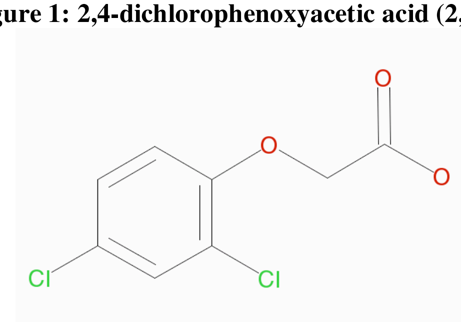
EFFECTS OF 2,4-D ON ANTHOCYANIN PIGMENTS IN RED NORLAND POTATOES (SOLANUM TUBEROSUM)

Shannon Engelman 2006

Introduction

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), shown in Figure 1, has been found to enhance the red coloration in Red Norland potatoes. This herbicide is popular amongst farmers because consumers are more inclined to buy potatoes with enhanced red coloration (1). Red coloration in potato periderm is due to the presence of anthocyanin pigments, which have antioxidative properties (2). Research suggests that 2,4-D may increase anthocyanin pigments in potatoes because of an observed color difference; however, there has been no published work on the role of 2,4-D in anthocyanin accumulation.

Figure 1: 2,4-dichlorophenoxyacetic acid (2,4-D)



Determining how 2,4-D enhances red coloration in potato periderm is of interest because there are health considerations associated with 2,4-D. The chemical has an LD-50 of 1500 mg/kg and has potential health effects, such as respiratory tract irritation, delayed pulmonary edema, liver and kidney damage, and reproductive and fetal effects (3). Although 2,4-D is harmful, it has not been shown to have negative health effects on consumers when applied properly. A study by Nelson, *et al*. showed that 2,4-D residues in potatoes themselves are negligible— only 15 to 110 ppb (4).

My study had three specific goals. The first goal was to use a colorimeter to determine how the following three aspects of visual color were changed with 2,4-D application: light intensity, which is the amount of reflected light; chroma, which is the saturation of a particular area with color; and hue angle, which is the basic color. The second goal was to quantitatively measure anthocyanin accumulation resulting from 2,4-D application. The third goal was to determine how 2,4-D application affected UFGT accumulation in the anthocyanin biosynthetic pathway by comparing UFGT concentration with actin concentration, which is unaffected by 2,4-D (5).

Figure 2 shows the last two steps in the anthocyanin biosynthetic pathway. These steps involve the conversion of leucoanthocyanin to anthocyanidin by leucoanthocyanidin dioxygenase (LDOX), and the conversion of anthocyanidin to anthocyanin by UDP-glucose: flavonoid 3-*O*-glucosyltransferase (UFGT). A study by Kobayashi, *et al*. found that UFGT is the gene in the anthocyanin biosynthetic pathway responsible for expressing all anthocyanin pigments (6). There are two types of anthocyanins believed to be primarily responsible for the dark red pigmentation in

Red Norland potatoes. Those are petunidin-type anthocyanins that appear purple and pelargonidin-type anthocyanins that appear red (5).

Figure 2: Anthocyanin Biosynthetic Pathway

LDOX UFGT

leucocyanidin → *anthocyanidin* → *anthocyanin*

The methods for my study were modeled after a study by Rosen, *et al*. (2004). The Rosen study found that at vine kill[1], light intensity and hue angle decreased, chroma was not significantly changed, and anthocyanin accumulation increased with 2,4-D application (7).

My hypotheses were based on the Rosen study and several other studies documenting visual red color enhancement with 2,4-D application. My first hypothesis was that chroma would be unaffected by 2,4-D application, but light intensity and hue angle would decrease. My second hypothesis was that anthocyanin accumulation would increase with 2,4-D application, because 2,4-D was found to enhance the red coloration of Red Norland potatoes. My third hypothesis was that UFGT accumulation would increase with 2,4-D application.

Methods

Potato Harvesting: Twelve potato plants were grown in a greenhouse maintained at 21 °C in daylight and at 13-16 °C at night. Half of the plants were sprayed with the ester form of 2,4-D at increasing concentrations [30 mL/acre, 44 mL/acre, and 59 mL/acre] at one-or two-week intervals between flowering and vine kill. Spraying was done outside. Laboratory gloves, protective clothing, and goggles were worn at all times when handling 2,4-D and 2,4-D treated potatoes. Vines were cut from all plants at vine-kill. Three treated plants and three untreated plants were harvested. Methods of growth in the greenhouse study were modeled after those of the Rosen, *et al*. study.

Color Analysis: Harvested potatoes weighing over 500 mg were washed and dried. A colorimeter was used to measure light intensity, chroma, and hue angle of each potato.

Anthocyanin Accumulation Measurement: Five, 7-mm radius disks of periderm were peeled from three potatoes that had been treated with 2,4-D and from three control (untreated) potatoes. The disks were placed into acidified methanol for 24 h at 4 °C. Then, absorbency was measured at 513 nm.

RNA Isolation: All potatoes weighing over 50.00 g were peeled, and the tissue was placed in aluminum foil and flash-frozen, using liquid nitrogen. The samples were stored at –80 °C. Next, 50.00 mg of each tissue sample were transferred to a mortar and ground with liquid nitrogen. The tissue was decanted into 2-mL collection tubes, and 200 µL of buffer RLT from the Qiagen RNeasy Plant Mini Kit were added. The buffer and ground tissue were put into a QIAshredder column. Then, the RNeasy Mini Protocol for Isolation of Total RNA was followed. The optional RNase-Free DNase Set was used for DNase digestion.

Reverse Transcription: A solution was prepared with 1 µL oligo$(dT)_{20,}$ 1 µg mRNA, 1 µL 10 mM dNTP [10 mM each dATP, dGTP, dCTP and dTTP at neutral pH], and 10 µL dH_2O and mixed. The mixture was heated at 65 ºC for five minutes, incubated on ice for a few minutes, and

[1] Vine kill is an agricultural method, where vines are cut approximately 6-8 weeks after germination to ensure that periderm sets properly.

briefly centrifuged. Then, 4 µL 5X first-strand buffer, 1 µL 0.1 M DTT, and 1 µL SuperScript™ III RT were added, and the mixture was incubated at 50 °C for 50 min. The reaction was inactivated by being heated at 70 °C for 15 minutes.

PCR: To prepare samples for PCR, 40 µL PCR-grade water, 5 µL 10X buffer, 1 µL cDNA, 2 µL primer mix, 1 µL 50X dNTP Mix, and 1µL 50X Taq polymerase were mixed. Primers for actin, as well as UFGT, were used. If means of control and treatment data differed by more than the least significant difference (LSD) of 0.10, they were considered statistically significant.

Transcript levels relative to actin were found by dividing calculated UFGT RNA concentration by calculated actin RNA concentration. A t-test was done to compare transcript levels of treated and control samples. The significance level was set at 0.05*****

Results

Table 1 shows colorimeter results for potatoes at vine kill, including light intensity (L), chroma (C), and hue angle (H) results. Anthocyanin accumulation (A) is also shown. Analysis of variance (ANOVA) with two degrees of freedom showed that there was a significant difference between those that were treated with 2,4-D and those that were not, except for chroma and anthocyanin accumulation in the greenhouse study. In the field study, light intensity and hue angle decreased significantly ($p<0.01$ for both) in 2,4-D treated potatoes, while chroma and anthocyanin accumulation increased significantly ($p<0.01$ and $p<0.05$, respectively) in 2,4-D treated potatoes. In the greenhouse study, light intensity and hue angle decreased significantly ($p< 0.01$ and 0.05, respectively) in 2,4-D treated potatoes, while chroma and anthocyanin accumulation were not significantly changed in 2,4-D treated potatoes.

Table 1: Colorimeter and Anthocyanin Accumulation Results

	Field Study				*Greenhouse Study*			
	L	**C**	**H**	**A**	**L**	**C**	**H**	**A**
Control	47	28.1	12.1	0.24	51	25.7	8.1	0.17
Treatment	45	30.5	7.4	0.52	45	28.8	0.1	0.18
Analysis of Variance p-value	**	**	**	*	*	NS	**	NS
LSD (0.10)	1.4	1.2	1.5	0.05	3.6	—	3.6	—

NS= Non significant; *, **= Significant at 5% and 1% respectively

Figure 3 shows UFGT transcript levels relative to actin in two controls and two samples treated with 2,4-D. Relative transcript levels in control samples were significantly higher than levels in treated samples ($p= 0.0094$). Figure 4 shows greenhouse samples that were analyzed the same way as the field samples (Figure 3). A t-test was done with averages of relative transcript level ratios. There was not a significant difference between control and 2,4-D-treated samples ($p= 0.0615$); however, the p-value is close to the level of significance.

Figure 3: Statistical Analysis of qPCR Field Data (#105 and #205 +2,4-D, #101 and #406 controls)

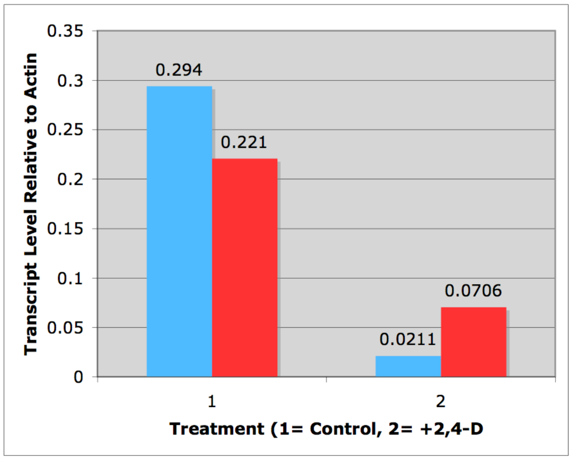
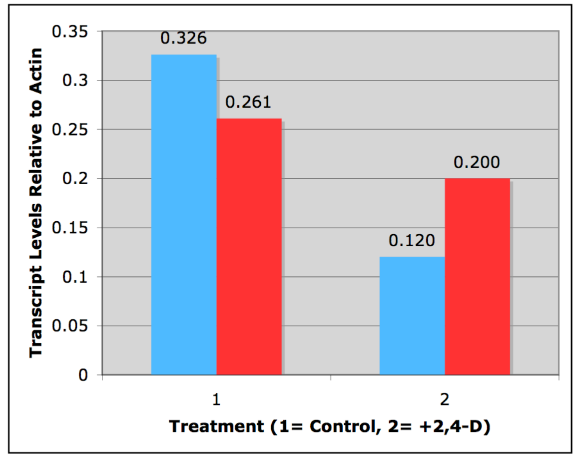


Figure 4: Statistical Analysis of qPCR Greenhouse Potatoes (#17 and #7 Controls, #13 and #11 +2,4-D)



Conclusion

The application of 2,4-D significantly affected the periderm coloration of Red Norland potatoes, giving treated potatoes a more purple and slightly darker appearance. In the greenhouse study, my first hypothesis that 2,4-D would decrease light intensity and hue angle but would not affect chroma was supported ($p<0.05$ and $p<0.01$, respectively). In the field study, my hypothesis was supported regarding light intensity and hue angle ($p<0.01$ for both), but my hypothesis that chroma would not be changed by 2,4-D application was refuted, because chroma significantly increased ($p<0.01$).

My hypothesis that 2,4-D application would increase anthocyanin accumulation was refuted in the greenhouse study ($p>0.05$) but accepted in the field study ($p<0.01$). My hypothesis that UFGT accumulation would increase with 2,4-D application was refuted in both the field and greenhouse studies, and results indicate a decrease in UFGT accumulation in 2,4-D treated samples. The decrease was significant in the field study ($p=0.0094$), but not significant in the greenhouse study ($p=0.0615$).

My results show that red coloration in Red Norland potatoes was enhanced with 2,4-D application, but results indicated that this color change was not due to an increase in anthocyanin pigments. Future work should be done to determine if the color difference that resulted from 2,4-D application was caused by increased production of petunidin-type anthocyanins rather than pelargonidin-type anthocyanins, since my study found that the color change is not due to an increase in anthocyanin pigments.

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