

**Phytoremediation Comparisons Between *Solanum lycopersicum* Wild-Type and brt Mutant Using Kelthane Miticide**

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**Introduction**

Biotechnological approaches to phytoremediation have, thus far, been the source of little research. There are two goals associated with genetic modification of plants for phytoremediation increasing ability and lowering cost (Singh, et. al., 2007). For increasing phytoremediation of metals, the key is to increase the number of water and nutrient uptake sites on the roots and raise the quantity of metal transporters in the xylem (Singh, et. al., 2007). Tomato plants, known hyperaccumulators of Cadmium, need to gain biomass in order to be effective phytoremediators (Setia, et. al., 2007; Cherian and Oliveria, 2005). Transferring genes or traits from bacteria or animal systems frequently improves remediation potential (Cherian and Oliveria, 2005). This was found to be true in genetic engineering selenium phytoremediators (Terry and Bañuelos, 2000). Terry proposed engineering the Indian mustard plant to overproduce enzymes and introduce additional metabolic pathways to remediate the selenium (Terry and Bañuelos, 2000).

Tomato plants and dicofol miticide (Kelthane) were used to complete this phytoremediation test. Tomato plants are not known for their phytoremediation abilities (Bush, n.d.). Research showed that mutated tomato plants may phytoremediate more effectively than regular tomato plants (Buch, n.d.). This may be due to modified root structure and veins.

A bushy root variety of tomato plant was selected from the University of California Davis Charles M. Rick Tomato Genetics Center for this experiment under the rationale that plants with larger roots could take up more chemical (Chetelat, 2010). Zobel (1971) located this mutation and notes that "The root system is very highly branched...the root system branches profusely within one day after emergence, in contrast to normal roots, which branch only after several days of growth" (Zobel, 1971). Zobel also notes that brt mutated tomato plants germinate more slowly than non-mutated plants (Volland and Zobel, 1988). This mutant also displays increased colonization of fungus on its roots (Zsogon, et. al., 2008). Increased fungus presence could contribute to phytoremediation abilities because of the plant's growing need for nutrients (Zsogon, et. al., 2008). There may be more microbial enzymes in the roots (Benedito, 2010). Over expression of root membrane proteins in Indian mustard plants led to an increase in phytoremediation ability for removal of selenium (Terry and Bañuelos, 2000). Peres (2010) noted that he has observed an increased concentration of Brix (sucrose) on the roots. Zobel (2010) confirms this observation by stating that there is an increase in starch at the base of the roots that could be duplicated by the presence of sucrose. This sucrose is likely located on the microbial chelators, which are

known to deliver nutrients to the plant, while sucrose probably is located on the top of the rizosphere (root shoot) (Gerhardt, et. al., 2009). In a 2010-2011 research project, the experimenter found the location for the bushy root mutant on the twelfth tomato chromosome at 19.8 cM (unit length of chromosome) or 95.8 cM. The gene at this location was TG296, a Lysr transcriptional regulator protein from bacteria that was placed in the castor bean plant before being extracted by Zobel at U.C. Davis (Zobel, 1971; Voland and Zobel, 1988).

Kelthane 50W (or WSP) Agricultural Miticide has been manufactured by Dow AgroSciences Canada Inc., Rohm and Haas Company, and Makhteshim-Agan and is “a miticide that provides a high initial kill and good residual (long lasting effectiveness) (MSDS: Kelthane, 2008; Rossi, 1998). A white to gray powder, it has an odor of fresh cut hay” (MSDS: Kelthane, 2008). Kelthane is composed of about 51 percent dicofol (Kelthane, 2005). Dicofol is “a nonsystematic acaricide (poisonous to mites) used to control mites that damage cotton, fruit trees, and vegetables” (Qiu, et al., 2005). Dicofol is similar in composition to DDT and, therefore, is classified a Persistent Organic Pesticide (Eckley, 2001). DDT is actually an intermediate substance in the forming of dicofol (Sánchez, 2010). These two pesticides are often used interchangeably and results in a dicofol experiment should apply to DDT (Garber and Peck, 2009). The EPA notes several important distinctions between DDT and dicofol, chiefly that dicofol is more water-soluble than DDT (Rossi, 1998). Essentially, all results found for dicofol are worse for DDT and is considered less harmful than DDT (Rossi, 1998).

Phytoremediating dicofol and DDT has been studied on a limited basis and a procedure for the remediation has been developed (Thompson, 2010; Gao, et. al., 2000). The DDT begins to be remediated when it is taken from the soil through the roots of the plant (Gao, et. al., 2000). This uptake is limited by the fact that both DDT and dicofol are hydrophobic and they resist water travel (Gao, et. al., 2000). A concentration gradient is formed near the root epidermis that is semi-permeable and absorbs some of the pesticide, transporting it to the root xylem using transport proteins (Setia, et. al., 2008). Benedito (2010) suggests that there are likely increased transport proteins in the roots of the bushy root mutated tomato plants. This suggestion is confirmed through previous research that points to a transcriptional protein gene modification that would effectively produce more transport proteins to increase the amount of DDT that could be transported from the root epidermis into the xylem. Plant metabolism transforms the DDT and degrades it significantly, first into DDD, a less hazardous pesticide, and then catalyzes the DDD using naturally occurring reagents (Gao, et. al., 2000). DDT can also form DDE through a dehalogenation, removing both halogen and hydrogen from the DDT (Gao, et. al., 2000). However, the remediation procedure in tomato plants could be significantly different than the one described since it occurred in two types of grasses (Gao, et. al., 2000). Frequently, remediated pesticides or metals will be sequestered in the leaf or stem (Setia, et. al., 2008). Either a vacuole will form around the pesticide or it will be sequestered away from any vital cell or plant process (Setia, et. al., 2008).

Similar experiments have been conducted using different plants and different chemicals from this experimenter and others. A phytoremediation experiment in 2005

using rye grass to remove DDT was extremely effective (Greenberg, 2006). In fact, 30% of the DDT was removed within 90 days, but it is noted that there is no way to know “whether DDT is being degraded in the soil or in the plants,” an important consideration (Greenberg, 2006). Initially, phytoremediation of DDT was deemed impossible, but was proven possible in 1977 (Russell, 2005). Chu (2006) performed a hydroponic experiment using DDT, PCBs and remediated both with rye grass (Chu, et. al., 2006). Though this test used an extremely small (ng) sample of DDT, it was remediated at a fairly fast rate and the half life determined to be only two or three days for such a small amount of DDT added (Chu, et. al., 2006).

The hypothesis for this experiment focused on the ability of the mutated tomato plants to phytoremediate: Tomato plants that have been genetically mutated to increase root length and size will phytoremediate more effectively, with fewer negative health effects when 1.5 g of dicofol is applied than wild-type tomato plants that have not been mutated.

### **Materials and Methods**

Sixty 5 inch diameter biodegradable (Jiffy Pots) plant pots were used in this experiment. They were purchased with two 5/16 inch holes for drainage. These holes were covered with a piece of duct tape to prevent pesticide leakage and evaporation of the pesticide. There were ten samples in each of six test groups and controls. Group A contained neither dicofol nor plants. Group B contained wild-type tomato plants without dicofol. Group C contained bushy root mutant plants without dicofol. Group D contained dicofol, but no plants. Group E contained dicofol and wild-type tomato plants. Finally, Group F contained dicofol and bushy root mutant tomato plants.

Scotts Premium Topsoil that contained organic materials and peat moss was autoclaved. Miracle Grow Water Soluble Fertilizer was prepared and added to each pot of soil every ten to fifteen days. All test groups were watered with 50 ml of tap water three days a week or as needed.

Seeds used for this test included tomato seeds and mustard seeds (for bioassay). *S. lycopersicum* brt bushy root mutant tomato plants (LA2816) were obtained from the C.M. Rick Tomato Genetics Resource Center and the University of California Davis. These seeds were acid treated in 1 percent HCL. The wild-type tomatoes were Better Boy Hybrids from Burpee (Lot 1). Southern Giant Curled Mustard from Wetsel Incorporated (Lot 1185) was used for the bioassay. All tomato seeds were prepared before being transplanted into their soil pots. Forty of the 50 mutant seeds (quantity was limited) and 40 wild-type seeds were placed in 2.7 percent sodium hypochlorite (half-strength bleach) in a 500 ml beaker for 30 minutes. Seeds were then rinsed and placed in plant trays lined with five layers of paper towel that was moistened and covered with five additional layers. Plant trays containing the seeds were placed in a warm dark location until germination. Seeds were then transplanted into soil pots, with two seeds per pot, planted ¼ inch below the soil.

Two different pesticides were obtained for this experiment from Dr. R. Allen Straw at Virginia Tech. Six lbs of Kelthane 50 Agricultural Miticide (Lot L2603), manufactured by Rohm and Haas Company with 50 percent dicofol and 50 percent inert ingredients was actually used in the test. Five lbs of Thionex 50 W (Endosulfan) was also obtained as an alternative to dicofol. The Thionex contained 50 percent endosulfan and 50 percent inert ingredients and was manufactured by Makhteshim Agan of North America, Incorporated (Lot GM809016).

Pesticide (Kelthane 50) was applied at two different times for phytoremediation opportunity. In powder form, 0.5 g of Kelthane was added to each test pot. After one month, an additional 1 g of Kelthane was added aqueously. These two applications simulated a large presence of dicofol initially and then additional dicofol being dumped at the remediation site. Thirty grams of Kelthane were added to 300 ml distilled water. The solution was heated and 2 ml of acetone forced the solution to combine. The acetone evaporated and 10 ml of solution was added to each of the pots receiving pesticide. A pipette pump was used to apply the solution and it was placed under the top layer of soil near the roots to minimize evaporation of the pesticide.

Leaf area, chlorophyll concentration at  $A_{663}$  and  $A_{645}$ , Brix concentration, wet root mass, and dry plant mass were measured as health indicators.

The soil was analyzed to see how much of the pesticide exists when compared to the control with just the miticide. The method of bioassay was used because it was deemed reliable from previous testing.

To prepare the bioassay, a baseline test was conducted. Pots of soil were prepared as described above. This means that 3230 g of soil (170 g per pot) were autoclaved. Nineteen pots were used. Each pot was given varying amounts of Kelthane, from 0 grams to 1.8 grams, increasing by 0.10 grams. The pesticide was weighed and mixed in powder form into each sample of soil for one minute. Forty mustard seeds were added to each pot. Mustard seeds were chosen because they have been known to be effective indicators of DDT (extremely similar to dicofol) (Orcutt, 2010). The number of plants that germinated was measured for twelve days. The results were compiled and averaged and one equation for each day that was representative of the data was found to allow for estimation of the amount of dicofol in soil with relation to the number of seeds that germinated. Similar testing was repeated with the pots that had unknown amounts of Kelthane. Germination of mustard seeds was recorded and using the equations found above, an average estimated amount of dicofol remaining in the soil was obtained. A different standard equation was used for each day of germination. If the logistic curve did not fit the number of seeds germinated, results were extrapolated. For example, if the lower bound for the equation was ten plants and one pot had four plants, the pot would be recorded as having the maximum (1.5 g) of Kelthane. After recording the daily amount of Kelthane remaining, the pots that had no Kelthane were used to standardize the data. A difference was taken between the germination of the pots with no Kelthane and those with Kelthane to obtain an accurate amount of Kelthane remaining. These results were averaged and t-tests tests were run.

## Results and Discussion

The hypothesis that bushy root mutated tomato plants would remove more Kelthane than wild-type tomato plants, but have more negative health effects, was not supported. In fact, the exact opposite result occurred. Bioassay results showed that autoclaved soil alone removed 0.384 grams of Kelthane, while the mutated plants removed 0.537 grams, and the wild-type removed 1.140 grams out of the total 1.50 grams added. Wild-type plants removed significantly more than mutated plants and mutated plants removed more than soil alone, but not to a significant degree.

In terms of health, the mutant plants seemed to fair best. Mutant plants had a significantly greater percent increase in leaf area, 123% for those with Kelthane added, when compared to a -5.16% increase for wild-type plants undergoing phytoremediation. Plants that were not phytoremediating increased leaf area at a steadier 41-61% rate. Percent change in plant height showed a similar that mutant plants grew taller without Kelthane (275 to 166%), while wild-type plants were significantly taller when phytoremediating (279 to 234%). Though not significant, mutant plants had more chlorophyll (0.458 without Kelthane and 0.182 g with Kelthane) when compared to wild-type plants (0.203 and 0.177 g). Mutant plants also had the highest Brix concentrations (121 and 3.61%), though the wild-type without Kelthane was significantly higher in Brix than the wild-type with Kelthane (39.1 and -5.63%). With plant dry mass, the mutant with no Kelthane had the highest mass (0.232 g) followed by the mutant with Kelthane (0.101 g). Finally, the mutant plants had the highest root masses (2.02 and 1.59 g) when compared to the wild-type plants (0.777 and 1.28 g).

## Conclusions

This experiment represents a much more comprehensive look at the phytoremediation of Kelthane when compared to three previous years of research. The amount of time that the plants grew was extended by a factor of eight and the amount of Kelthane was raised to more typical levels. Bioassay testing was also much improved, with additional precision.

Mutated tomato plants were healthier, sometimes statistically so, when compared to wild-type plants and test groups without Kelthane were healthier than those undergoing phytoremediation. Russell (2005) supports this conclusion and notes that plants must have phytotoxicity, or ability to withstand the presence of dicofol, a factor that wild-type tomatoes typically do not have. Weaver (2010) warns that tomato plants are usually fairly phytotoxic and are used as bioindicators meaning that their health will be adversely affected by the presence of pesticides like Kelthane.

The major finding from this experiment was that more effective phytoremediation occurred in wild-type tomato plants when compared to mutated tomato plants. "The root system is very highly branched...the root system branches profusely within one day after emergence, in contrast to normal roots, which branch only after several days of growth" (Zobel, 1971). This phenomenon may have actually hurt phytoremediation ability since the root branching causes stringier and less developed roots. Zobel also

notes that brt mutated tomato plants germinate more slowly than non-mutated plants (Volland and Zobel, 1988). The increased Brix concentration found in mutated plants seems to have contributed to plant health, but may have made enzyme transport more difficult (Peres, 2010). Relationships between the number of microbial enzymes and their effect on phytoremediation are currently being investigated (Benedito, 2011). Research to better explain the reason that wild-type plants are more effective phytoremediators of Kelthane is still ongoing.

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