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Herbicide Residues in Composts: pH and Salinity Affect the Growth of Bioassay Plants

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Herbicide residues have raised new concern since 1999 when damage to plants appeared to result from composted turf clippings (Bary et al. 2001; Rynk 2000). Other case reports of some incidents suggested that other unknown sources of phytotoxicity were present in composts used in gardens (Nielson 2003). Several plant bioassays have been reported to test composts for herbicide residues (SPU 2002; WERL 2003; Fauci 2003; Fauci et al. 2002; KCSW 2002) and in Washington one standardized procedure has been developed (WA-DOE 2003).

Plant bioassay techniques to detect and identify herbicide damage in field soils are normally run with 100% soil (Rashid et al. 2001). However, compost is not meant to be a soil substitute and it is understood that composts must be diluted for plant growth by mixing into soil or peat-based media (Blewett et al. 2005; WA-DOE 2003). Fauci et al. (2002) discuss bioassays with pinto beans, tomatoes and peas in 25:75 v/v compost:peat blends and Fauci (2003) describes a 50:50 v/v compost/commercial media mix, which Oregon DEQ employed during evaluation of composts for potential clopyralid residues (ODEQ 2003). Seattle Public Utilities (SPU 2002) recommends 25% blends of compost in peat media with added nutrients, while presently the standardized Washington bioassay (WA-DOE 2003) uses a 2:1 v/v compost: commercial potting mix, in other words a 67% compost blend. All of this variability in methodology is of concern because effects of varying compost on plant performance are not well characterized.

The handling of composts in herbicide residue bioassays can be critical to the development of accurate results as composts often contain high salt levels at concentrations known to inhibit plant growth and development (Bailey et al. 2003; El-Iklil et al. 2000). Recent reports have indicated other sources of phytotoxins in recycled composts (Morel and Guillemain 2004). Salinity in growing media has been shown to exacerbate harmful effects of xenobiotics in growing media for tomatoes and cucumbers (Alpaslan and Gunes 2001) which are frequently used as test plants for herbicide bioassays (Fauci et al. 2002; Brinton and Evans 2002). Woods End Research Laboratory (WERL) has found that elevated boron in compost media can produce plant damage symptoms which are similar visually to herbicide damage symptomology for beans, peas and cucumbers (Brinton and Evans 2003). Poor root development and stunting of

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plants are also mentioned in current bioassay literature as symptoms of herbicide contamination (Bugbee and Saraceno 1994; ODEQ 2003; SPU 2002) but can have other causes.

In this study the Washington Department of Ecology (WDOE) bioassay method to detect herbicides was compared to a newer method (WERL) that adjusts background salinity. This adjustment removes the effect of salinity while determining herbicidal influence on plants commonly used as bioassays in compost. The WDOE method utilizes a high rate (67%) of compost, while the WERL procedure uses a variable rate in order to maximize use of compost and yet achieve a non-phytotoxic salt-concentration (WA-DOE 2003; WERL 2003). The WDOE utilized peas, tomatoes and beans as test plants. WERL primarily utilized red clover, but has also used peas, beans and tomatoes. To date, methods employing red clover in Washington to evaluate herbicide residues in composts have been unsuccessful (M. Fauci personal communication March 2003). The Oregon DEQ bioassay study of herbicide residues in compost also reported that red clover seedlings failed in that procedure (ODEQ 2003). We hypothesize that the inability to use red clover in the Oregon and Washington protocols is due to uncontrolled intrinsic compost traits and the sensitivity of this species to soil conditions. It is thought that plant performance in the unmodified WDOE bioassay, or others similar to it, are likely to be affected by the uncorrected high salinity and thus, compromising the value of the bioassay.

MATERIALS AND METHODS

Composite blends of commercial composts that were free of clopyralid herbicide residues in GC/MS analysis and red-clover bioassay were prepared to produce two high and two low levels of electrical conductivity (EC), as measured by the saturated paste procedure (TMECC 2001). A preliminary survey of 32 commercial composts in Washington State indicated salinities ranged from 0.4 to 24.2 dS m^{-1} with a mean of 6.2 dS m^{-1}. A range of mixed composts were designated #1, #2 (high EC) and #3, #4 (low EC), each representing composts from different sources blended to be similar in EC. Limed and unlimed peat moss was used to adjust pH of the media, as determined by hydrogen-ion electrode in saturated paste extracts (TMECC 2001). After mixing, the two high EC blends had 16 dS m^{-1} $\pm$ 0.2 and the two low EC blends had 4.9 dS m^{-1} $\pm$ 0.5. pH values for all four averaged 7.6 $\pm$ 0.4. Each of these four compost treatments were then treated in accordance to either the WDOE or WERL procedure and planted, in triplicate, to four plant species often employed in herbicide bioassays: field pea, *Pisum sativum*, var. Maxim; snap bean, *Phaseolus vulgaris*, var. Provider; red clover, *Trifolium pratense*, var. Mammoth; curly cress, *Lepidium sativum.* After 5-7 days plants were thinned to 4 plants per cell in 0.5L pots with a surface area of 100cm^2 and kept under metal halide Gro-Lux lamps on 16-hr day-lengths at 25C, and irrigated daily. The appearance of plants were compared with those in the control media, and recorded on day 10 for the beans and day 13 for the peas, clover, and cress. The plants were then harvested and biomass (fresh weights) recorded.

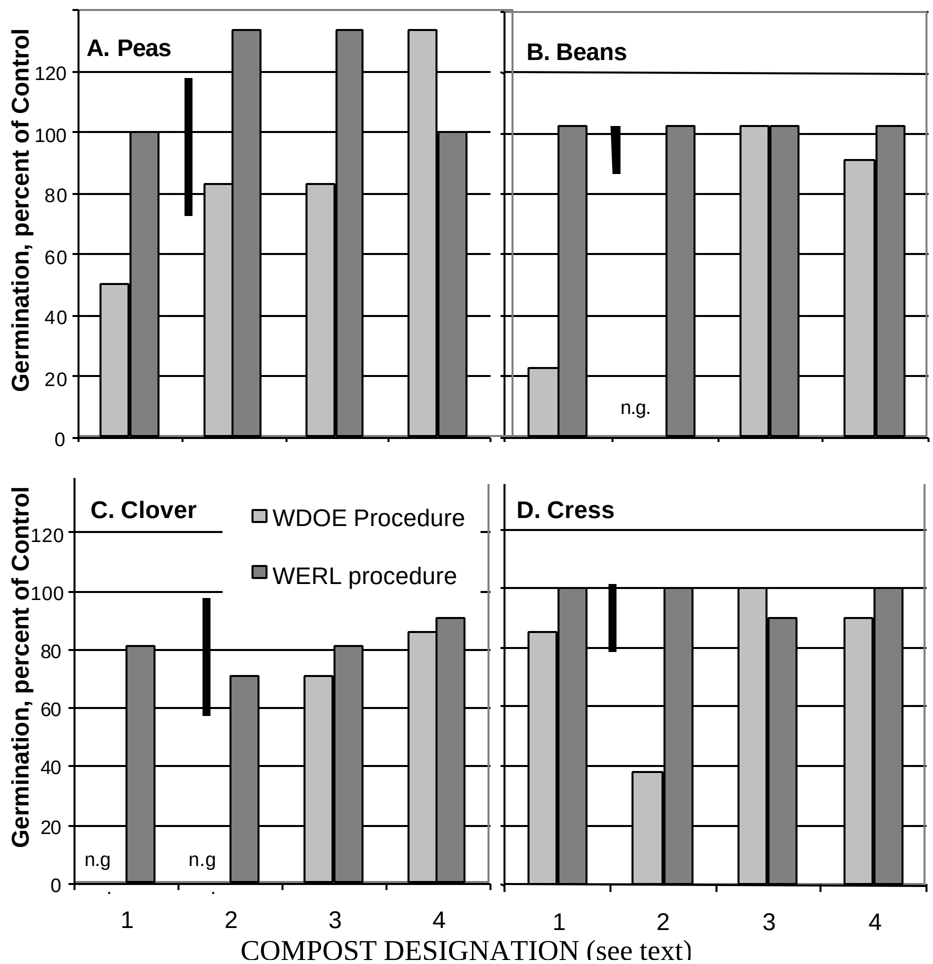

Figure 1. Effect of WDOE and WERL bioassay procedures on seed germination rate, in four composts of varying salinities. Germination rate is expressed as percent of control medium. n.g. = no germination. Vertical bars represent for $p < 0.05$.

RESULTS AND DISCUSSION

Pea germination was significantly lower in the WDOE procedure than in the WERL procedure for both high-EC composts and also in one of the two low-EC composts (Figure 1). Bean germination was unaffected by low-EC compost in both procedures. In the high-EC composts, germination was severely inhibited in WDOE but not in WERL media. We observed significant growth inhibition of beans for compost blend #1 and total loss of beans with compost blend #2 (Figures 1 and 2).

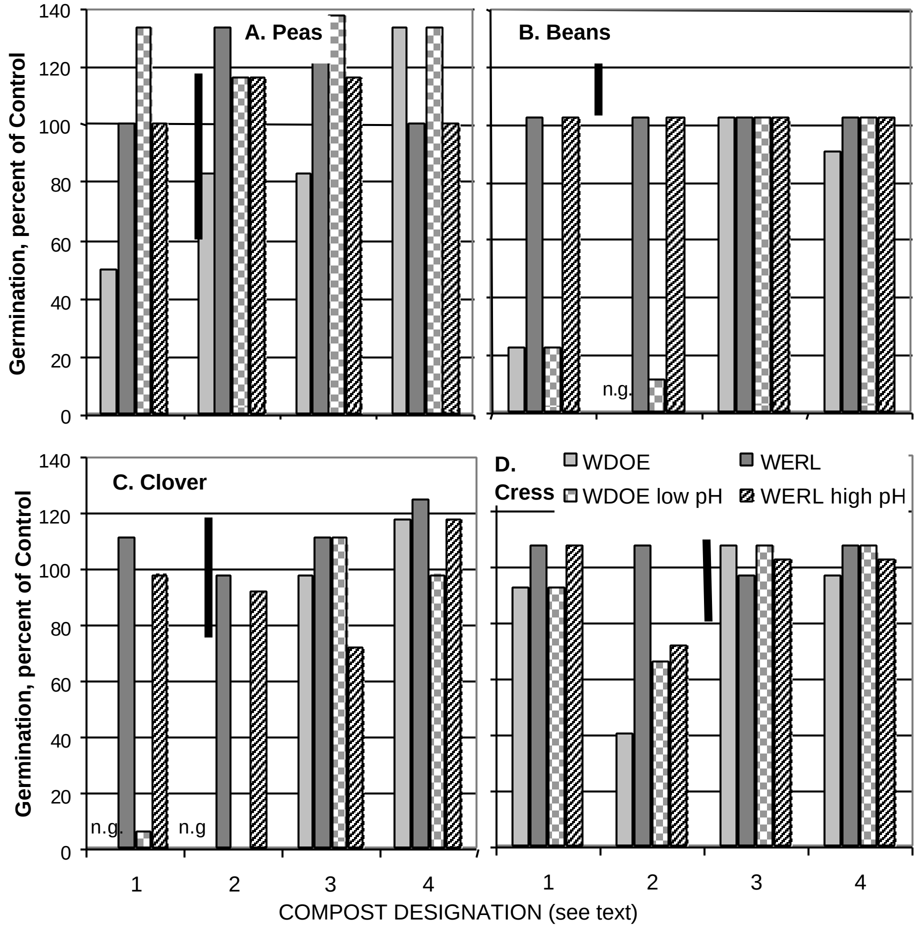

Figure 2. Effect of standard and pH-modified bioassay procedure on seedgerm-Ination rate for four composts of varying salinities. Germination is rate expressed as percent of control. n.g. = no germination. LSD for $p < 0.05$.

This indicated that these composts possessed confounding factors that are likely to negatively influence seedling growth and development if used for bioassays. Red clover germination was high and unaffected by low-EC compost in both WDOE and WERL procedures. With high-EC compost, clover germination was severely inhibited in WDOE but not in WERL, and behaved similarly to beans in the composts #1 and #2.

Cress germination was high and unaffected by either low-EC or high-EC compost in WERL media but was severely inhibited by compost #2 in WDOE media. This compost blend also had the highest ammonia levels, a sign of compost immaturity (data not shown). Figure 2 shows the effects of modifying pH levels on both

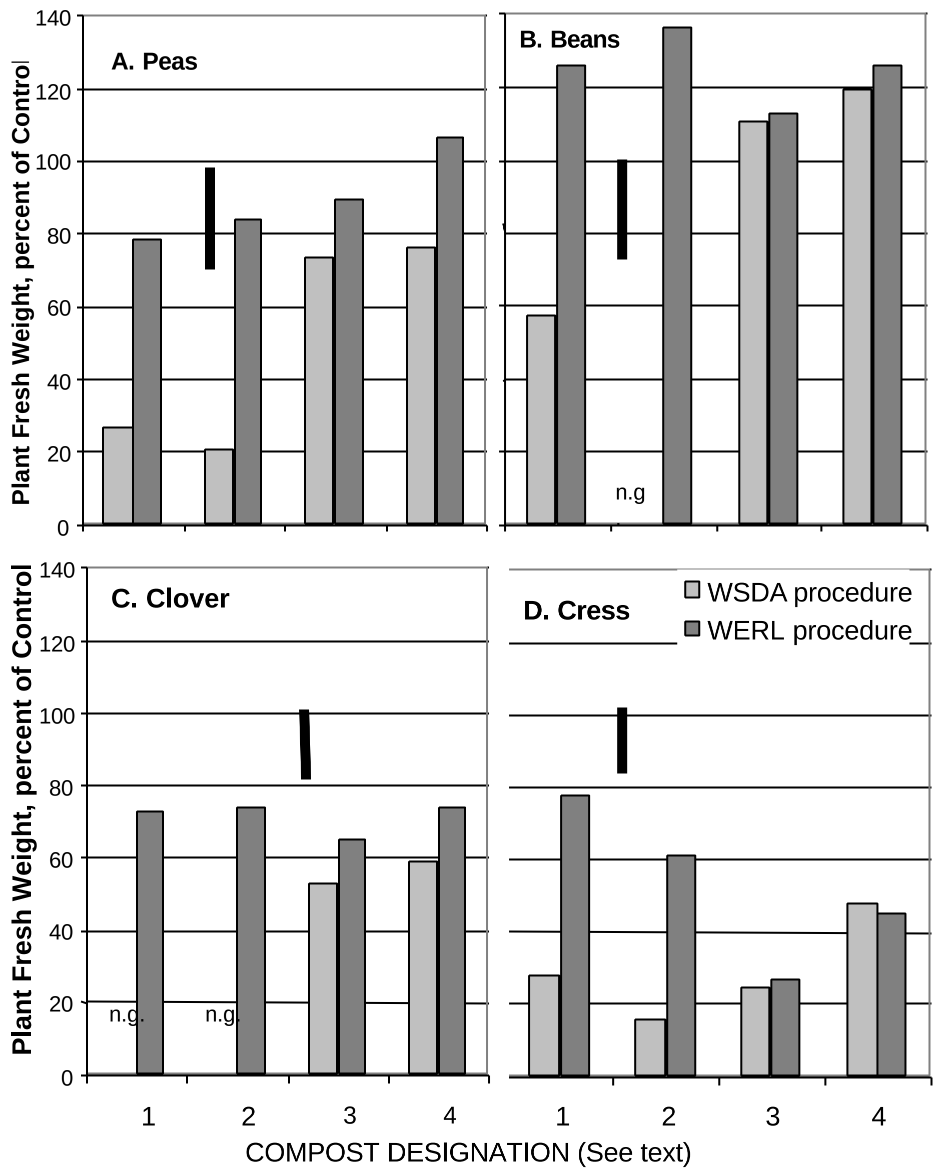

Figure 3. Effect of standard WDOE and WERL bioassay procedures on plant yield in four composts. Weight is expressed as percent of control medium. n.g. = no germination. Vertical bars represent LSD for $p < 0.05$.

procedures. There were no uniform differences observed in germination rates between the standard and pH-modified WDOE and WERL procedures, however, a significant Procedure x pH interaction was found. Pea germination in WDOE with composts #1 and #3 was significantly improved when the compost was blended with unlimed peat to alter the pH. In contrast, there were no differences

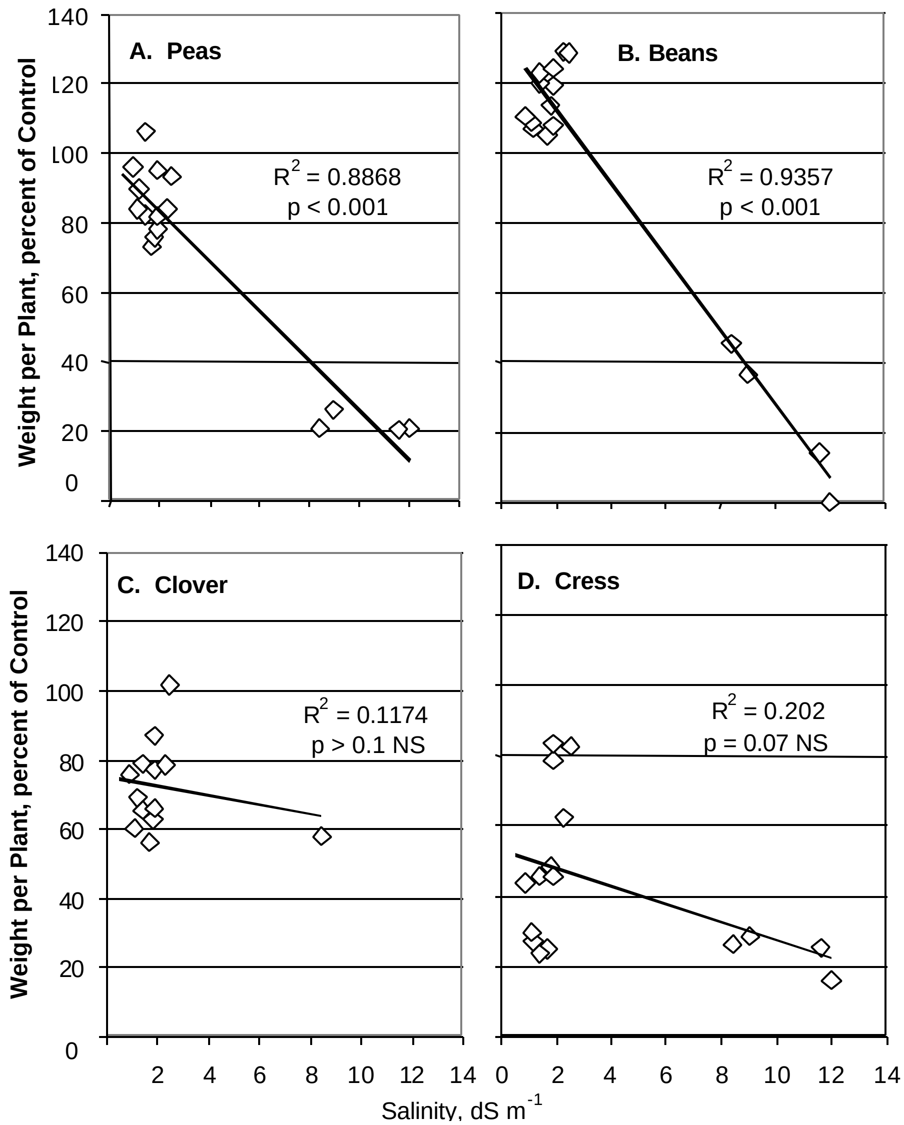

Figure 4. Relationship of test media salinity and seedling biomass (relative Fresh weights.) for test species across all salinity conditions.

in pea germination between the standard and modified WERL procedures. Peas are a popular plant for bioassays (Bary et al. 2003; SPU 2002). These data suggest that variations in pH and salt in one procedure may severely impact field peas used for herbicide bioassays, causing a misdiagnosis as to the phytotoxic agent. Fresh plant yields were also examined for the various treatments. In low-EC composts (compost #3 and 4) there was a range of yield effects with pea and bean growth being the same as controls, while both clover and cress were significantly smaller than controls (Figure 3).

For cress, dilutions used for the WERL procedure resulted in significantly better growth and are probably related to dilution of other non-herbicide phytotoxins. For all four plant types there was no difference between WDOE and WERL media with low-EC composts. With the two high-EC composts, however, plant growth of all four plant types was much smaller in the WDOE than in the WERL procedure. With WERL, pea, bean and clover growth was no different with the high-EC composts than it was for the low-EC composts. Clover growth inhibition in both procedures was slight with the low-EC composts (composts #3 and #4). With high-EC composts (#1 and #2) there was no germination in WDOE, while clover growth in WERL was equal to the controls. Cress growth inhibition in both procedures was severe with the low-EC composts. These plants were also chlorotic, indicating that they may have suffered from a nutrient deficiency.

With the high-EC composts (#1 and #2) there was severe inhibition in WDOE, but only slight inhibition in WERL. There was a species-specific relationship between salinity and plant biomass production (Figure 4). A significant correlation existed between pea and bean fresh weight and EC ($p<0.001$) for all media and composts. However, no correlation existed for clover because clover did not emerge in 3 out of 4 WDOE tests employing high EC compost.

When media with EC values greater than 3.0 were excluded from the correlation analysis, pea, beans and clover growth showed weak or no correlations with EC. The relatively weak overall correlations and low slopes for the field peas, beans and clover grown in media adjusted to be less than 3 dS m^{-1} indicated that the WERL procedure reduced salinity toxicity and resulted in more uniform growth across otherwise broad ranges of compost properties. This uniformity should be a prerequisite for a useful and reproducible bioassay to measure phytotoxicity from herbicide residues in composts.

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