PARADOX DIVERSITY CROWN GALL SCREEN - SET 1 PRELIMINARY RESULTS

Jim McKenna, Beth Teviotdale, Tom Turini, Sandie Uratsu, and Gale McGranahan

INTRODUCTION

The bacteria, *Agrobacterium tumefaciens*, are the causal agent of the disease crown gall. The host range of Agrobacteria includes nearly one quarter of the plant kingdom. The bacteria are ubiquitous in soil worldwide, and are commonly associated with plants, living on the surface of or in the vicinity of roots (the rhizosphere). They cause disease when they enter plants through natural or artificial wounds and inject a plasmid (small piece of DNA) into plant cells which then becomes incorporated into the plants own DNA. Infected plant cells then express the bacterial genes and produce both nutritious compounds for the bacteria (opines), and plant hormones (auxin and cytokinin) which lead to the formation of the tumor or gall. Walnut is, in general, susceptible to crown gall and Paradox hybrids in particular, are very susceptible. Both walnut nurseries and walnut growers feel the economic impact of this disease since nurseries must destroy finished trees when galls are discovered, and galled orchard trees cause growers to suffer reduced growth and yield.

This crown gall screen is designed to evaluate the host-plant resistance of the diverse collection of walnut genotypes within the Paradox Diversity Study (PDS). In 1996 and 1997, methods of inoculating walnut were studied along with procedural details to devise a standard inoculation and evaluation method for the PDS crown gall screen. This year, we inoculated 1200 PDS Set 1 trees in the field, comprised of 55 different sources, using the best methods developed the two previous years. The data from the trial will not be fully collected until the middle of January 1999 and we are thus unable to report the complete results. Instead, we are presenting data on 2 standard sources and the specific methods followed this year. In addition, we are presenting data on the natural infection of the Set 1 PDS sources. This information will be valuable for assessing the validity of the results of artificially infecting sources in the crown gall screen.

OBJECTIVES

To determine the host-plant response of PDS walnut sources to crown gall.

MATERIAL AND METHODS

The walnut sources in the crown gall screen are listed in Table 1 of the PDS report (see report this volume). Additional sources were included to function as resistant controls and included 3 Chinese wingnut (*Pterocarya stenoptera*) sources: 'Pomology Hybrid', 'Dairy Road' and 'Mini Center', and one pistachio (*Pistacia atlantica*) rootstock source. All trees were planted at the Kearney Agricultural Center, Parlier, CA, March 6-8, 1998. The trees were planted as described in last years report and the plot was designed as a randomized complete block with 4 blocks. Six trees per source were included in each block except when a source was limited by low numbers of Paradox. Sources that had only 12 trees available were included as "partial" sources which have
only 3 trees per block. All of the partial sources were paired to keep the blocks even. The plot was surrounded by 2 rows of NCB on the east and west sides and 1 row of Paradox on the south side. The north side was left open. The trees were spaced 15 feet between rows and approximately 2 feet within rows. The plot was kept weed free by a combination of herbicides and cultivation, and was flood irrigated. Fertilizer (15-15-15) was broadcast 3 times during the growing season. In May, all trees were pruned to remove suckers and to leave 3 branches.

The plot was inoculated over the course of 4 days, July 13-16, 1998. Three *Agrobacterium tumefaciens* strains were combined and used as inoculum: 2516, 18W-7A, and 18W-19C. These strains were initially isolated from natural walnut galls by Dr. Milt Schroth. The strains were stored at UC Davis in an -80°C freezer, and were cultured on agar plates and grown the week prior to inoculating. Inoculum was prepared fresh each morning before inoculating the trees. Equal amounts of each of the Agrobacteria strains were combined in a buffered solution and brought to a final concentration of $10^7$ cfu's (colony forming units/ ml). The actual concentration of bacterial cells in the inoculum was measured (Table 1).

Table 1. Concentration of *Agrobacterium tumefaciens* as determined by dilution plating in the PDS Set 1 inoculum solution.

<table>
<thead>
<tr>
<th>Date</th>
<th>Transmittance</th>
<th>start</th>
<th>finish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>time plated</td>
<td>cfu/ml</td>
</tr>
<tr>
<td>13 July</td>
<td>75.5</td>
<td>10:45 am</td>
<td>$1.34 \times 10^7$</td>
</tr>
<tr>
<td>14 July</td>
<td>75.1</td>
<td>6:15 am</td>
<td>$1.75 \times 10^7$</td>
</tr>
<tr>
<td>15 July</td>
<td>75.6</td>
<td>6:30 am</td>
<td>$3.20 \times 10^7$</td>
</tr>
</tbody>
</table>
| 16 July | 75.0          | 8:00 am  | $1.87 \times 10^8$ | 11:15 am | $1.53 \times 10^9$

\(^y\) Transmittance determined by Campbel Scientific Spectronic 21 spectrophotometer set at wavelength of 600 nm.

\(^z\) The medium used at the end of the day on 16 July was prepared at KAC, the rest of the media was prepared at UC Davis.

The inoculum was then dispensed into 500-ml glass bottles, which were covered with aluminum foil. The bottles were placed into ice buckets to keep the bacteria cool (daytime high temperatures exceeded 100°F during the four days of the experiment).

On the north side of the trees, wounds were made on both the new wood and 1-year wood using a dove-tail hand saw fitted with a wooden stop so that the saw would cut to a prescribed depth of 1.8 mm. Immediately after wounding, inoculum was applied with a dropper to saturate the cut. Once inoculated, the wound was covered with parafilm and then wrapped with white flagging tape. A total of 10 wounds were made on each tree; 5 on the main stem (1-year wood) and 5 on a current-year branch (new wood). At the beginning and end of each day, a sample of the inoculum was plated onto sterile plates in the lab to check for live bacteria. In addition, Kalanchee plants, clonally propagated, were inoculated to serve as indicator plants to check for bacterial virulence. These plants were potted and were kept in a lathhouse at Kearney. Twenty-four NCB and 16 Paradox seedlings in the guard rows were used as walnut controls to determine the amount of natural wound callus associated with the experimental method. Control trees were treated identical to experimental trees with water substituted in place of inoculum.

At the end of the growing season in December, the galls were measured. Each inoculation site was measured twice with a vernier caliper; first to measure the greatest outward projection of the gall and stem; and second, to measure the stem alone, directly above or below the gall. The
gall size is calculated as the difference of these two values. For each tree, the gall size is based on the average of all 5 inoculations per wood type.

Because each individual tree is tagged in the PDS, it was easy to keep track of galled trees by source at digging time. The standard practice is to rogue out galled trees as soon as they are discovered and burn them. As this was done, the tree tag was removed and saved as a record of the galled tree.

RESULTS AND DISCUSSION

Few results are available but overall, we are confident that this first screen has been very successful. Last year we reported that 75-80% of our inoculations developed galls. This year, from casual observations in the field, it appears that 95% of our inoculations developed galls. Figure 1 shows the results of 2 standard sources this year; 'AW' which is the variety *J. hindsii* 'Rawlins' and the clonal Paradox 'Vlach'. The gall sizes for 'Vlach' last year on 1-year and new wood were 13.0 and 14.3 mm respectively. This year, they were 14.7 mm for 1-year wood and 14.9 mm for new wood. The consistency of these results is a good indicator that real differences among sources may be detected. The 'Rawlins' Paradox seedlings formed much larger galls than 'Vlach' and 'Rawlins' NCB seedlings were not as resistant as the random NCB seedlings tested last year. Last year, the NCB seedlings gall size for 1-year wood averaged only 2.2 mm, which is too small to be considered a gall. There was no difference between wood type for the 'Rawlins' NCB seedlings.

The Kalanchoe indicator plants, much like the walnuts, showed a good gall response (Figure 2). Every inoculation resulted in a gall and the galls were quite consistent. Interestingly, the gall sizes tended to be larger at the end of the day, despite the fact that the bacterial concentration in the inoculum declined slightly (Table 1). The wound callus of Kalanchoe controls was similar in size to walnut controls. There were no galls observed in either the Kalanchoe or black walnut controls but one wound on a Paradox control produced a crown gall.

Figure 3 shows the natural infection of all PDS Set 1 sources to crown gall in the nursery. This data is based on all trees of each source and includes black seedlings along with hybrids. Nearly all of the galls observed formed on the tap root or first order lateral roots. Only 2 trees formed galls at the crown. The wide variation in values for 'QZ', 'BZ', and 'NX' reflect the small number of seedlings these sources produced. The only 2 sources that produced galls at all 3 nursery locations were 'JX' and 'PX'. Because these data only represent one year, it is premature to draw any conclusions regarding source susceptibility.

ACKNOWLEDGEMENTS

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Figure 1. Gall size of Paradox and NCB (J. hindsii) controls with inoculated Paradox, NCB seedlings of the 'AW' source (J. hindsii 'Rawlins'), and the clonal Paradox 'Vlach'. Values are the average of 12-24 seedlings inoculated 5 times for each type of wood +/- the standard deviation of the mean.

Figure 2. Gall size of Kalanchoe indicator plants inoculated with the inoculum used for the PDS crown gall screen. Values are the average of 2 plants, each inoculated 3 times, at the start of the day and at the end of the day between July 13-16, 1998.
Figure 3. Natural infection of Set 1 PDS sources to crown gall. The data represent the number of trees per source (Paradox and black) identified with at least one gall after digging. The values are the average of the 3 locations +/- standard errors of the mean.