CANOPY DECLINE ASSESSMENTS IN AMERICAN ELM AFTER INOCULATION WITH DIFFERENT DOSES OF *OPHIOSTOMA ULMI* AND *O. NOVO-ULMI*

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Abstract.—Restoration of American elm (*Ulmus americana* L.) in natural and urban landscapes necessitates the development of new selections that not only exhibit Dutch elm disease (DED, caused by the fungal pathogen *Ophiostoma novo-ulmi* and *O. ulmi*) tolerance, but also an increase the genetic variability of tolerant elms. Toward this end, our program tests DED tolerance of large survivor American elms, crosses between DED-tolerant American elms, and crosses between large survivor and DED-tolerant elms. Accurate phenotyping is critical to accurately assess DED-tolerance. This study examined 1) the effect of different DED pathogen doses; 2) American elm responses to two inoculation timings; and 3) the 8-week DED-induced canopy decline response of 29 American elms selections planted at the Delaware, OH, Forestry Sciences Laboratory. Results suggest a significant dose effect in which the treatment group receiving high levels of DED inoculum exhibited significantly more DED-induced foliar symptoms relative to trees receiving low dosage rates. Furthermore, there is considerable variability in the DED-induced canopy decline ratings associated with the timing of the inoculation. Finally, we observed differences in DED-induced canopy decline between selections of large survivor trees collected around the Midwest, indicating that unique tolerance mechanisms may be present in the natural elm population.

Introduction

The American elm (*Ulmus americana* L.) was once widely distributed throughout the eastern United States before the arrival of Dutch elm disease (DED), caused by the fungal pathogens *Ophiostoma ulmi* (Buisman) C. Nannf. and *O. novo-ulmi* Brasier. American elm's tall height coupled with its vase-like shape provides for a uniquely graceful tree that was commonly planted along city streets and boulevards. The crowns of mature elms spanned countless roadways, houses, and recreation areas, where they provided the benefits of cleaner air and cooler temperatures. American elm is one of the few native tree species capable of thriving in the harsh urban environment, where extreme summer temperatures, air pollution, and road salt are common. Before the invasion of DED, elm was an ecologically important tree species in riparian areas and bottomlands, stabilizing riparian slopes against seasonal flooding and enriching soils through the production of rapidly decomposable nutrient-rich leaf litter. Finally, its seeds were an important source of food for song birds and other early migratory birds, as elm seeds matured in the spring before most other seeds are available.

The DED fungal pathogen *O. ulmi* was introduced into the United States in 1930 and its spread has devastated North American species of elm, severely reducing the use of American elm as an urban shade tree. In Illinois in the 1940s the Eurasian race of *O. novo-ulmi* appeared causing a second wave of elm mortality. Research on American elm from the 1970s to the present has focused on the identification of American elm selections that could withstand the

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DED pathogen. Of the more than 100,000 American elm trees tested for resistance to DED, very few selections exhibited adequate levels of DED tolerance. While a few selections are commercially available, most of the elms purchased in the United States are 'Princeton' elms. The widespread use of few DED-tolerant clones presents the risk of another wave of elm mortality due to attacks by other pests/pathogens or mutation of the DED pathogen. Additional DED-tolerant selections representative of the genetic diversity of native American elm populations and suitable for both urban and forested settings are needed to ensure the long-term stability of DED-tolerance among American elm populations. Toward this goal, several research programs have carried out work on the selection and breeding of American elms (Schreiber and Domir 1994; Sherald 1993; Smalley et al. 1993; Smalley and Guries 1993; Townsend 2000; Townsend et al. 2005, 1995), though all have largely ended due to retirements and limited funding.

We are engaged in an ongoing study to identify and generate additional American elm selections that can tolerate DED pathogens. Our approach is twofold: to test DED tolerance of large surviving American elm trees, and to cross these elms with known DED-tolerant elms in order to develop genetically diverse and regionally adapted DED-tolerant American elm populations. This paper describes the results from three complementary experiments: 1) a DED inoculation trial of American elm selections with low, high, and very high doses of a mixture of *O. ulmi* and *O. novo-ulmi*; 2) an experiment investigating differential responses of American elm selections (Kuhar 1 and 2) inoculated in the early summer (June) and late summer (August); and 3) a test of the DED tolerance of 29 American elm selections.

Materials and Methods

To test the response of American elm selections to different DED pathogen dosage rates, six American elm clones from each selection were clonally propagated. Five of these selections (ND104, NR496, NV17, NR521, and NV463) are from DED-tolerant × DED-tolerant crosses and the sixth (SL32) is from a large survivor tree from Michigan (n=137, between 22 and 26 per selection). Elms were planted in two tree orchards at the Delaware, OH, U.S. Forest Service Forest Science Laboratory between 2005 and 2011. Elm trees were inoculated with a 50–50 mixture of *O. ulmi* and *O. novo-ulmi* spores on June 7 and 8, 2016. The inoculum was prepared a week in advance from frozen cultures of *O. ulmi* (strain PG442) and *O. novo-ulmi* (strain H961) as described in Pinchot et al. (in press). Trees in field plots received either a low DED dose of 6×10^5 *O. ulmi* and *O. novo-ulmi* spores, or a high dose of 1.2×10^6. A cordless drill with a 0.47-cm-diameter brad point bit was used to drill a 1.3-cm deep hole 30 cm from the base of trees, and the fungal spores were pipetted into the hole. The canopies of field-grown elms were cleared of any dead branches at the time of inoculation. As such, all trees had baseline measurements of 0 percent canopy decline. Each tree was remeasured 8 weeks post-inoculation. Canopies were rated at 5 percent decline classes (i.e., 0, 5, 10…95, 100 percent) for DED symptoms. Typical DED symptoms consist of foliar yellowing, wilting (flagging), and eventual browning as a branch dies. Because a subset of the trees was split between two tree orchards, we tested differences in the tree canopy decline ratings between the orchards with an analysis of variance (ANOVA) with orchard and selection (n=3) as the main factors. As the canopy decline of three well-replicated selections were similar between two tree orchards (ANOVA; P=0.082) this factor was excluded from all future analyses. Following this, we tested for a DED dosage treatment effect using a mixed model ANOVA with dose (low vs. high) and selection (ND104, NR496, NV17, NR521, NV463 and SL32) as our main factors and a dose*selection interaction. Differences within main factors were analyzed using post-hoc Tukey's honestly significant difference tests (α=0.05).

To test the response of American elms to the timing (and rate) of DED-inoculation, 10 Kuhar (1 and 2, n=5 each) trees were inoculated with a 50–50 mixture of *O. ulmi* and *O. novo-ulmi* spores. Trees were 9 and 12 years old depending on the time of inoculation; d.b.h.: 7.15 ± 0.56 cm [mean ± SE]. The methods outlined above were followed for the elms inoculated June 8, 2016. For the elms inoculated August 13, 2013, a total of 16×10^6 spores were placed into three equidistant holes drilled at a height of 1 m from the base of the tree. In each year, canopy decline was measured as described above at 8 weeks. To analyze differences between the foliar responses of Kuhars inoculated with DED at different times in the summer, we first utilized a t-test to analyze for differential decline between Kuhar 1 and Kuhar 2. No significance was found (P>0.05) and we compiled all data from each time for a two-tailed t-test of foliar decline between August 2013 and June 2016.

Finally, as part of a large-scale DED screening efforts, we clonally propagated large survivor American elm trees (n=29 selections) found in Michigan, Ohio, Illinois, and Indiana, in addition to American elm generated from DED-tolerant selections (cross progeny trees) as described above. These trees (n=497) were planted in replicated blocks and ranged in diameter from 1.2 to 13.9 cm at breast height (diameter at 1.27 m from ground; d.b.h.) and in height from 1.4 to 9.96 m. On June 7, 2016 (as described above) elms we inoculated with the low dose of DED inoculum (6×10^5 spores) to test differential responses to DED exposure. Again, we measured canopy decline after 8 weeks and compared the percentage canopy decline between selections using an ANOVA model with block nested within plot and selection as main effects, and d.b.h. as a covariate. Post-hoc pairwise comparisons of canopy decline between selections were conducted using Bonferroni adjustments (α=0.05).

Results & Discussion

We observed significantly lower rates of DED-induced canopy decline between trees inoculated with a low level of DED (6×10^5 spores, 14.4 percent foliar symptoms) relative to those inoculated with a high level (1.2×10^6 spores, 26.5 percent foliar symptoms) (Figure 1A; ANOVA P<0.001). As expected, we observed differential decline between the selections with SL32 (>45 percent foliar symptoms) and NV463 (25 percent) exhibiting the highest level of DED-induced foliar symptoms (Fig 1B; P<0.001). No significant interaction was observed, suggesting similar responses across all selections to the increased dose (P=0.079). Despite the lack of a significant interaction effect, the selections which exhibited the lowest levels of DED tolerance (NV463 and SL32) performed worse under the high DED inoculation rate relative to the low rate. Interestingly, there was not an enhanced decline in NV17 or NR521 to the increased DED dosage rate, suggesting opportunities for future exploration. Considering ongoing DED inoculation trials, the implications of this dosage effect suggest that researchers should consider rates such as the 1.2×10^6 spores used above to elicit stronger responses in elms. It should be considered however that the optimal dose may vary with DED strain and the ratio of *O. ulmi*: *O. novo-ulmi*.

It has long been suspected that there is a seasonal effect of DED and that exposure during the early summer (in part because of growth, acropetal water and nutrient transmission, and general physiology) may be more harmful to elms than a late summer/fall exposure (Pomerleau 1965, Smalley and Guries 1993). Our results indicate that early June exposure results in significantly enhanced canopy decline relative to late summer/fall exposure, even despite the difference in dosage rates within the study (6×10^5 in spring vs. 16×10^6 in fall, Figure 2). While our replication was low, these results suggest that when undertaking DED tolerance testing, care should be taken to challenge elms during the period when they are most susceptible.

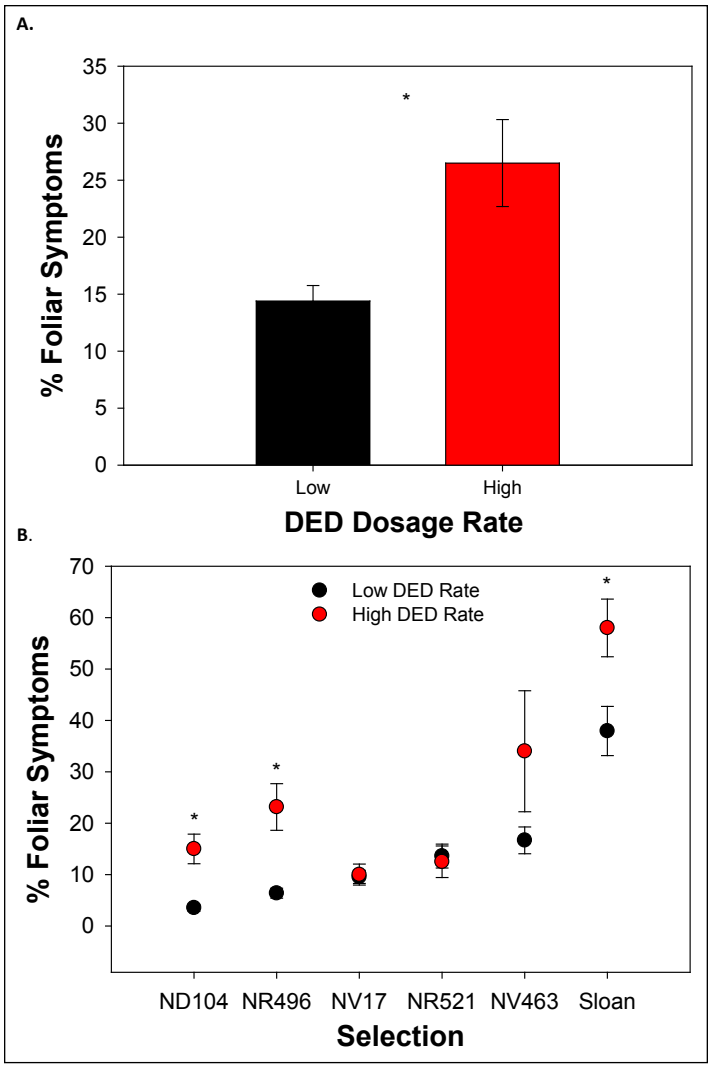

Figure 1.—A. Elm foliar symptoms 8-weeks following inoculation with low (black) and high (red) rates of *O. ulmi* and *O. novo-ulmi*. B. Differential elm foliar symptoms 8-weeks following DED inoculation in six selections exposed to low (black) and high (red) DED inoculation rates. Values represent means ± SE. Asterisk denotes significant difference between the low and high rate ($P<0.05$).

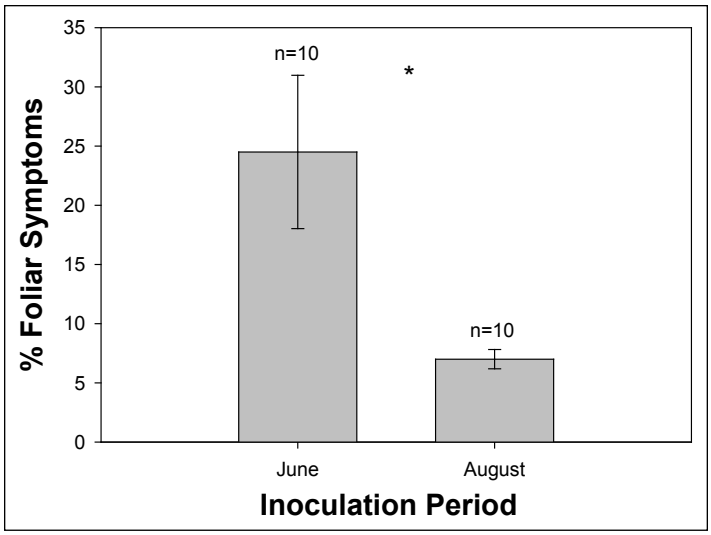

Figure 2.—Eight-week foliar symptoms following DED inoculation of Kuhar (1 & 2). Values represent means ± SE, asterisk denotes significant difference between June and August ($P=0.015$).

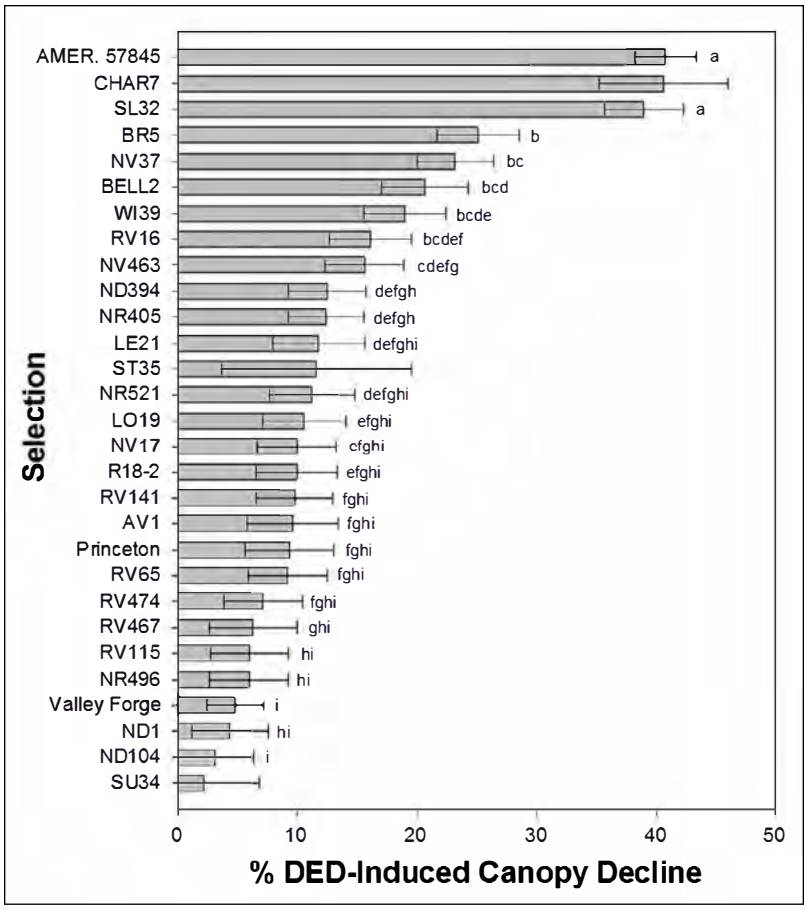

Figure 3.—Eight-week foliar symptoms following DED inoculation of American elm selections. Superscripts denote significant differences between cultivars (P<0.05), cultivars without letters were excluded from the model because of insufficient replication across blocks.

Results of our large-scale tree screening trial indicates considerable variability in canopy decline between the selections 8-weeks post-inoculation (Figure 3; ANOVA, P<0.001). Canopy decline ranged from <5 percent decline (in SU34, ND104, ND1, and 'Valley Forge') to ~40 percent (known susceptible Amer. 57845, SL32 and CHAR7). Furthermore, several selections performed as well as existing commercial cultivars ('Valley Forge' and 'Princeton'). The variability in performance highlights that moderate DED tolerance is exhibited in many selections and that continued breeding may enhance tolerance levels by stacking genes associated with tolerance mechanisms within new selections.

In summary, these results highlight the variability in decline symptoms that can be observed during DED inoculations conducted under differing conditions. To make DED-inoculation data cross comparable between studies, care must be taken to inoculate individuals at a similar time of year and with a consistent amount of inoculum. Findings herein suggest that the high inoculation rate (1.2×10^6) elicits a higher decline rate (relative to the low rate), and thus produces a more stringent tolerance test. More testing should be conducted to compare different strains and investigate a strain x dose interaction. The seasonal effect described herein should be used to guide optimal inoculation times for tolerance trials and suggests that early season inoculations elicit a higher response. Finally, results from the 2016 elm screening indicate considerable variability in the DED tolerance and that several large surviving elms performed as well as the commercially available American elms ('Valley Forge' and 'Princeton').

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