detected by DAPI tests. Positive, although weak, reactions were noted in two specimens, while controls remained negative. This result supports the interpretation based on DNA hybridization that the MLOs in velvet ash Zion Park are AshY MLOs closely related to those in other ash species and lilacs in the East.

The other promising approach to MLO identification involves RFLP (restriction fragment length polymorphism) analysis of MLO DNA amplified from extracts of total DNA of MLO-infected plants by PCR (polymerase chain reaction) (Ahrens & Seemüller 1992, Kirkpatrick et al. 1992, Lee et al. 1992). In brief, PCR is an enzymatically catalyzed exponential replication of DNA, primed by small synthetic oligonucleotides that have sequences corresponding to tiny portions of the DNA of interest. Through PCR, otherwise undetectable fragments of the DNA of interest, in this case MLO DNA, may be amplified up to quantities that are readily detectable and suitable for genetic analysis. DNA amplified by PCR can be digested with restriction endonucleases, resulting in characteristic numbers and sizes of smaller fragments. These numbers and sizes vary with the nucleotide sequence

of the parent fragment and the enzyme(s) used. Analysis of this variation (RFLP analysis) permits identification of the amplified fragment.

Our collaborator, Dr. I.-M. Lee, has designed oligonucleotide PCR primers that permit the amplification of a 1.2-kb fragment of the 16S ribosomal RNA gene of any MLO (Lee et al. 1992). Sufficient variation exists in the nucleotide sequence of this gene to permit the differentiation, through RFLP analysis, of MLOs that are known from previous research to represent different groups. Thus, AshY MLOs can be distinguished from all others (I.-M. Lee et al., unpublished). We have successfully used Lee's primers to amplify MLO DNA from MLO-infected white ash but not yet velvet ash.

SUSCEPTIBILITY OF WHITE ASH TO MLOS FROM VELVET ASH

In 1991 and again in 1992, segments of roots of diseased velvet ash saplings growing in Zion Canyon were used as sources of bark patches that were grafted into stems of 3- to 5-yr-old white ash (*F. americana*) seedlings in a greenhouse at Ithaca. Roots rather than stems were chosen as MLO sources because the titer of MLOs is likely to be higher in roots than stems of ash (Sinclair et al. 1992a). Unions formed between all bark patches and recipient stems. Up to the time of writing, however, only one transmission has been detected. The diseased white ash seedling died before the MLO strain in it could be propagated further.

SUSCEPTIBILITY OF VELVET ASH TO MLOS FROM WHITE ASH

Two experiments like those described in the preceding paragraph but involving attempted transfer of New York MLOs from white ash into velvet ash seedlings are underway. At the time of writing, one velvet ash seedling is confirmed as infected, but no external symptoms had been observed.

MYCOPLASMAL INFECTION IN PLANTS OTHER THAN ASH IN ZION PARK

Further search in May and August, 1992, for MLO-infected plants other than velvet ash in Zion Canyon was fruitless. Immunofluorescence tests with anti-AshY and anti-aster yellows monoclonal antibodies obtained from Guo and Chen were performed in 1992 on dried healthy and MLO-infected rabbitbrush collected in 1990. Results were negative. Thus, the MLO in rabbitbrush is probably are not an AshY MLO. PCR amplification and RFLP analysis of MLO DNA from the dried plants will be attempted for further information.

VECTOR DETECTION AND IDENTIFICATION

We collected leafhoppers from sticky traps in Zion Canyon during one week in August. As before, relatively few insects were obtained. They are preserved in acetone, awaiting processing for MLO detection. One identified species, *Scaphoideus lobatus*, is of special interest because it has also been trapped on sites of AshY occurrence in New York State.

CHEMOTHERAPY FOR SUPPRESSION OF ASHY

The experiments begun in 1991 were completed with negative results and were reported in an abstract (Sinclair & Griffiths 1991), the text of which is reproduced in part below.

Plavsic et al (1988) reported remission of symptoms caused by MLOs in tomato and potato plants after treatment of the plants with solutions of the cytokinin growth regular kinetin. We therefore tested kinetin on potted periwinkle (*Catharanthus roseus*) and white ash, healthy or infected with ash yellows MLOs. Periwinkle is an experimental host of many MLOs. Experiments were designed as randomized complete blocks. For white ash there were 2 concentrations of kinetin (and boiled distilled water as a control) x 3 methods of application (spray, soil drench, stem infusion) x 3 replications each of diseased and healthy plants. For periwinkle there were 3 concentrations of kinetin (and water control) x 2 methods of application (spray and drench) x 5 replications each of diseased and healthy plants. Solutions (0, 0.1, 1, or 5 μM kinetin) were applied as sprays (with c. 100 μL Tween 20 per liter as a wetting agent) or soil drenches daily for 7 days or were injected into stems of ash once. All plants remained unchanged in appearance for 2 months after treatment. Diseased ash and periwinkle had stunted shoots and leaves. Microscopic examination (DAPI tests) revealed no effect of treatment on populations of MLOs in periwinkle or ash phloem. The white ash trees then underwent a cycle of dormancy and

regrowth. All but one initially infected ash remained stunted and again tested positive with DAPI, while healthy plants showed no changes associated with treatments. Thus, kinetin is not useful for therapy of ash or periwinkle infected with ash yellows MLOs."

♦ CONTINUING STUDIES

ASHY INCIDENCE IN NORTH CREEK AND PARUNUWEAP CANYONS

Root samples collected in August, 1992, will be processed for MLO detection and determination of incidence levels.

IDENTIFICATION OF ASH YELLOWS MLOS IN ZION PARK

RFLP analysis will be performed on PCR products obtained by amplification of DNA from MLO-infected and healthy velvet ash. This procedure will yield a third and conclusive line of evidence about the relationship of MLOs in ash in Zion National Park to those in other ash species in the East.

VECTOR DETECTION AND IDENTIFICATION

The leafhopper collection from Zion Canyon will be used for attempts to detect AshY MLOs by immunofluorescence microscopy with an AshY-specific monoclonal antibody and by means of RFLP analysis of PCR-amplified MLO DNA.

SUSCEPTIBILITY OF VELVET ASH TO MLOS FROM WHITE ASH, AND VICE VERSA

Four greenhouse experiments will be evaluated in spring, 1993.

SUSCEPTIBILITY OF SINGLELEAF ASH TO ASHY MLOS

We will try to grow singleleaf ash seedlings from seed for grafting with diseased scions for possible transmission and evaluation of resistance.

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