

# Generation of American Elm Trees with Tolerance to Dutch Elm Disease Through Controlled Crosses and Selection

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## Abstract

The goal of our research and development efforts is to generate new and/or improved selections of the American elm (*Ulmus americana* L.) with tolerance/resistance to Dutch elm disease (DED). The approaches we are taking for this effort include: 1) controlled breeding using known DED -tolerant selections, 2) controlled breeding using DED-tolerant selections and local escape/survivor trees from specific geographic areas to generate site-adapted and DED-tolerant selections, 3) screening of escape/survivor trees for tolerance to DED, and 4) identification of additional American elm escape/survivor trees across its range. Our efforts in these areas are described. Progeny trees generated from crosses of Delaware 2 x New Harmony, Valley Forge x New Harmony, R18-2 x New Harmony, and Valley Forge x R18-2 exhibited a significantly lower mean of foliar symptoms at 8 to 9 weeks compared to the susceptible control.

*Key words:* American elm, *Ulmus americana*, Dutch elm disease resistance, tolerance

## Introduction

Dutch elm disease (DED) has devastated North American species of elm, nearly eliminating the use of American elm (*Ulmus americana* L.) as an urban shade tree. There is a need for multiple, genetically diverse, American elm cultivars with superior tolerance to DED to be used by arborists and nurseries for urban plantings as shade trees and for the restoration of the American elm in forests. A few American elm selections have been identified and released to the nursery industry. The Princeton cultivar is the most sold cultivar by far in the United States (Sherald 1993, Smalley et al. 1993, Townsend and Douglass 2001, Townsend et al. 1995, Townsend et al. 2005).

Previous attempts at selective breeding of American elm resulted in low levels of resistance (Lester 1969a, 1969b). In Wisconsin, a group led by Smalley and Guries (1993) crossed 29 trees yielding more than 3,000 progeny. After inoculation screenings of vigorous individuals multiplied for clonal trials, six trees were selected to comprise the American Liberty Multiclone and one of these was patented as the Independence elm. Unfortunately, the DED tolerance of American Liberty has subsequently been extensively tested and found to be unexceptional (Townsend and Douglass 2001, Townsend et al. 1995, Townsend et al. 2005).

Several American elm clones have tested with higher DED tolerance than the American Liberty. Valley Forge showed the best tolerance of all clones, but significant tolerance was also measured for New Harmony, Princeton, R18-2, and Delaware 2 (Townsend and Douglass 2001, Townsend et al. 1995, Townsend et al. 2005). The different levels of foliar symptoms and crown dieback may suggest that multiple distinct genes are involved in conferring tolerance to DED. If complementary DED tolerance genes from both parental trees were placed in a progeny tree through crossing, the progeny could exhibit greater tolerance to DED than the parents.

There have been about 80 years of DED pressure on American elms in the landscape. Consequently, there is an increasing likelihood that the remaining large trees (0.9 to 1.2 m diameter at breast height) have tolerance to DED and the screening of these trees could be fruitful.

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## Materials and Methods

Controlled breeding was carried out in the laboratory with cut branches and/or with field-planted trees. The selections listed in table 1 have been used to date.

**Table 1—DED tolerant, escape/survivor, and elm yellow escape/survivor selections used**

Known DED tolerant	Escape/survivor trees on the Chippewa NF	Escape/survivor trees in Massachusetts & Connecticut
Princeton (Pr)	Black Duck (BD)	Hadley (HA)
Valley Forge (VF)	SO 1	Rainbow Bridge 1 (RB1)
New Harmony (NH)	SO 2	Goff (GF)
Delaware 2 (Del2)	Walker (WR)	Rainbow Bridge 2 (RB2)
R18-2 (18-2)		
8630 (elm yellows survivor)		

Pollination bags were placed on branches on trees in the field prior to flower bud opening. Branches from trees were collected, brought into the laboratory, and pollen was collected after pollen drop. Pollen was used to pollinate flowers within bagged branches on trees in the field and flowers on branches in the laboratory. The controlled pollinations performed to date include:

VF ♀ x 18-2 ♂, 18-2 ♀ x VF ♂, VF ♀ x SO 2 ♂, 18-2 ♀ x SO 2 ♂, 18-2 ♀ x GF ♂  
 VF ♀ x NH ♂, NH ♀ x Del2 ♂, VF ♀ x WR ♂, Del 2 ♀ x BD ♂, 18-2 ♀ x RB 2 ♂  
 R18-2 ♀ x NH ♂, VF ♀ x 8630 ♂, 18-2 ♀ x SO 1 ♂, Pr ♀ x SO 2 ♂, VF ♀ x HA ♂  
 Del2 ♀ x NH ♂, VF ♀ x BD ♂, 18-2 ♀ x WR ♂, 18-2 ♀ x HA ♂, VF ♀ x RB 1 ♂  
 Pr ♀ x VF ♂, VF ♀ x SO 1 ♂, 18-2 ♀ x BD ♂, 18-2 ♀ x RB 1 ♂, VF ♀ x GF ♂  
 VF ♀ x Pr ♂, VF ♀ x RB 2 ♂, Pr ♀ x RB 1 ♂, Pr ♀ x HA ♂, Pr ♀ x GF ♂  
 Pr ♀ x RB 2 ♂

Progeny generated from controlled pollinations of Del2 x NH, VF x NH, R18-2 x NH, and VF x R18-2 were established in field plots at the Delaware, Ohio Forestry Sciences Laboratory, Northern Research Station, using a randomized complete block design. The plot design consists of 10 blocks of 3.05 m x 3.05 m plantings with a total of 100 progeny of each of the four crosses; ramets of each parent and a susceptible clone, NA57845; and unselected seedlings (total n = 720). When trees reached 5 to 7 years old, each was inoculated with *O. novo-ulmi* strains PMP1 and H961, and the *O. ulmi* strain PG442, using a mixed inoculum consisting of equal counts of conidia of the three strains. The mixed inoculum resolves degrees of tolerance among American elm clones better than a single strain inoculum (Townsend, ARS, US National Arboretum, retired, personal communication). An aliquot of approximately 0.15 ml of an aqueous stock suspension with a concentration of 4 million spores/ml was applied to a hole drilled with a 0.2 cm diameter bit to a 2.54 cm depth in the lower third of the trunk, and the hole was wrapped with parafilm. Foliar symptoms of DED include wilting, chlorosis, and necrosis. For each tree, the combined percentage of the crown exhibiting wilting, chlorosis, or necrosis was visually estimated (consensus rating by a pair of evaluators) to the nearest 5 percent at 8 to 9 weeks, 1 year, and 2 years after inoculation. In addition to the 5 percent intervals, an additional interval of < 5 percent was used to indicate trace symptoms that were significantly less than 5 percent.

## Results and Discussion

### Controlled Crosses of Chippewa National Forest, Connecticut, and Massachusetts Escape/Survivor American Elms

The results of field pollinations performed in 2011 are shown in table 2. The number of seeds generated per flower ranged from 2.1 to 19.0. The control bags produced essentially no viable seeds. The germination rate of seed from the crosses ranged from 70 to 85 percent.

**Table 2—Results of controlled pollinations performed in 2011**

Cross	Seeds/Flower Bud	Cross	Seeds/Flower Bud
18-2 ♀ x HA ♂	7.2, 8.9, 6.0	Pr ♀ x HA ♂	10.1
18-2 ♀ x RB 1♂	3.2, 8.0, 2.2	Pr ♀ x RB 1♂	8.5, 11.4
18-2 ♀ x GF ♂	7.4, 6.4, 4.6	Pr ♀ x GF ♂	8.7
18-2 ♀ x RB 2 ♂	4.6, 6.9, 2.1	Pr ♀ x RB 2 ♂	10.5
VF ♀ x HA ♂	2.1	18-2 ♀ control	0, 0, 0.3, 0
VF ♀ x RB 1♂	13.4, 12.2	VF ♀ control	0.1, 0, 0, 0
VF ♀ x GF ♂	10.0, 8.8	Pr ♀ x 18-2 ♂	10.2
VF ♀ x RB 2 ♂	3.0	18-2 ♀ x VF ♂	9.9, 19.0

Seedlings generated from crosses between known DED-tolerant and Chippewa National Forest elm trees, and known DED-tolerant and Massachusetts and Connecticut trees, have been planted in test sites at the Chippewa National Forest and in Connecticut. When the trees are approximately 5 to 7 years old they will be inoculated with *O. ulmi* and *O. novo-ulmi* to test for DED tolerance.

### DED Testing of Progeny Trees From Controlled Crosses of DED Tolerant Selections

The Del 2 x NH, VF x NH, R18-2 x NH, and VF x R18-2 crosses yielded seedlings with lower-mean foliar symptoms at 8-9 weeks compared to the susceptible control. The distributions of symptom severity for the progeny trees of the VF x R18-2 cross and the clonally propagated DED susceptible control (NA54845) are shown in fig. 1. The progeny trees from the other crosses exhibited a similar symptom response and distribution compared to the progeny trees from the VF x R18-2 cross (data not shown). Valley Forge x R18-2 yielded the highest number of individuals with few to no symptoms compared to the other crosses (data not shown). The crosses showed no correlation between tree height or caliper and foliar symptoms (data not shown). These results show that it may be possible to generate new American elm clones with high levels of DED tolerance using these selected crossings. Fifteen progeny trees that showed little or no DED symptoms were clonally propagated, the clones were established in field plots, and the trees will be challenged with *O. novo-ulmi* as described above in 2 to 3 years.

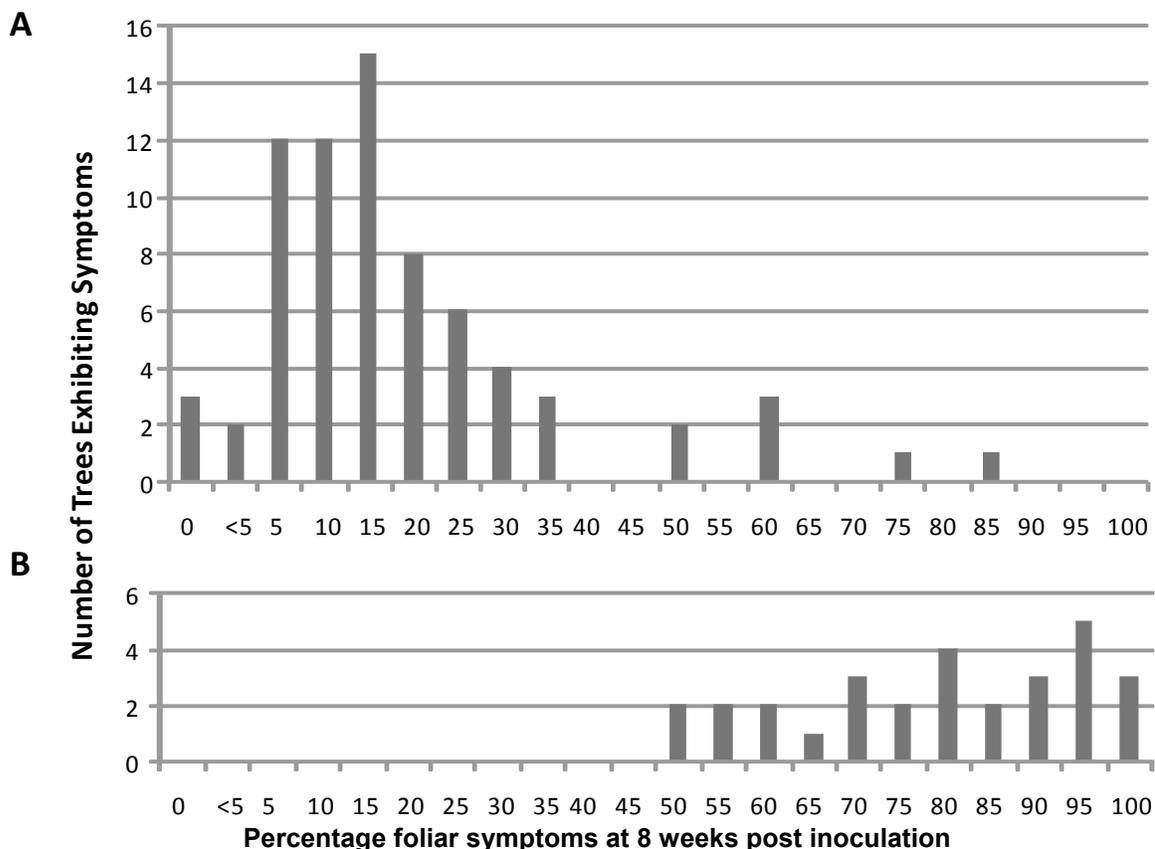


Figure 1—(A) Dutch elm disease symptoms on progeny trees from the R18-2 x Valley Forge cross, and (B) on clonally propagated susceptible control NA54845.

### Identification of Large American Elm Escape/Survivor Trees

Twenty-five large escape/survivor American elms have been identified in Michigan, Ohio, Indiana, and Illinois. These trees were clonally propagated, established in replicate blocks at Delaware, Ohio, and will be tested for DED tolerance in 2 to 3 years. Additional escape/survivor trees are being identified with the help of interested foresters and others as discussed below.

A new effort was initiated to identify additional large surviving American elms through the generation of a website where information on these trees can be submitted (<http://nrs.fs.fed.us/SurvivorElms>). Approximately 605 elm trees throughout the region have been submitted as of June 5, 2012. We are asking for the help of state foresters, park employees, and the interested public to identify large American elm trees on their landscapes.

Potential DED-tolerant elm trees identified by cooperators (fig. 2) will be compiled in a database and prioritized for sampling. When funding becomes available, scion wood will be collected from the identified high-priority trees and used to clonally propagate these trees initially through bud grafts and then vegetative cuttings. When the trees reach susceptible size, they will be screened for DED tolerance, and selections with tolerance will be released to state tree nurseries for generation of elm seed orchards and to the commercial nursery trade. In addition, seed collected from the seed orchards created at the Delaware, Ohio Forest Service laboratory will be used in landscape restoration efforts.

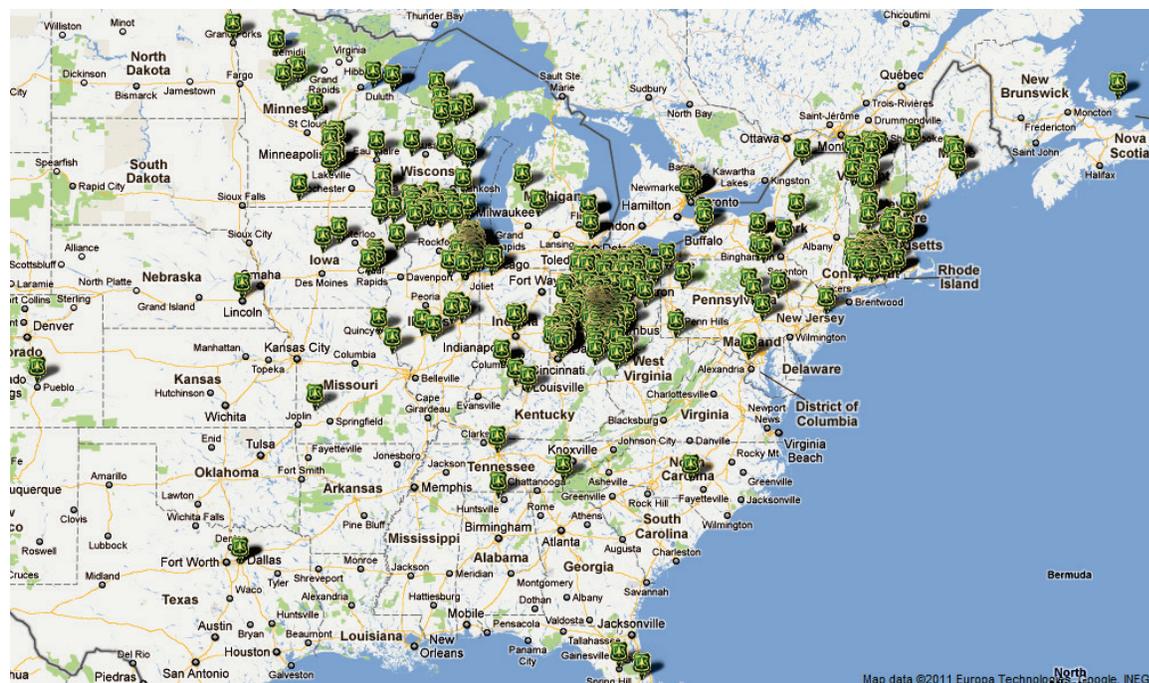


Figure 2—Locations of submitted escape/survivor American elm trees as of August 17, 2011.

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