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Effects of Triffid on Prunus Americana and Surrounding Rhizosphere

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Triffid is an organic fertilizer composed of bacterial and fungal synergists in a growth hormone laden nutrient complex. We investigated the effects of Triffid on nine parameters of Prunus americana: physical parameters of trunk diameter, early budding, and leaf development, as well as chemical parameters of the rhizosphere: water retention, organic matter, microbial activity, pH, and concentrations of potassium, phosphorus, and nitrate. Of the parameters tested, soil pH was the only one that showed a significant difference in means (p = 0.045) between control (6.22) and Triffid plots (6.55); the higher pH in the Triffid plots is consistent with results from application of nitrogen and potassium to soil (Kissel, D.E and P. F. Vendrell, 2004). However, results of the other parameters do not support the hypothesis that the added fungal relationship stimulated growth, affected overall soil nutrient levels including microbial activity, or decreased effects of environmental stressors. Although the sample size was small, the general trends for many of the parameters do not support the claims made by Triffid, perhaps due to the concentrations used in this study.

Introduction

All plant species rely on the accessibility of nine basic macronutrients to grow productively and help form and maintain organic compounds that make up the plant's structure. Out of the 9 macronutrients, nitrogen, potassium, and phosphorus are the most readily absorbed, which makes them highly scarce in untreated soil (Campbell et Al., 2008). For this reason, farmers, gardeners, and the common planter rely on the use to fertilizers to increase the yield of their crops, flowers, or shrubs.

Although many artificial fertilizers have been partly responsible for the high increase in crop production in the 20th century (Kane, 2010), there is an environmental cost to manufacturing these fertilizers. Organic fertilizers such as manure, compost, and fishmeal, already have the important macronutrients incorporated, eliminating the requirement to draw them from other sources.

One organic fertilizer, Triffid, claims to increase the soil microbes by 20 percent in 14 days through the use of an active component, 1-Tricontanol (Ford, n.d.). Triacontanol, a saturated long-chain alcohol in crystalline form isolated from alfalfa meal and chloroform extracts, has been found to stimulate plant growth.

Triacontanol has increased the dry weight, chlorophyll content, and net photosynthesis substantially in rice seedlings. It was also found to stimulate shoot growth, early flowering, and synthesize an essential component in plant tissue growth and development (Chen et al., 2005, Chen, et. al., 2002, and Dyson and Hall, 1972). What makes the use of triacontanol very practical is its ability to stimulate production at low concentrations. Laughlin, et al. (1983) found that growth increased at a dosage application of 1 nanogram per cubic decimeter with a maximum effective application of approximately 100 nanograms per cubic decimeter.

Triffid has been approved to be used on agricultural plants including *Prunus americana,* American plum tree, which is the subject of this experiment. Plum trees have an added mutualistc relationship with endo- and ectomycorrhizal fungi, which the application of Triffid should stimulate, causing an increase in secondary growth, nutrient content and microbial activity of the soil, and a decrease in the affect of environmental stressors.

Methods and Materials

Twenty six *Prunus americana* planted in 2006 in LeSuer Nature preserve near Monmouth, IL were designated into two groups: control and Triffid, with a buffer group in between (Appendix: Figure 1). The Triffid group was treated with an application of 10mL of concentrated Triffid (containing 0.33μL of triacontanol) in 1.89L of distilled water.

Three sampling dates at the beginning (June), middle (July), and end (September) of the growing season were analyzed for physical parameters of *Prunus americana* and chemical parameters of the rhizosphere. On each sampling date (pre- (June) and post-Triffid application (July and September)), the samples and data collected included: observation of the health of each tree, primary and secondary growth, and soil samples. The trees were observed for vibrancy of leaves, herbivore damage, leaf and shoot growth, discoloration of leaves and trunk, and surrounding weed growth. Secondary growth was measured as trunk diameter 12.5cm from the soil line with a micrometer.

Four soil samples were taken from each plot on each sample date. The collected soil samples, 5 x 13cm plugs (O and A horizon), from randomly selected trees near the root bulb were labeled and frozen for later analysis. One sample was typed for texture (sand: silt: clay) (Soil Types and Testing, n.d). The chemical parameters tested on these samples included: soil pH, organic matter, microbial activity, water retention, and concentrations of potassium, phosphorus, and nitrate.

Soil pH was measured using a 1:2 ratio of soil to 0.01M $CaCl_2$ (200g soil: 0.444g $CaCl_2$ in 500mL distilled water) (U.S Department of Agriculture, n.d). The soil water retention was calculated as the water loss of the weighted bulk sample after oven drying at 104.4°C. Soil organic matter was the difference in material lost after incineration at 1780°C. Soil microbial activity was measured with a CI-340 portable infrared gas analyzer which read the difference of CO_2 released (by measuring CO_2 in and CO_2 out) from the soil pellet (d=10.2mm, h=1.9mm). Additionally, the nitrate, potassium, and phosphorous were extracted from the soil and analyzed using a Hach DREL/2000 spectrophotometer.

Results

The soil type was found to be loamy: 48% sand, 29% silt, and 24% clay. The other parameters were statistically analyzed as two main groups due to the small sample sizes: 1) control vs. Triffid for September and 2) control vs. Triffid regardless of sampling date. This meant that any samples not receiving the Triffid application, including Triffid plots before application, were considered a control sample.

Out of the nine parameters examined in September between the control and Triffid groups, only the extracted potassium concentration from the soil was found to have significantly lower means in the Triffid group samples than the control ($P = 0.013$) (Table 2). However, this parameter was only found to be significant (2-sample T-test) after an outlier in the Triffid sample data set was removed. A non-parametric test was also run on the data (Mann-Whitney) which also found the potassium concentration to have a significantly lower mean in the Triffid samples than the control ($P = 0.021$) but only if the outlier was removed.

Out of the nine parameters examined disregarding the sampling date, soil pH was found to have a significantly different mean between the two groups ($P = 0.045$), without removing the outlier (Table 1). Using the Mann-Whitney non-parametric test, the soil pH was found to be marginally significant ($P = 0.081$), again only before outlier removal. The trunk diameter was also found to be marginally significant (p-value: 0.053) between the means of the Triffid and control groups using a 2-sample T test. The Triffid group was found to have a higher average trunk diameter; however, the significance dropped to a P of 0.145 when tested non-parametrically. Additionally, in the spring, both the control and Triffid groups had the same average number of leaf buds.

Conclusion:

Regardless of the statistical analysis used, our results were not consistent with expectations claimed for the Triffid fertilizer. When comparing all parameters, with the exception of soil pH and trunk diameter, the control group (in both the September group and the group excluding the sampling date) had a higher mean and median than the Triffid group. This included seeing a higher average concentration of nitrate, potassium, and phosphorous in the control soil samples than in the Triffid soil samples. This could have been a result of increased NPK nutrient uptake by *Prunus americana* from the soil. However, we cannot answer that for sure without more physical parameters to indicate the health of the tree or chemical parameters of the leaves or roots to indicate nutrient absorption. In addition, if this is true, it would suggest that the fertilizer would need to be continually applied to increase productivity over a long period of time, seeing that nutrients are lost more quickly than in the control plots.

When comparing the control and Triffid groups without differentiating by sampling date, the soil pH was found to be higher in the Triffid group than the control, supporting research by Kissel and Vendrell (2004) that pH increases when nitrogen and potassium are added to the soil. However, the September control samples had a higher mean and median pH than the Triffid samples that was marginally significant ($0.20<p>0.10$). But if the trees were absorbing more nutrients from the soil by the end of the growing season, then a lower pH in the Triffid plots would be expected. However, observations made over the course of the growing season did not show any difference between the recoveries of the treated *Prunus americana* to environmental stressors than the control. A possible reason for this could have been due to the intensity of the environmental stressor including herbivore grazing, disease, insect damage, and sun exposure due to grass coverage.

With the mixed results, further research can be done to determine if there is increased absorption of nutrients by the leaves and roots of the trees after application of Triffid. Also, due to the larger number of environmental stressors that the plum trees were exposed to during the course of the experiment, comparison of stress-related proteins produced by the trees to combat those stressors may also be useful in determining the effects Triffid has on *Prunus americana*.

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Appendix

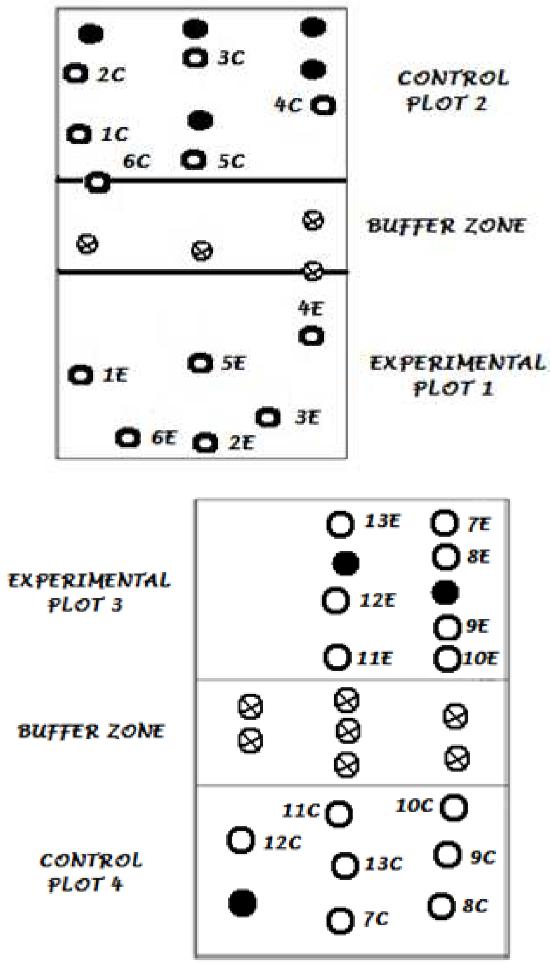

Figure 1: Plot set up in Le Suer Nature Preserve. The pairs of plots were located in the same area, about 80 feet apart. Experimental plots: 1 and 3, and control plots: 2 and 4, include 13 trees. A buffer zone of non-treated soil (11 trees) was to prevent fertilizer spread. Black dots represent the dead or damaged trees.

Table 1: The results of 9 parameters tested, comparing all the control samples to all the Triffid samples regardless of sampling date. Assuming normal distribution, p-values were determined using a 2-sample t-test. (P-value: after/before indicates after or before removal or an outlier; ^ means outliner removes). * Soil pH is significant ($p<0.05$) before the outlier is removed

	Triffid	Control	
Parameters (sample size=Triffid/Control)	mean/st. dev	mean/st. dev	P-value after/ before
Soil pH (8/14)	6.550/0.314	6.352/0.211	0.143^/0.045*
Microbial activity (ppm CO_2 released) (7/10)	127.59/52.26	138.68/39.5	0.645
Organic Material (g) (12/16)	0.332/0.079	0.389/0.153	0.210
Water Retention (g) (8/14)	10.98/1.36	11.36/1.88	0.607
Potassium (ppm K) (12/16)	1558.33/651.51	1768.75/537.55	0.373
Phosphorus (ppm P) (12/15)	11.75/10.55	15.9/13.8	0.381^/0.193
Nitrate (ppm N) (11/15)	2.73/0.82	5.29/1.4	0.177^/0.217
Number of buds (13/13)	26/28	26/38	0.958
Trunk Diameter (mm) (Difference: Sept-June) (12/9)	1.08/0.73	0.60/0.27	0.053

Table 2: Chemical parameters of soil samples from September sampling date. Assuming normal distribution, the p-values were determined using a 2 sample t -test. (P-value: after/ before indicates either before or after outlier removal: ^ means outlier was removed). *Potassium found to be significant

	Triffid	Control	
Parameters (sample size)	mean/st.dev	mean/st.dev	P-value (after/before)
Soil pH (n=4)	6.35/0.172	6.66/0.267	0.107
Organic Matter (g) (n=10)	0.335/0.083	0.384/0.120	0.302
Microbial Activity (ppm CO_2 released) (n=5)	-145.66/50.75	-166.45/52.56	0.571
Water Retention (g) (n=6)	10.97/1.59	10.53/2.04	0.690
Potassium (ppm K) (Control=10)(Triffid=9)	1356/313	1850/450	0.013^*/0.135
Phosphorus (ppm P) (n=10)	13.1/11.09	16/15.52	0.637
Nitrate (ppm NO_3) (n=10)	10.6/4.38	11.3/5.44	0.755