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## The Trojan Horse Experiment: Understanding Reservoirs of Boxwood Blight Inoculum

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Boxwood blight is a foliar disease of boxwood caused by the fungal pathogen *Cylindrocladium buxicola* (*=Calonectria pseudonaviculata*). Prior research has shown that the popular boxwood cultivars *Buxus sempervirens* 'American' and 'Suffruticosa' (English) are highly susceptible to the pathogen. In the summer of 2012, we performed a study in order to identify boxwood cultivars with resistance to boxwood blight (<u>http://go.ncsu.edu/boxwood\_blight\_links</u>). Several commercial cultivars were identified as partially resistant (or tolerant) to the disease, due to minimal symptom and disease development. When exposed to *C. buxicola*, these cultivars remained healthy and expressed limited leaf lesions, stem cankers, and dieback.

The Trojan Horse study was conducted in order to determine the ability of these partially-resistant boxwood cultivars to serve as sources of inoculum for the pathogen, thus being capable of initiating infection in blocks of susceptible boxwood cultivars. We decided to call this the 'Trojan Horse Experiment' because we wanted to identify if boxwood blight could be introduced to a landscape or production area disguised like the Greeks in the infamous Trojan horse story. We hypothesized that apparently healthy, partially resistant cultivars with *C. buxicola* infection could serve as inoculum reservoirs which would contribute to infections and boxwood blight outbreaks in more susceptible cultivars.

The trial was conducted in a self-contained, shaded lathe house with overhead irrigation in Raleigh, NC during April and May 2013. The partially resistant test plants included *B. harlandii, B. microphylla* 'Golden Dream', *B. microphylla* 'John Baldwin', *B. microphylla* var. japonica 'Green Beauty', *B. microphylla* 'Winter Gem', *B. sinica* var insularis 'Nana', and *Buxus* 'Green Gem'. The test plants in the study were exposed to the fungus in two different ways: 1) prior exposure to *C. buxicola* by both direct spray (1,000 spores per ml) and indirect (test plants were placed next to heavily infected 'Suffruticosa' plants) inoculation during the summer of 2012; and 2) recent exposure to *C. buxicola* on 11 April by direct spray-inoculation of 10,000 spores per ml until run-off. The study included 'Suffruticosa' positive control plants which were direct spray-inoculated until run-off with either 10,000 spores per ml, 1,000 spores per ml, or water. Healthy, uninfected 'Suffruticosa' plants. Disease evaluations were performed on 2 May, 10 May, and 28 May using a modified Horsfall-Barratt rating scale. Partially-resistant test plants which were recently inoculated with the pathogen in April 2013 were generally more effective at transmitting the pathogen to healthy 'Suffruticosa' plants that were exposed to the pathogen during the summer of 2012.

Between 18 May and 23 May, the Raleigh area received over two inches of rain with an average daily temperature of 72°F, therefore the conditions were extremely conducive for boxwood blight over this time period. Due to the climate, high inoculum production on infected plants in the experiment, and inoculum transmission via wind-driven rain, some of the uninoculated 'Suffruticosa' negative control plants (spaced 3 ft. from infected reservoir plants) were also infected with *C. buxicola*. Inter-plot interference from the inoculated 'Suffruticosa' positive control plants may have contributed to some disease development on the negative controls and the disease indicator plants. However, our data suggests that the partially-resistant boxwood cultivars we tested with minimal boxwood blight symptom development may be capable of transmitting the pathogen to healthy, susceptible cultivars.



Fig. 1 (left): The center plant, partially resistant cultivar *Buxus harlandii*, was direct inoculated with 10,000 *Cylindrocladium buxicola* spores per ml until runoff on 11 April. Then healthy, uninfected *B. sempervirens* 'Suffruticosa' were placed next to the test plant. This photograph was taken 28 May.

Fig. 2 (right): Up close photograph of the partially resistant cultivar, *Buxus harlandii*, from Fig. 1. The dark circular lesions with yellow halos are boxwood blight symptoms. This photograph was taken 28 May. Although this cultivar produces minimal box blight symptoms after infection, the fungus can still produce spores on the plant.