Development of New Dutch Elm Disease-Tolerant Selections for Restoration of the American Elm in Urban and Forested Landscapes[1]

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Abstract

The goal of our research and development efforts is to restore American elm (*Ulmus americana*) as a species in both natural and urban landscapes. Accomplishing this goal requires identification/generation of additional American elm cultivars that are tolerant to Dutch elm disease (DED) caused by *Ophiostoma ulmi* and *O. novo-ulmi*, and development of methods to reintroduce American elm along the rural to urban gradient. To accomplish our goal we are screening large survivor trees for DED tolerance, generating DED-tolerant/site-adapted cultivars, generating three regional seed orchards, establishing experimental American elm restoration sites, using elm in the Appalachian Regional Reforestation Initiative, performing operational trials using elm seed along the Mississippi River, comparing local vs. DED-tolerant enriched seedlings, investigating fungal transmission in DED-tolerant selections, and investigating the basis for cold-hardiness in the American elm and the genetic as well as metabolic basis of DED tolerance. In the spring of 2016, branches containing flower buds were collected from large surviving American elm trees in New England. Pollen was collected from the flowers and was used in controlled pollinations with the DED-tolerant American elm cultivars 'Delaware 2', 'Princeton', R18-2, and 'Valley Forge'. The mean numbers of seeds produced per cross for each DED-tolerant mother tree ranged from 0.1 to 23 per flower. Scion wood was collected from the branches and used to graft buds and scion wood to potted American elm root stock using several types of grafting techniques. Veneer and top grafting had the highest success rates (30 percent and 22 percent, respectively), followed by bottle grafts (12 percent) and bud grafts (6 percent). Top grafts, veneer grafts, and bud grafts produced the greatest growth and number of cuttings. Plants growing from successful grafts were used to generate clones by vegetative cuttings using the rooting hormone indol-3-butyric acid at varying concentrations. Sixty-five percent of the cuttings had rooted by early August, and 42 percent of the trees that rooted had also produced shoots. Clonally propagated American elm selections generated from crosses among DED-tolerant cultivars, clones of large survivor trees found in Michigan, Ohio, Illinois, and Indiana, and susceptible and tolerant controls were inoculated with a mixture of *O. novo-ulmi* and *O. ulmi* spores. For each tree, the combined percentage of the crown exhibiting wilting, chlorosis, or necrosis was visually estimated to the nearest 5 percent. The percentage of the crown exhibiting DED symptoms in trees from the Midwest and New England inoculated trees ranged from 0 to 35 percent at 4 weeks post-inoculation. Control trees (inoculated with water) in both studies exhibited from 0 to 5 percent canopy decline. Trees with high levels of DED tolerance will be released to the tree nursery industry and retained in test plots to constitute a seed orchard. Over the next 6 years approximately 16,000 elm trees—clones of survivor trees and site-adapted progeny from crosses between DED-tolerant elms and elms from the upper Midwest and New England states—will be inoculated.

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Introduction

The American elm (*Ulmus americana*) was once widely distributed throughout the eastern United States before the arrival of Dutch elm disease (DED) caused by the fungal pathogens *Ophiostoma ulmi* and *O. novo-ulmi*. 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 within 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, bottomlands, and the urban environment, serving to enrich soils through the rapid decomposition of its nutrient-rich leaf litter. Its seeds were an important source of food for song 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, nearly eliminating 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 focused on the identification of American elm cultivars that could withstand the DED pathogen. Of the over 100,000 American elm trees tested for resistance to DED, only nine cultivars exhibited adequate levels of DED tolerance. While a few cultivars are commercially available, about 90 percent of the elms purchased in the United States are the 'Princeton' cultivar. The widespread use of one DED-tolerant cultivar presents the risk of another wave of elm mortality due to attacks by other pests/pathogens or the mutation of DED. Additional DED-tolerant cultivars 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 and Guries 1993, Smalley et al. 1993, Townsend et al. 1995, Townsend 2000, Townsend et al. 2005), though all have largely ended due to 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 to clonally propagate survivor elm trees, cross them with known DED-tolerant selections and to test the clones and crosses for tolerance to DED. This paper focuses on methods used for collection of pollen, controlled pollinations in the field, clonal propagation through grafting and vegetative cuttings, inoculation of American elm selections—both orchard trees (5 to 7 years old) and small potted trees (1-year-old)—with *O. ulmi* and *O. novo-ulmi*, and evaluation of foliar stress metabolite concentrations as an early screening marker for DED tolerance. The results of the controlled pollinations, preliminary results of vegetative propagation, foliar symptoms observed at 4 weeks post-inoculation with DED, and pre-inoculation concentrations of foliar stress metabolites are described.

Polyamines (PAs) are a group of metabolites including a diamine putrescine (Put) whose cellular concentrations fluctuate significantly over and above baseline background variations when plants are under stress - making it an ideal metabolite for early detection monitoring of stress (Minocha et al. 2014). Two other common polyamines are spermidine, and spermine. These are small, positively charged, organic molecules that are found in all living organisms. Their simple structure, universal distribution in all cellular compartments, and involvement in various physiological processes is why fluctuations in their cellular concentrations are often related to varied responses of plants to different forms of stress. A strong positive relationship between abiotic stress and foliar putrescine has been proposed as a potential biochemical marker of persistent environmental stress in several species of trees where phenotypic symptoms of stress were not yet visible. Various types of stress lead to a modulation of putrescine levels, suggesting Put is a reliable indicator of cellular functional adjustments. An increase in putrescine indicates resistance to a stress and a decrease upon reversal of stress means amelioration from stress (Minocha et al. 2010, Wargo et al. 2002). Despite continued interest in polyamine metabolism in plants

subjected to abiotic stresses, work on polyamine metabolism relative to plant-pathogen interactions lags behind (Walters 2000, 2003). In several preliminary, yet unpublished, studies conducted in the Minocha laboratory using different tree species, higher concentrations of putrescine were found to be associated with tolerance to biotic stress at different forested sites (fig. 1). Metabolic markers respond to environmental and microsite soil chemistry differences related to infestation and tolerance.

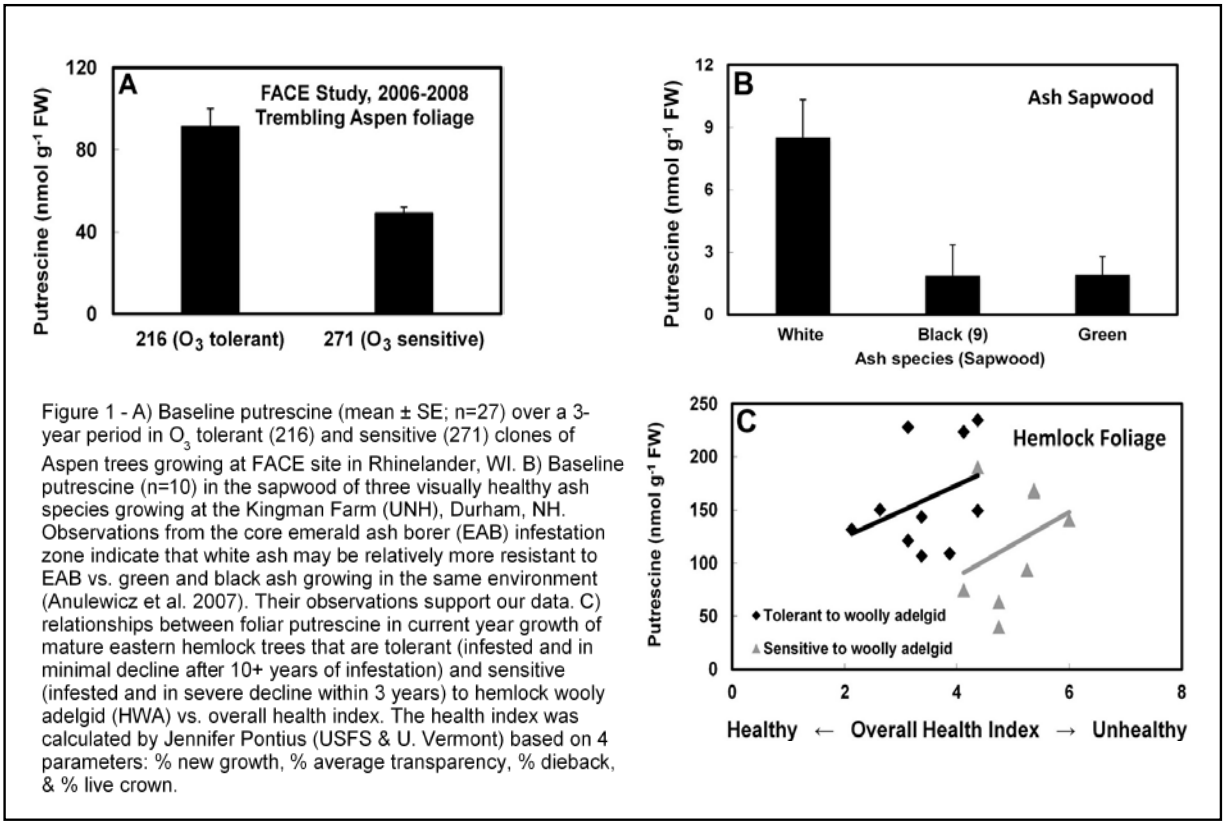

Figure 1 - A) Baseline putrescine (mean ± SE; n=27) over a 3-year period in O_3 tolerant (216) and sensitive (271) clones of Aspen trees growing at FACE site in Rhinelander, WI. B) Baseline putrescine (n=10) in the sapwood of three visually healthy ash species growing at the Kingman Farm (UNH), Durham, NH. Observations from the core emerald ash borer (EAB) infestation zone indicate that white ash may be relatively more resistant to EAB vs. green and black ash growing in the same environment (Anulewicz et al. 2007). Their observations support our data. C) relationships between foliar putrescine in current year growth of mature eastern hemlock trees that are tolerant (infested and in minimal decline after 10+ years of infestation) and sensitive (infested and in severe decline within 3 years) to hemlock wooly adelgid (HWA) vs. overall health index. The health index was calculated by Jennifer Pontius (USFS & U. Vermont) based on 4 parameters: % new growth, % average transparency, % dieback, & % live crown.

Thus, such markers will be very useful in following the physiological response of selected genotypes to disease tolerance (based on phenotype, e.g., historic survival – if available) as well as the development of management practices to ensure the survival of plantations. Changes in a biochemical marker due to disease infection appear much sooner than the appearance of visual symptoms, making its detection a faster way to assay tolerance than rating foliar symptoms. Additionally, markers can be used to detect differences in tolerance levels in the absence of infection, eliminating the need to inoculate the trees. Microsite factors, such as soil moisture and light availability will also affect levels of biochemical markers, however an effective marker will distinguish susceptible vs. tolerant elms even with variation caused by site differences. In situations where an identified genetic marker of resistance turns out to be a functional gene, epigenetic changes could affect its expression, and in that case the phenotype may no longer match with the presence of this genetic marker. Under such conditions, and in situations where no genetic markers of resistance have yet been identified, we may have to rely on additional metabolic markers to differentiate between ranges of tolerance to a disease among individuals within a clone or even within a species; the clonal material provides unique opportunities in this regard.

Materials and Methods

Pollen Collection, Controlled Pollinations, and Seed Production

During the spring of 2016, branches with flower buds from exceptionally large surviving American elms in New England states were collected and shipped to Ohio (table 1). We attempted to have elms

represented from a large geographic area, where we defined populations in terms of watersheds because American elm is primarily a riparian species in New England. In selecting trees, we used size as a proxy for age, so by considering size and isolation from other elms we hoped to select trees that were likely exposed to Dutch elm disease multiple times over their lives. We collected from elms only where we had permission from the landowner and only if the tree had sufficient flower buds at the time of collection (in early March just before flower buds open). Elms growing in the milder climate of the Lower Connecticut River region were taller and younger at the same diameter than elms from the Upper Connecticut River region. The 21 elms selected varied considerably in easily observable traits like branch architecture and bark, probably reflecting genetic diversity.

Table 1—Summary of American elms from which scions were collected (DBH = diameter at breast height (i.e.1.4 m (4.5 ft) above ground level (Isolation codes are: 0 for trees <30 m (100 ft), 1 for trees <91 m (300 ft), and 2 for trees >91 m (300 ft) from adjacent elms (live or dead), if present. Lower Connecticut River is defined as downstream of Turner's Falls Dam in MA. Upper Connecticut River is defined as upstream of Wilder dam in VT)

Number	Population	State	DBH cm (in)	Height m (ft)	Isolation
30	Housatonic River Valley	MA	94 (37)	22 (72)	0
29	Housatonic River Valley	CT	74 (29)	17 (56)	0
42	Lake Champlain Valley	VT	131 (52)	22 (72)	0
43	Lake Champlain Valley	VT	107 (42)		1
44	Lower Connecticut River	MA	128 (51)		1
45	Lower Connecticut River	MA	114 (45)	25 (82)	1
36	Lower Connecticut River	CT	107 (42)	26.5 (87)	1
32	Lower Connecticut River	MA	93 (36)	24 (79)	1
38	Lower Connecticut River	MA	75 (30)	26.5 (87)	0
31	Lower Connecticut River	MA	73 (29)		1
37	Lower Connecticut River	MA	61 (24)	30.7 (100)	0
33	Middle Connecticut River	VT	186 (73)	24 (79)	1
27	Middle Connecticut River	MA	111 (44)		1
25	Middle Connecticut River	MA	107 (42)	23 (76)	0
28	Middle Connecticut River	MA	80 (31)	17.5 (57)	0
34	Middle Connecticut River	VT	77 (30)	22.5 (74)	0
35	Middle Connecticut River	VT	75 (30)	20.5 (67)	1
26	Middle Connecticut River	MA	40 (16)		0
41	Upper Connecticut River	NH	91 (36)	23.5 (77)	2
39	Upper Connecticut River	VT	90 (35)	17 (56)	2
40	Upper Connecticut River	VT	81 (32)	16.6 (55)	0

To prevent seed predation by insects, a mixture of dormant oil and carbaryl was prepared using the manufacturer's specifications, were sprayed on the elm branches and allowed to dry. Prior to spraying, scion wood was collected from branch tips for use in grafting. The cut end of the branches were recut while submerged in water to prevent embolism, then disinfected by dipping in 10,000 ppm activated chlorine dioxide (Bio-Cide International), and placed in flasks containing water (fig. 2). Flasks were placed on tables covered with dry wax paper sheets and enclosed with a wood frame covered with plastic sheeting (to reduce airborne pollen transmission). The released pollen was collected by scraping the wax paper sheets with razor blades, placing the pollen in plastic vials that were then placed in 50 ml plastic tubes containing a desiccant. Individual flowers were counted on select branches of mature DED-tolerant elms cultivars ('Delaware 2', 'Princeton', R18-2 and 'Valley Forge'). Pollination bags were placed over these branches on March 2nd and 3rd with the assistance of a canopy lift. Pollen was added to the bags on March 9 through March15 (fig. 2), depending of the stage of flower development. A small hole was made at the top of the pollination bag and an inflator needle was inserted into the bottom of the pollination bag.

The nozzle was attached to the controller of an air compressor which was set to 138 kPa (20 PSI). Upon addition of pollen through the top hole, bursts of air were used to distribute the pollen to the flower heads within the pollination bags, and duct tape was applied to the bags to cover the holes. Pollination bags were collected on April 19th and 20th using a pole pruner. Seeds were removed from the bags, inspected for damage, and counted.

Figure 2 - American Elm pollen collection station (left) and pipetting pollen collected from survivor American elm into pollination bag (right). Photo credit: K. Lehtoma

Clonal Propagation of Trees Using Grafting and Cuttings

Branches with at least three vegetative buds were dipped in wax, placed in plastic bags, and refrigerated for 2 to 4 weeks. One-year-old containerized dormant elms from multiple selections of unrelated trees were used as root stock. Five root stocks were used for each of the 21 survivor tree selections, with multiple grafts attempted on each root stock. Multiple grafting techniques, including top (cleft) grafting, veneer grafting, bottle grafting, and bud grafts (chip budding) were used to propagate the elm selections in March 2016 (Garner 2013, Winieski 1959). A top grafting tool was used to make identical “V” cuts in the root stock and scion; care was taken to match the diameter of the stems (fig. 3). Veneer grafts and bud grafts were performed using a grafting knife, with the cambium layer aligned on at least one side of the graft junction. Bottle grafts were performed using a grafting knife using an “approach grafting” technique and tubes of water to support the grafted scion. Bud grafts were affixed with budding tape. The other grafts were tied with grafting bands and painted with wax. In mid-April, the budding tape was removed. When the grafts were well calloused, grafting bands were removed to prevent girdling on rapidly expanding branches. Growing grafts were supported with stretch fabric bands to prevent them from breaking. Pots were kept in the greenhouse, watered regularly, and supplied with 20-20-20 fertilizer (diluted to 350 ppm) once the scion was growing. Greenhouse pests and diseases were controlled using insecticide sprays and antifungal drenches as needed.

Figure 3 – Photographs depicting a top graft (left), tan buds on a branch indicating its suitability for taking cuttings (center), and rooted American elm cuttings (right).

Most of the grafts that calloused grew rapidly. When the buds on the new growth began to turn tan (fig. 3), we took cuttings from the new growth (leaving some buds to grow). Cut branches were submerged in water and cut into sections with three to four buds per section of stem. At least half of each leaf was cut off to reduce transpiration. The bottom of each section was stripped of bark in at least two areas, dipped in rooting hormone, and placed in Oasis Growing Medium Wedge Strips® (#5656) in a mist bed. For each selection, we treated some cuttings with each of three different concentrations of powdered indol-3-butyric acid hormone: 0.3 percent, 0.8 percent, and 1.6 percent (Hormex®). After a few weeks in the mist beds, cuttings were supplied with 10-30-20 fertilizer (diluted to 350 ppm). After 3 weeks, cuttings were checked weekly for roots (fig. 3). Rooted cuttings were potted into 7 cm x 25 cm pots containing a mixture of potting soil and fertilizer (1 bag Fafard 3B® potting mix, 300 g Osmocote®, 61 g Micromax® nutrients) and supplied weekly with 10-30-20 fertilizer (diluted to 350 ppm). Cuttings usually rooted in 3 to 4 weeks and developed shoots several weeks thereafter.

Inoculation of American Elm Trees in Pots and in Field Plots

Survivor American elm trees from Illinois, Indiana, Ohio, and Michigan were clonally propagated between 2006 and 2010 and planted in replicated plots at the U.S. Department of Agriculture Forest Service (USDA FS) Delaware, Ohio laboratory. In 2015, rooted cuttings from each tree were generated and transplanted into 3.8 liter (1 gal) pots. Results of inoculation experiments on planted and potted trees of the same genotypes will be compared to determine whether potted trees can be used in an early screening for DED. The trees generated from clonal propagation and crosses from New England survivor elm trees, described in the earlier sections of this paper, will be screened using these techniques in 5 to 7 years.

American elm trees, including susceptible and tolerant controls, were inoculated with a 50/50 mixture of *O. ulmi* (PG442 strain) and *O. novo-ulmi* (H961 strain) spores on June 7 and 8, 2016. The inoculum was prepared a week in advance as follows: frozen cultures of *O. ulmi* (strain PG442) and *O. novo-ulmi* (strain H961) were thawed and spread on separate potato dextrose agar plates, 50 µl/plate, and nine plates/isolate. The plates were kept dark and at room temperature. Fungal spores were harvested after 11 days of growth by addition of sterile deionized water to the plate surface. The surface was scraped gently with a bent glass rod and the spores of each isolate were removed to a separate sterile 50 ml conical tube. Fungal spore concentrations for each isolate were determined using a hemocytometer. The final 50/50 concentration of spores was adjusted to a volume appropriate for the inoculation of trees. Trees in field

plots received either 6×10^5 or 1.2×10^6 *O. ulmi* and *O. novo-ulmi* spores and potted elms were inoculated with a total of 2.8×10^4 spores. A cordless drill containing a 0.5 cm (3/16 inch) diameter brad point bit was used to drill a 1.3 cm (1/2 inch) deep hole 30 cm (12 inch) from the base of trees located in field plots, and the fungal spores were pipetted into the hole. A 0.2 cm (1/16 inch) diameter bit was used to drill a 0.6 cm (1/4 inch) deep hole 15 cm (6 inch) from the base of potted trees and the fungal spores were pipetted into the hole.

Canopy Decline Measurements

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 re-measured 4 weeks post-inoculation (early July). 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 the branch dies.

Analysis of Polyamines

Foliage was collected from several DED-tolerant and -susceptible elms in two replicated field plots 7 days before inoculation with DED and processed as described previously (Minocha and Long 2004, Minocha et al. 2000). Undamaged leaves from the sun-exposed upper canopy were sampled between 8:30 am to 5:30 pm. Both treatment plots were sampled at the same time for each clone. Samples (100 mg) collected in 5 percent perchloric acid (PCA) were extracted by three freezing and thawing cycles using procedures described previously (Minocha et al. 1994, Minocha et al. 2000) and were stored at -20 ˚C until they were analyzed. For analyses of PAs, the supernatant of the PCA extracted samples was subjected to dansylation and quantitation by HPLC (PerkinElmer Inc., Waltham MA) using previously published protocols (Minocha and Long 2004, Minocha et al. 2000). We have used these procedures for polyamine analysis in over 30 species including algae, fungi, plant cell cultures, herbaceous plants, young and old trees, and animal cells.

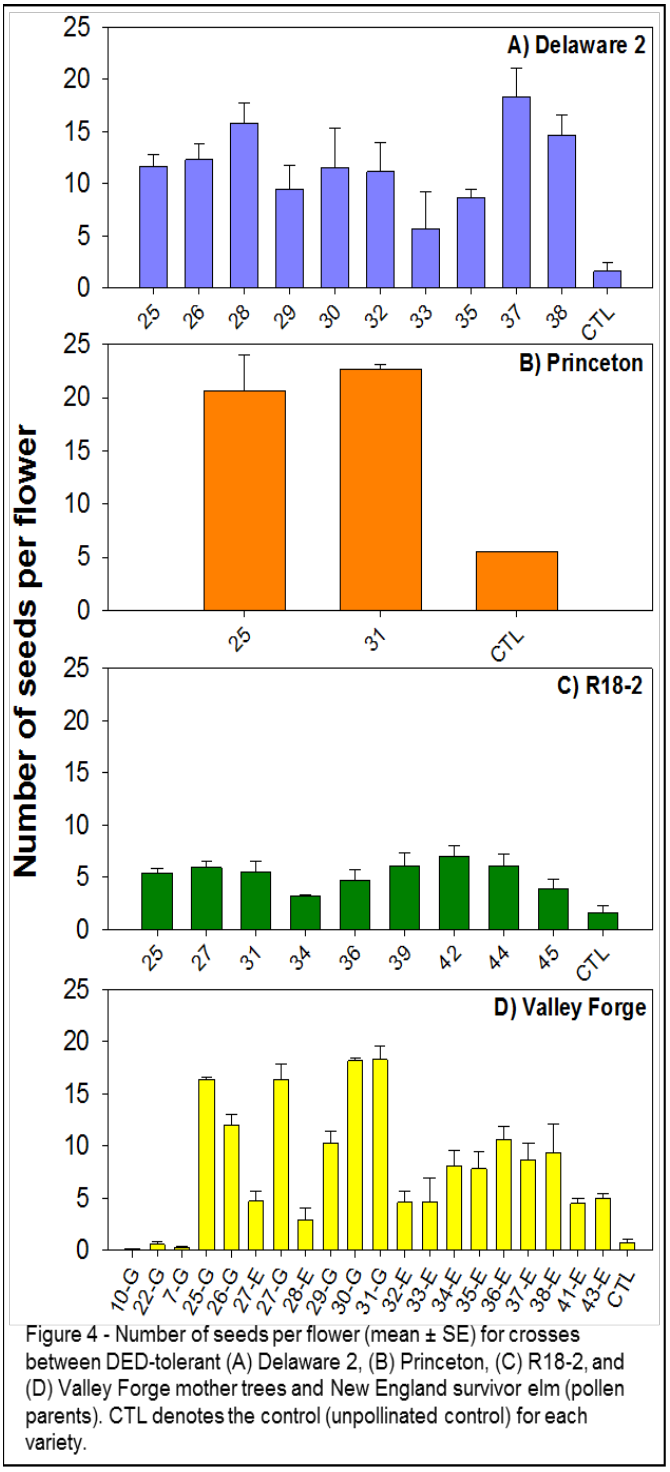

Figure 4 - Number of seeds per flower (mean ± SE) for crosses between DED-tolerant (A) Delaware 2, (B) Princeton, (C) R18-2, and (D) Valley Forge mother trees and New England survivor elm (pollen parents). CTL denotes the control (unpollinated control) for each variety.

Results and Discussion

Pollen Collection, Controlled Pollinations, and Seed Production

Between 0.5 and 8 mL of pollen was collected from cut branches of each of the 21 survivor trees. A total of 37 crosses made with four DED-tolerant mother trees and 20 pollen parents yielded approximately 42,000 seeds. The number of seeds produced by 'Delaware 2' × New England survivor tree crosses ranged from about 5 to18 seeds per flower. In contrast, unpollinated bagged controls averaged 1.6 seeds per flower. Each of the crosses performed with 'Delaware 2' (with 10 New England survivor elm pollen parents) was successful and produced viable seed (fig. 4A). The 'Princeton' × New England trees crosses produced between 20 to 23 seeds per flower, the greatest of all performed crosses (fig. 4B). The high number of seeds produced by these crosses was likely due to placement of the pollen bag over the branches a day late as evidenced by the high seed count in the control bags. The number of seed produced by the R18-2 × New England survivor tree crosses was in general lower than the other crosses (fig. 4C) except a few of the 'Valley Forge' × New England survivor tree crosses (7, 10, and 22; fig. 4D). The low seed production for those crosses was likely due to the use of pollen that was 3 years old. In previous years we have successfully used 2-year-old pollen that was stored in the refrigerator in a tube with desiccant. Seed generated by the 'Valley Forge' mother tree growing at the G site generally yielded more seed compared to 'Valley Forge' trees at the E site (fig. 4D).

Clonal Propagation of Trees Using Grafting and Cuttings

Thirteen out of 21 survivor tree selections were successfully grafted. Some of the unsuccessful grafts had poor quality scion wood, including small diameter twigs, dead twigs, and scale insect infestation (which we removed with floss). Grafts of unsuccessful trees will be attempted again in future years. All grafting techniques produced some successful grafts. Grafting success with different techniques varied among those doing the grafting. Overall, top grafting and veneer grafting were the most successful techniques (table 2). These two grafting techniques had also produced the largest number of cuttings per graft by July 21 (table 2). The bud grafts were initially slower to produce cuttings, but by mid-July they were growing rapidly and will likely produce cuttings as successfully as top graft and veneer graft techniques by later in the season. The bottle graft technique was not as successful as hoped. We were unable to get as good a graft union with this technique, and the water in the bottle may have grown fungus or bacteria that rotted the scion.

Table 2—Success of different grafting techniques attempted on survivor elm selections

	Bottle graft	Bud graft	Top graft	Veneer graft
Number of grafts attempted	33	338	93	44
Number of grafts that took	4	20	20	13
Grafting success rate	12%	6%	22%	30%
Number of cuttings	7	121	168	96
Average cuttings per graft	1.8	6.1	8.4	7.4

Grafted scions were at the right stage to begin taking cuttings by mid-May to early June for most selections (table 3). We produced rooted cuttings from all 13 of the survivor tree selections that were successfully grafted. A total of 581 cuttings were made and placed in mist beds. By August 2, 380 of these had produced roots, and 160 of the rooted cuttings had produced shoots (table 3). We expect that many more of the cuttings will produce roots and shoots by the end of the growing season. Some of the selections have produced many more cuttings than others because these selections had more successful grafts or because their grafts grew faster.

Metabolic Marker

Preliminary data from the Minocha laboratory on the effects of biotic stress on polyamine metabolism indicated that relatively higher disease/environmental stress tolerant aspen (*Populus*) clones (fig. 1A) as well as ash (*Fraxinus*) (fig. 1B) and hemlock (*Tsuga*) species (fig. 1C) had higher concentrations of foliar putrescine. This encouraged us to further investigate the relationship between foliar putrescine and biotic stress tolerance.

Table 3—Status of cuttings from 13 selections of survivor trees as of August 2 2016

Selection	Cuttings stuck (N)	Date of first cuttings	8/2 rooted (N)	8/2 shooted (N)
26	27	26-May	13	5
28	100	26-May	44	5
28a	74	26-May	42	2
30	37	23-May	30	2
32	11	9-Jun	11	10
33	23	2-Jun	16	3
34	47	26-May	38	22
35	79	12-May	47	34
36	5	17-Jun	5	1
39	25	23-May	20	12
41	9	26-May	5	2
42	37	26-May	15	15
44	57	17-Jun	56	13
45	50	17-Jun	38	34
TOTAL	581		380	160

Preliminary data from the pre-inoculation samples analyzed for putrescine show differences in the cellular concentrations of Put among different clones (fig. 5). However, there were no significant differences among trees sampled from control or DED designated plots at time zero. The samples for a few clones were taken from replicated plots for two DED-tolerance studies (known as cross progeny and Lesser) but these data were pooled since there were no significant differences by location of the trees within a single treatment (data not shown). Further analyses of samples collected each week post-inoculation will reveal whether these inherent differences among clones are further modified with exposure to DED or remain the same as shown in fig. 5. At this point, however, there seems to be some relationship of these data with observed percent decline in these clones at week 4 post-inoculation, indicating that there may be a correlation between homeostatic levels of cellular Put and disease tolerance (fig. 5). Although only four selections were examined in this study, results suggest that disease tolerance may be correlated with higher putrescine concentrations.

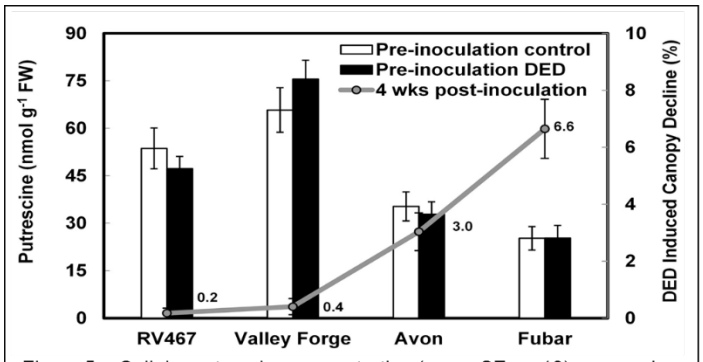

Figure 5 – Cellular putrescine concentration (mean SE; n=10) one week pre-inoculation in 4 different American elm clones. Overlaying this bar graph is the % canopy decline observed in these clones at 4-weeks post-inoculation. Whereas Fubar is known to have low tolerance to DED exposure, Valley Forge and RV467 have high tolerance. Avon's tolerance level in the long-term is yet to be determined.

Canopy Decline Measurements

Dutch elm disease-induced canopy decline results 4 weeks post-inoculation show considerable variation in the susceptibility of the different elm varieties under cultivation at the USDA FS laboratory in Delaware, Ohio (fig. 6). Several varieties (RV141, ND104, RV467, 'Sunfield', RV65, NR405, RV16 and ND1) perform comparably to known DED-tolerant varieties ['Princeton' (PRN) and 'Valley Forge' (VF)]. 'Sloan', 'Kuhar 2', 'Charlotte' and 'Braun' selections had higher canopy symptoms than the susceptible control used in this study (Amer. 57845; fig. 6). Additional readings will be obtained at 8 weeks and 1 year post-inoculation. Preliminary results from potted elm inoculations indicate that there are no differences in height or root collar diameter growth between DED-tolerant and susceptible elms, nor between elms inoculated with DED or water. Measurements of xylem discoloration suggestive of DED-infection will be collected later this growing season and may be more informative regarding potential DED tolerance.

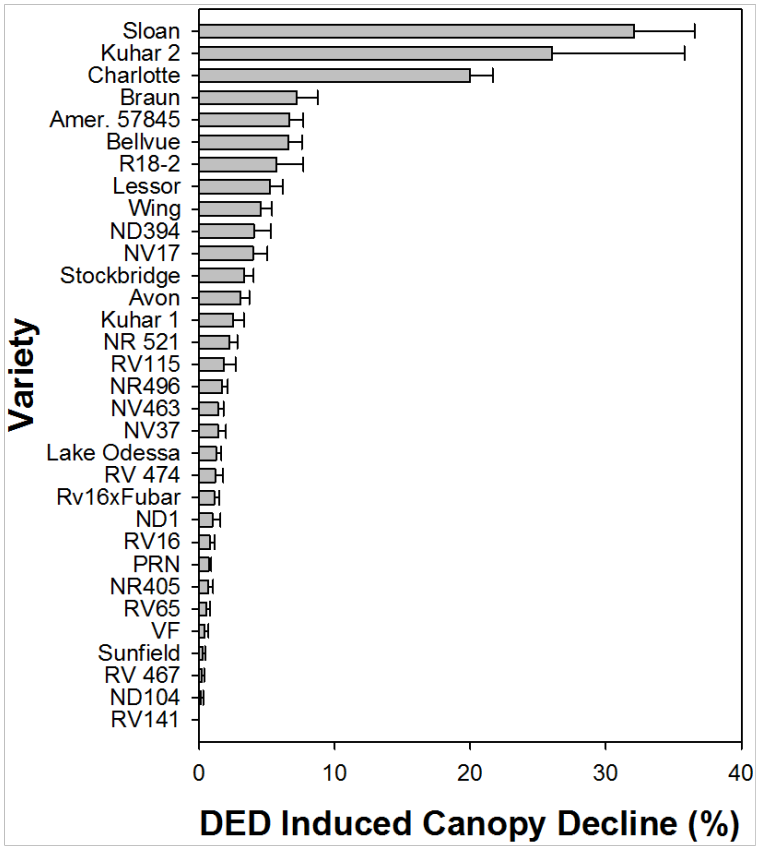

Figure 6 - DED-induced canopy decline (%) across different elm varieties (mean ± SE).

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