

2,4-Dichlorophenoxyacetic Acid Increases Peonidin Derivatives in Red Norland Periderm

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Abstract Studies were conducted to determine the effects of selected plant growth regulators on red color development of Red Norland potato periderm, and the mechanism by which color changes occurred. Two derivatives of the auxin 2,4-dichlorophenoxyacetic acid (2,4-D ester and 2,4-D amine), S-abscisic acid, n-propyl dihydrojasmonate, and the ethylene synthesis inhibitor aminoethoxyvinylglycine were foliar applied at 4, 6, and 8 weeks after emergence. Only the 2,4-D ester and 2,4-D amine significantly improved tuber periderm red color in two seasons of evaluation. Red color faded during storage regardless of treatment. Anthocyanin content at vine kill was significantly increased by 2,4-D treatment in 2004 but not 2005. The 2,4-D treatments increased the ratio of peonidin derivatives to pelargonidin derivatives compared with controls. This change in ratio may explain the observed change in hue angle with 2,4-D treatment compared to controls.

Resumen Se realizaron trabajos para determinar los efectos de reguladores de crecimiento selectos sobre el

desarrollo del color rojo del peridermo de papa de Red Norland y el mecanismo por el cual ocurrieron los cambios de color. Dos derivados de la auxina 2,4-ácido diclorofenoxyacético (2,4-D éster y la amina 2,4-D), S-ácido abscísico, n-propil dihidrojasmonato y el inhibidor de la síntesis del etileno aminoetoxivinilglicina se aplicaron a las 4, 6 y 8 semanas después de la emergencia. Solo el éster 2,4-D y la amina 2,4-D mejoraron significativamente el color rojo del peridermo en dos temporadas de evaluación. El color rojo disminuyó durante el almacenamiento en todos los tratamientos. El contenido de antocianina en el momento de la destrucción del follaje aumentó significativamente con el tratamiento de 2,4-D en el 2004, pero no en el 2005. Los tratamientos con 2,4-D incrementaron la relación de derivados de peonidina a derivados de pelargonidina comparado con los testigos. Este cambio en la relación explicaría los cambios observados en el ángulo de matiz con el tratamiento 2,4-D comparado con los testigos.

Keywords Anthocyanin · Color · Pelargonidin · Peonidin

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Abbreviations

2,4-D	2,4-dichlorophenoxy acetic acid
ABA	abscisic acid
AVG	aminoethoxyvinyl glycine
DTPA	diethylene triamine pentaacetic acid
PDJ	n-propyl dihydrojasmonate
HPLC	high pressure liquid chromatography

Introduction

Enhancement or maintenance of bright skin color in red potatoes (*Solanum tuberosum* L.) is a primary factor associated with attaining a high price for this crop. Red color in tuber periderm is due to the presence of anthocyanins. Anthocyanins are glycosylated flavonoids,

which are biosynthesized via the shikimic acid pathway (Holton and Cornish 1995). In many plant species, anthocyanin biosynthesis is limited by the transcription of the biosynthetic genes (Mol et al. 1998). Factors other than RNA transcription, such as amounts and types of anthocyanins; the presence of metal ions and copigments, like flavonols and flavones; cell shape; and pH of the vacuoles where anthocyanins accumulate, also affect the color of anthocyanins (Mol et al. 1998).

To help maintain bright red skin color, growers apply 2,4-D, a growth regulator, during the growing season as a foliar spray. While 2,4-D applied at low concentrations has been used with some success for many years (Fults et al. 1950; Nylund 1956), periderm color still tends to fade with storage. The mechanism of 2,4-D enhancement of red color in potato periderm is not known. In addition, there is a need to find alternative compounds that may be more effective and perhaps more benign environmentally than 2,4-D in enhancing and maintaining red periderm color. Studies have shown that other plant growth regulators can induce anthocyanin biosynthesis, so may be worth testing on potatoes. For example, abscisic acid (ABA) has been shown to enhance red coloration of grapes (Cantín et al. 2007; Peppi et al. 2006). Kondo et al. (2000) showed that a jasmonic acid analog, propyl dihydrojasmonate (PDJ) increased anthocyanin accumulation when sprayed preharvest on apple fruit. The overall objectives of this study were to 1) evaluate two types of 2,4-D (ester and amine) and other plant growth regulators for their effects on periderm color and yield of 'Red Norland' potatoes and 2) determine the possible mechanisms by which 2,4-D enhances red coloration of potato periderm.

Materials and Methods

Field Studies

These studies were conducted in 2004 and 2005 at the Sand Plain Research Farm in Becker, Minnesota on a Hubbard loamy sand. Selected soil chemical properties (0–6") prior to planting were (2004/2005): organic matter, 2.4/2.3%; pH, 6.0/6.5; Bray P1, 26/43 mg kg⁻¹; ammonium acetate extractable K, Ca, and Mg, 104/127, 174/157, and 913/875 mg kg⁻¹, respectively; and DTPA-extractable Zn, 1.4/1.0 mg kg⁻¹.

Three, 6-m rows were planted for each plot with the middle row used for sampling and harvest. Whole "B" seeds were hand planted in furrows in late April. Spacing used was 90 cm between rows and 25 cm within the row. In the first year six treatments were evaluated, while in the second year 12 treatments were evaluated.

The six treatments tested in 2004 were:

1. Untreated check
2. N-propyl dihydrojasmonate (PDJ) 1×, 100 mg L⁻¹ in 3 applications
3. PDJ 5×, 500 mg L⁻¹ in 3 applications
4. Abscisic acid (ABA), 500 mg L⁻¹ in 3 applications
5. 2,4-D, 2-ethylhexyl ester in 3 applications (75 mL ha⁻¹, 112.5 mL ha⁻¹, 150 mL ha⁻¹)
6. 2,4-D dimethylamine in 3 applications (75 mL ha⁻¹, 112.5 mL ha⁻¹, 150 mL ha⁻¹)

The twelve treatments evaluated in 2005 were:

1. Untreated check
2. Aminovinyl glycine (AVG), 500 mg L⁻¹
3. AVG, 1,000 mg L⁻¹
4. PDJ, 100 mg L⁻¹
5. PDJ, 100 mg L⁻¹, foliar applied 3 weeks after plant emergence
6. PDJ, 100 mg L⁻¹, foliar applied 3 and 5 weeks after plant emergence
7. PDJ, 100 mg L⁻¹, foliar applied at 4, 6, and 8 weeks after emergence
8. ABA, 500 mg L⁻¹ with 0.05% vol/vol Tween 20, foliar applied at 28, 42, and 56 days after emergence plus one application prior to vine kill
9. ABA, 75 mL ha⁻¹, 112.5 mL ha⁻¹, 150 mL ha⁻¹ with 0.2% vol/vol NuFilm, foliar applied at 28, 42, and 56 days after emergence
10. 2,4-D dimethylamine, 75 mL ha⁻¹, 112.5 mL ha⁻¹, 150 mL ha⁻¹ with 0.2% vol/vol NuFilm, foliar applied at 28, 42, and 56 days after emergence
11. 2,4-D, 2-ethylhexyl ester, 150 mL ha⁻¹, 225 mL ha⁻¹, 300 mL ha⁻¹ with 0.2% vol/vol NuFilm, foliar applied at 28, 42, and 56 days after emergence
12. 2,4-D dimethylamine, 150 mL ha⁻¹, 225 mL ha⁻¹, 300 mL ha⁻¹ with 0.2% vol/vol NuFilm, foliar applied at 28, 42, and 56 days after emergence.

The AVG used was VBC-30012 (5% wt/wt), provided by Valent BioSciences Corporation, Long Grove, Illinois. The PDJ was VBC-30050 (5.35%), provided by Zeon Corporation, Tokyo, Japan, and the ABA was VBC-30025 (90% S-ABA), provided by Valent BioSciences Corporation, Long Grove, Illinois. The 2,4-D dimethylamine was Clean Crop 4CA, 46.5% ai. The 2,4-D, 2-ethylhexyl ester was AGSCO 400, 71% ai.

For three of the 12 treatments in the second year (treatments 2, 3, and 4), tubers were dipped in a solution containing the specified treatment for 1 hour prior to planting. Each treatment was replicated four times in a randomized complete block design. Admire was applied in-furrow for beetle control. Weeds, diseases, and other insects were controlled using standard practices. A banded fertil-

Table 1 Effects of 2,4-D and experimental compounds on tuber color at vine kill, 2004

Treatments	Color	Anthocyanin			
		Metric lightness	Metric chroma	Metric hue angle	
1	Untreated check	47.0 a	28.1 c	12.1 a	0.242a
2	PDJ, 100 mg L ⁻¹	45.8 ab	29.7 b	11.8 a	0.268ab
3	PDJ, 500 mg L ⁻¹	46.1 ab	29.4 bc	11.5 a	0.277abc
4	ABA, 500 mg L ⁻¹	45.6 ab	29.5 bc	11.2 a	0.307bc
5	2-4-D Ester	45.0 bc	30.5 ab	7.4 b	0.323c
6	2-4-D Amine	43.8 c	31.2 a	6.9 b	0.311bc

Means followed by the same letter are not significantly different at $P=0.05$

izer application of 896 kg ha⁻¹ of 8N-16P-16K was made at planting. Post-plant N applications were supplied as ammonium nitrate and cultivated in at emergence or incorporated by the hilling operation. Fertilizer at 111 kg N ha⁻¹ was applied at emergence and at 34 kg N ha⁻¹ at hilling about 14 days later.

All other treatments were applied using a CO₂ backpack sprayer connected to a boom spanning the width of three rows. The boom was held by two people, one person at each end. Treatments were applied to the foliage of each plot by calibrating a walk to deliver the appropriate amounts. Treatments were applied based on timing. Applications were performed 21, 28, 35, 42, and 56 days after emergence starting in mid-June, and the week of vine kill in early August. All products were applied with 143 L of water per ha. In 2004 and 2005, the 2,4-D treatments were applied at the following rates:

- 75 mL ha⁻¹: 18 June 2004 / 21 June 2005
- 113 mL ha⁻¹: 2 July 2004 / 6 July 2005
- 150 mL ha⁻¹: 16 July 2004 / 15 July 2005

In 2005, an additional 2,4-D amine treatment at double the rates listed above was included. Tubers were about 1 cm in diameter on the first spray application date. NuFilm (an adjuvant) at 300 mL ha⁻¹ was applied with all 2,4-D applications.

Vines were sprayed with Diquat on 29 July 2004 and 5 August 2005 and plots were machine-harvested on 20 August 2004 and 29 August 2005. Total tuber yield, graded yield, tuber number (2005 only), and the incidence of hollow heart and brown center were measured after harvest. Tubers were stored at 4°C and then reevaluated after 3 months.

Greenhouse Studies

Twelve potato plants were grown in a greenhouse maintained at 21°C in daylight and at 13–16°C at night. Plants were grown in Hubbard loamy sand obtained from the Sand Plain Research Farm in 19 liter pots. Admire was applied to the exterior of tubers at planting at a rate mimicking that in the field (30 uL per ml dH₂O). Eight g of fertilizer (8N-

Table 2 Effects of 2,4-D and experimental compounds on tuber color at vine kill, 2005

Treatments	Color	Anthocyanin			
		Metric lightness	Metric chroma	Metric Hue angle	
1	Control	41.6 a	28.8 ab	16.8 a	
2	AVG, 500 mg L ⁻¹ dip	43.0 a	29.6 ab	14.4 ab	
3	AVG, 1,000 mg L ⁻¹ dip	42.2 a	28.7 ab	16.9 a	
4	PDJ, 100 mg L ⁻¹ dip	41.3 a	29.2 ab	16.3 a	
5	PDJ, 100 mg L ⁻¹	44.1 a	27.0 b	16.3 a	
6	PDJ, 100 mg L ⁻¹	41.5 a	29.7 ab	15.5 a	
7	PDJ, 100 mg L ⁻¹	43.0 a	28.4 ab	16.0 a	
8	ABA, 500 mg L ⁻¹	42.2 a	29.2 ab	15.7 a	
9	ABA, 500 mg L ⁻¹	41.8 a	29.5 ab	15.2 a	
10	2-4-D Amine 337.5 mL ha ⁻¹	42.3 a	29.0 ab	11.5 bc	
11	2-4-D Ester 337.5 mL ha ⁻¹	42.1 a	29.0 ab	11.0 dc	
12	2-4-D Amine 675 mL ha ⁻¹	40.9 a	30.6 a	8.1 d	

Means followed by the same letter are not significantly different at $P=0.05$

Table 3 Effects of 2,4-D and experimental compounds on tuber color at harvest, 2004

Treatments	Color				Anthocyanin
	Visual rating	Metric lightness	Metric chroma	Metric hue angle	
1 Control	2.3 b	42.9 a	28.6 a	18.9 a	0.162ab
2 PDJ, 100 mg L ⁻¹	1.8 b	42.2 a	29.4 a	17.5 a	0.136a
3 PDJ, 500 mg L ⁻¹	1.8 b	42.7 a	28.4 a	19.1 a	0.147a
4 ABA, 500 mg L ⁻¹	2.3 b	42.9 a	28.5 a	18.3 a	0.154ab
5 2-4-D Ester	3.3 a	40.1 b	31.1 a	11.5 b	0.198b
6 2-4-D Amine	3.3 a	39.3 b	31.6 a	11.7 b	0.180ab

Visual red color rating: 1 (pale red/pink) to 5 (dark red)

Means followed by the same letter are not significantly different at $P=0.05$

19P-30K) was applied to soil at planting. Ammonia nitrate (33N-0P-0K) was applied at emergence (1.62 g) and hilling (0.6 g).

Half of the plants were sprayed with the ethylhexyl ester form of 2,4-D at increasing concentrations (75 mL ha⁻¹, 110 mL ha⁻¹, and 147.5 mL ha⁻¹) at 7-or 14-day intervals between flowering and vine kill. The ester form of 2,4-D is registered for use on potatoes, and the amine form is not, so only the ester form was used in the greenhouse studies. Vines were cut by hand from all plants at vine-kill. Tubers from three treated plants and three control plants were harvested. Fourteen days later, tubers from the rest of the plants were harvested.

Another set of 18 plants was started 14 days after the first planting, and grown in the same way as the first planting.

Color and Anthocyanin Measurements

Harvested potatoes were washed with tap water and air-dried. A Minolta CR-200 colorimeter was used to measure

light intensity, chroma, and hue angle of each potato. For field-grown tubers, 25 tubers per plot (100 tubers per treatment) were evaluated for color using a Minolta CR-200 colorimeter just prior to vine kill, after harvest, and after 4 months in storage at 4°C. At harvest, in addition to using the colorimeter, 25 tubers from each treatment were also rated visually for red color. The color of all greenhouse-grown tubers >500 mg was also measured using the Minolta colorimeter.

For total anthocyanin measurements, five 7-mm diameter 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 (10% concentrated HCl, v/v) methanol for 24 h at 4°C after which absorbance at 513 nm of the solution was measured.

High Pressure Liquid Chromatography (HPLC)

Anthocyanins were extracted from two periderm samples of control or 2,4-D-treated tubers following the methods

Table 4 Effects of 2,4-D and experimental compounds on tuber color at harvest, 2005

Treatments	Color			
	Visual rating	Metric lightness	Metric chroma	Metric hue angle
1 Control	1.6 b	44.0 ab	22.8 a	27.1 a
2 AVG, 500 mg L ⁻¹ dip	1.5 b	45.4 a	22.7 a	25.9 a
3 AVG, 1,000 mg L ⁻¹ dip	1.5 b	44.9 ab	23.0 a	27.2 a
4 PDJ, 100 mg L ⁻¹ dip	1.4 b	43.5 ab	24.3 a	26.8 a
5 PDJ, 100 mg L ⁻¹	1.5 b	42.0 ab	23.1 a	27.5 a
6 PDJ, 100 mg L ⁻¹	1.6 b	44.6 ab	23.7 a	25.6 a
7 PDJ, 100 mg L ⁻¹	1.3 b	44.5 ab	23.0 a	26.1 a
8 ABA, 500 mg L ⁻¹	1.3 b	44.2 ab	22.8 a	27.7 a
9 ABA, 500 mg L ⁻¹	1.1 b	44.9 ab	22.9 a	25.9 a
10 2-4-D Amine 337.5 mL ha ⁻¹	3.0 a	42.2 ab	24.1 a	19.5 b
11 2-4-D Ester 337.5 mL ha ⁻¹	3.3 a	40.9 b	24.6 a	19.2 b
12 2-4-D Amine 675 mL ha ⁻¹	3.4 a	42.0 ab	24.5 a	17.5 b

Visual red color rating: 1 (pale red/pink) to 5 (dark red)

Means followed by the same letter are not significantly different at $P=0.05$

Table 5 Effects of 2,4-D and experimental compounds on tuber color after storage, 2004

Treatments		Color		
		Metric lightness	Metric chroma	Metric hue angle
1	Control	42.9	23.1	25.5
2	PDJ, 100 mg L ⁻¹	44.6	20.9	28.2
3	PDJ, 500 mg L ⁻¹	46.3	21.9	26.0
4	ABA, 500 mg L ⁻¹	45.6	21.0	26.6
5	2-4-D Ester	43.6	21.7	22.1
6	2-4-D Amine	43.5	22.7	20.3
Significance		NS	NS	NS

NS = Non significant at $P=0.05$

described by Lewis et al. (1998). Field samples were from the 2004 vine kill. Due to a lack of tissue from the 2,4-D ester treatments from the field experiments, the 2,4-D amine treatment was used for HPLC. Greenhouse samples were from tubers harvested at vine kill. HPLC analysis of anthocyanins was performed on a system fitted with a 5 μm Spherisorb (4.6 mm \times 250 mm ODS2) C₁₈ column, Waters 2,690 Separations Module, and a Waters 996 Photodiode Array Detector setup to scan wavelengths 260–600 nm. Solvent and gradient conditions followed methods described by Lewis et al. (1998) or Rodríguez-Saona et al. (1998). Resolution of anthocyanin peaks was significantly improved by following solvent and gradient methods described by Rodríguez-Saona et al. (1998). For the Lewis et al. (1998) method, solvent A was 10% (v/v) aqueous acetic acid and solvent B was acetonitrile. A linear gradient from 0 to 30% solvent B in 30 minutes with a flow rate of 1 mL min⁻¹ was used. Samples dissolved in solvent A were injected. For the Rodríguez-Saona et al. (1998) method, solvent A was 100% acetonitrile and solvent B was 1%

phosphoric acid, 10% acetic acid, 5% acetonitrile and water. A linear gradient from 0 to 30% solvent A in 30 minutes with a flow rate of 1 mL min⁻¹ was used. Replicate 50 μL volumes of samples dissolved in solvent B were injected. An eluted fraction from 24.5 to 27 minutes was collected for mass spectral analyses.

Mass Spectroscopy

Low resolution mass spectroscopy was performed using electrospray ionization. The instrument used was a Thermo Electron LCQ Classic Ion Trap Mass Spectrometer equipped with an ion spray source (source voltage = 4,500) and a 5 μL injection loop. Three replicates of each collected HPLC fraction were injected directly onto the system.

Statistical Analyses

Statistical analyses were performed using the general linear model procedure (SAS Institute 1986). Mean separations

Table 6 Effects of 2,4-D and experimental compounds on tuber color after storage, 2005

Treatments		Color		
		Metric lightness	Metric chroma	Metric hue angle
1	Control	43.2	20.7	33.4
2	AVG, 500 mg L ⁻¹ dip	43.1	20.7	34.1
3	AVG, 1,000 mg L ⁻¹ dip	40.9	23.3	32.8
4	PDJ, 100 mg L ⁻¹ dip	42.9	20.3	36.2
5	PDJ, 100 mg L ⁻¹	43.0	20.6	33.4
6	PDJ, 100 mg L ⁻¹	43.2	19.3	36.6
7	PDJ, 100 mg L ⁻¹	41.1	21.5	33.8
8	ABA, 500 mg L ⁻¹	43.8	19.8	36.0
9	ABA, 500 mg L ⁻¹	43.4	19.6	36.5
10	2-4-D Amine 337.5 mL ha ⁻¹	42.7	19.7	29.5
11	2-4-D Ester 337.5 mL ha ⁻¹	38.8	21.8	26.9
12	2-4-D Amine 675 mL ha ⁻¹	42.3	19.2	26.9
Significance		NS	NS	NS

NS = Non significant at $P=0.05$

Table 7 Effects of 2,4-D ester on color and anthocyanin accumulation in periderm of greenhouse-grown tubers at vine kill

Treatments	Vine kill			Harvest				
	Metric lightness	Metric chroma	Metric hue angle	Antho	Metric lightness	Metric chroma	Metric hue angle	Antho
Control	51	25.7	6.1	0.17	55	22.3	12.3	0.21
2,4-D Ester	45	28.8	0.1	0.18	48	26.8	1.8	0.31
Significance	*	NS	**	NS	*	NS	**	NS

Antho = anthocyanin, NS = Non significant; *, **, = significant at 10% and 1%, respectively

were done using least square differences (color and anthocyanin data) or the Waller-Duncan Bayesian procedure with a k-ratio set at 100 (yield and size data).

Results and Discussion

Field Studies

Total and graded yields were not significantly affected by treatment in either year (data not shown). In 2004 at vine kill, the 2,4-D treatments resulted in tubers with the darkest, most intense, and most purplish-red color based on lesser lightness, greater metric chroma, and lesser metric hue angle readings, respectively (Voss 1992) (Table 1). Anthocyanin accumulation per given surface area was increased by the 2,4-D and ABA treatments. In 2005 at vine kill, the 2,4-D treatments exhibited lesser hue angle, indicating that they had a more purple color than control tubers (Table 2), but there were no significant differences in lightness or chroma. Although PDJ increased chroma in 2004, in general, compounds other than 2,4-D did not significantly affect color in either 2004 or 2005. At harvest in both years, the red color had changed in all treatments compared with the color detected at vine kill based on higher metric hue angle values (Tables 3 and 4). However, tubers from the 2,4-D treatments were visually redder than those of the control and other treatments. There was a good correlation between visual assessment and metric hue angle readings. In 2004, there were no significant differences at harvest in anthocyanin content of the periderm. After 3 months of storage, the red color continued to fade in all treatments, although there was a trend for the 2,4-D treatments to result

in redder tuber periderm than the others based on lesser hue angle measurements (Tables 5 and 6).

2,4-D Effects on Greenhouse-Grown Tubers

The application of 2,4-D significantly affected the periderm coloration of greenhouse-grown 'Red Norland' potatoes, as it did in field-grown potatoes, giving treated potatoes a more purple-red and slightly darker appearance than controls (Table 7). In the greenhouse study, light intensity and hue angle were significantly less in the 2,4-D treated potatoes compared with the controls at both vine kill and harvest, while chroma and anthocyanin accumulation were not significantly different. There were no significant differences between controls and 2,4-D treatments in total anthocyanin content per given surface area at either vine kill or harvest.

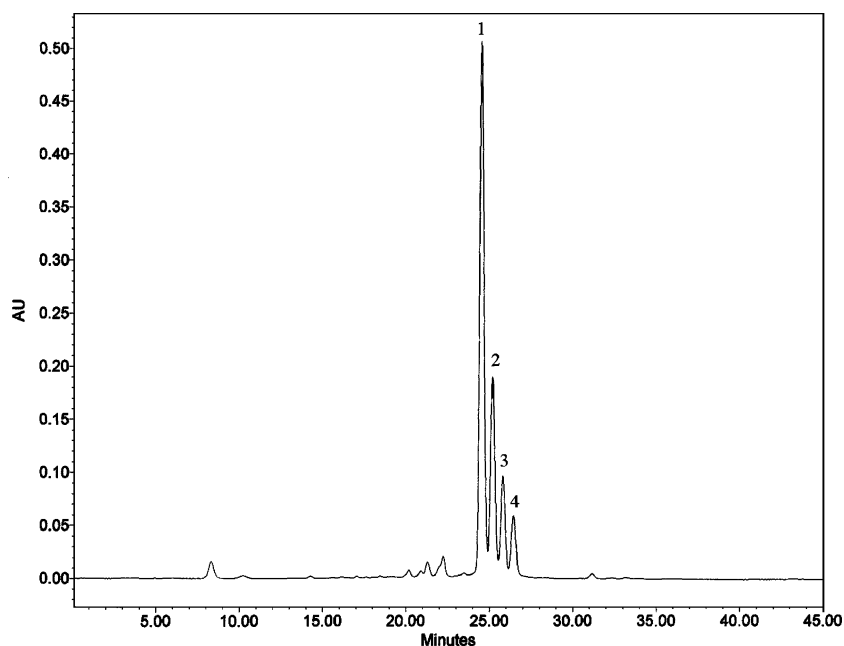
Anthocyanin Analyses

The total peak area of a single, predominant anthocyanin peak (following methods described by Lewis et al.) was four times greater (comparing peak areas at 500–530 nm) in the field-grown 2,4-D treatment than in the control, and two and a half times greater in the greenhouse-grown 2,4-D treatment compared with the control in 'Red Norland' periderm (Table 8). The greater anthocyanin levels in tubers of greenhouse-grown, 2,4-D treated plants over controls were discernable in the HPLC samples and not in samples measured spectrophotometrically (Table 7). This was probably due to the use of different anthocyanin extraction methods. Samples used for spectroscopy were extracted in acidified methanol overnight at 4°C and in darkness, then

Table 8 Anthocyanin retention times and peak areas

Location	Treatment	Retention time (min)	Peak are (500–530 nm)
Field	Control	27.80	5,819,070
	2,4-D Amine	27.64	23,404,729
Greenhouse	Control	27.98	8,668,392
	2,4-D Ester	27.88	21,660,315

Fig. 1 Representative HPLC separation of anthocyanins (500 nm to 530 nm) indicating four major peaks. See Table 9 for peak identities



measured the next day. Samples subjected to HPLC were extracted three times sequentially with acidified methanol, then dried to completion and stored at -80°C and in darkness prior to separation. Samples that were not dried to completion were noticeably lighter in color after 24 h, suggesting that oxidation of anthocyanins occur if kept in solution. Thus, the small differences between 2,4-D treatments and controls were more discernable in the HPLC samples than in the spectroscopy samples.

Improved resolution obtained by following HPLC methods described by Rodríguez-Saona et al. (1998) resulted in detection of four major anthocyanin peaks accounting for approximately 90% of the total peak area (Fig. 1). The four major peak levels exhibited more significant variation among samples than other relatively small anthocyanin peaks observed and were chosen for further spectral characterization (Fig. 2).

UV-visible spectra of anthocyanins is a useful tool in determining anthocyanin structural characteristics (Harborne 1958) where the maximum absorbance in the 500 to 530, 310 to 330 and 400 to 460 nm ranges provide information regarding parent pigment, acylation, and glycosylation, respectively. Maximum visible absorbance of 505 nm, $E_{440}/E_{\lambda_{\text{max}}}$ of 22% and $E_{\text{acyl}}/E_{\lambda_{\text{max}}}$ of 76% and 79% in peaks 1 and 3 (Table 9) were consistent with a hydroxycinnamic acid monoacylated, 3,5-glycosylated pelargonidin (Harborne 1958; Giusti et al. 1999). Acylation with coumaric or ferulic acid were determined by absorbance maxima of 312 and 327 nm, respectively. Spectral characteristics of peaks 2 and 4 showed maximum visible absorbance of 520 nm, $E_{440}/E_{\lambda_{\text{max}}}$ of 15% and $E_{\text{acyl}}/E_{\lambda_{\text{max}}}$ of 86% and 81% that is consistent with a similar derivative of peonidin.

Mass spectral results (data not shown) of the aggregate fraction of the four peaks confirmed m/z values based on relative abundances of peaks compared to HPLC peak levels as well as showing product ions corresponding to

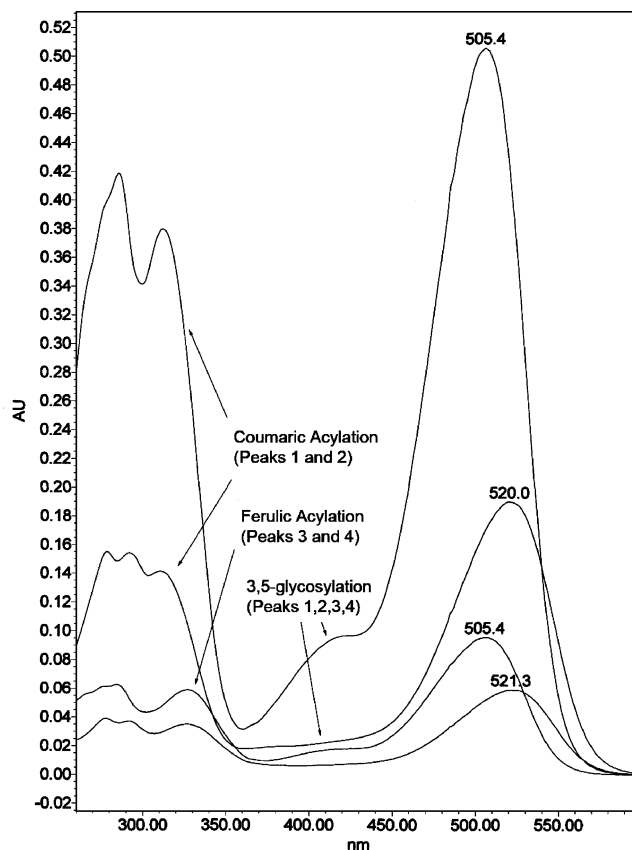


Fig. 2 UV-Visible spectral characteristics of four major HPLC peaks indicating glycosylation and monoacylation patterns

Table 9 Identification of four major potato anthocyanin peaks; Pg = pelargonidin, Pn = peonidin, coum = coumaric, feru = ferulic, rut = rutoside and glc = glucoside

Peak	λ max (acyl, visible)	$E_{440}/E_{\lambda_{max}}$ (%)	$E_{(acyl)}/E_{\lambda_{max}}$ (%)	M/z	Proposed identification
1	312, 505	22	79	887	Pg-3-coum-rut-5-glc
2	312, 520	15	86	917	Pn-3-coum-rut-5-glc
3	327, 505	22	76	918	Pg-3-feru-rut-5-glc
4	327, 521	15	81	888	Pn-3-feru-rut-5-glc

pelargonidin and peonidin. The anthocyanin peak assignments 1 to 4 are as follows: 1 = pelargonidin-3-coumaroyl-rutinoside-5-glucoside, 2 = peonidin-3-coumaroyl-rutinoside-5-glucoside, 3 = pelargonidin-3-feruloyl-rutinoside-5-glucoside, and 4 = peonidin-3-feruloyl-rutinoside-5-glucoside. This is also in order of relative quantity. These results are consistent with previous studies that indicate that the primary anthocyanins in red-skinned, white-fleshed potatoes are pelargonidins (Lewis et al. 1998) with coumaryl and/or rutoside side groups. ‘Désirée’ tuber periderm also contained peonidin-3-(p-coumaroyl) rutoside-5 glucoside. ‘Norland’ potatoes contain both pelargonidin and peonidin anthocyanidins; pelargonidin is 4.5 times more abundant than peonidin in the periderm of mature tubers (Hung et al. 1997). The feruloyl derivatives have also been reported by Rodríguez-Saona et al. (1998).

Because commercial standards of these anthocyanins are not available, the percent total peak area contribution of each anthocyanin was calculated. Table 10 shows changes in anthocyanin profiles among treatments and growth environments. The 2,4-D treatment resulted in a 6% decrease in pelargonidin derivatives (λ_{max} =505 nm) and a 3% increase in peonidin derivatives (λ_{max} =520 nm) compared with the control in the field environment. A similar decrease of 3% in pelargonidin derivatives and 6% increase in peonidin derivatives were observed in the greenhouse environment. Beyond the increase in total anthocyanin levels, these results may play a role in the decreased hue angle observed in 2,4-D treatments where a bathochromic shift may be attributed to the shift from the orange-red color of pelargonidin derivatives to the red-blue color of peonidin

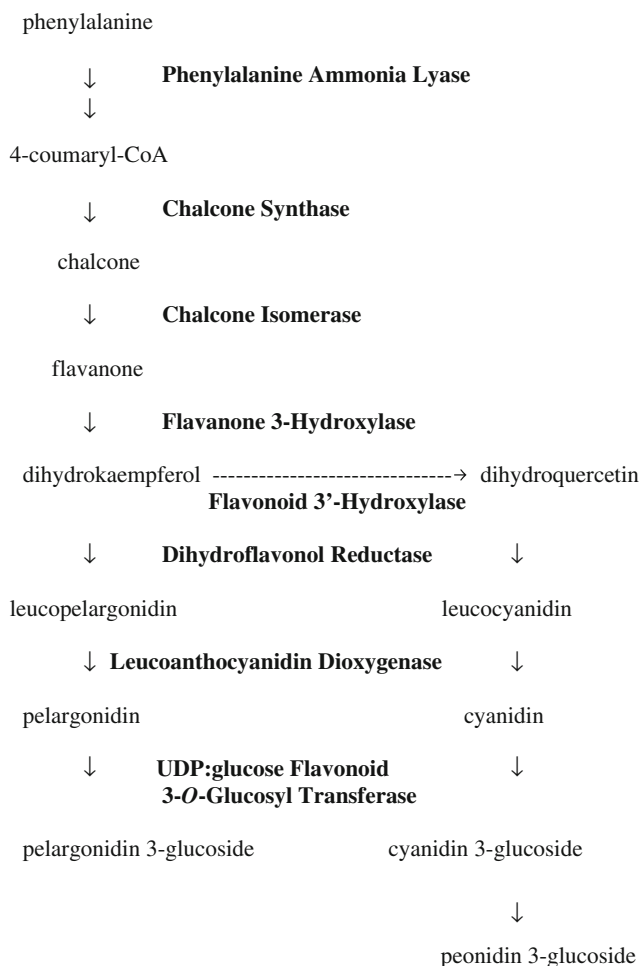
Table 10 Percent total peak area of four major anthocyanins comparing 2,4-D and control treatments in two growth environments

Peak	Field		Greenhouse	
	Control	2,4-D	Control	2,4-D
1	52	49	36	39
2	20	21	16	23
3	13	10	21	15
4	6	8	14	13
Total Pg derivatives	65	59	57	54
Total Pn derivatives	26	29	30	36

Pg = pelargonidin, Pn = peonidin

derivatives. Greenhouse grown potatoes also showed decreased levels of coumaric acylated pelargonidin and peonidin derivatives and an increase in ferulic acylated derivatives as previously reported by Rodríguez-Saona et al. (1998).

In conclusion, 2,4-D sprayed on ‘Red Norland’ potato vines produced tubers that were darker, more purple-red, and contained greater levels of anthocyanins than tubers from control plants or plants sprayed with alternative compounds. Results were similar for tubers grown both in the field and in the greenhouse, although effects with greenhouse-grown plants were not as statistically significant as with field-grown

**Fig. 3** Schematic of the anthocyanin biosynthetic pathway. Enzymes catalyzing a particular step are shown in bold lettering

plants. The observed change in hue with 2,4-D treatment can be explained by a slight increase in production of peonidin derivatives compared to controls. One possible mechanism that could explain this change is if 2,4-D increased the proportion of dihydroquercetin, the precursor of peonidin, compared to dihydrokaempferol, the precursor of pelargonidin, perhaps by increasing the activity of flavonoid 3'-hydroxylase (Fig. 3). A change in the activity of this enzyme has been shown to alter flower color. Ueyama et al. (2002) transformed torenia flowers in which flavonoid-3', 5'-hydroxylase activity was suppressed to constitutively over-express flavonoid 3'-hydroxylase. This would theoretically result in greater dihydroquercetin, cyanidin, and peonidin production, and less dihydrokaempferol, and pelargonidin accumulation. The transformants had redder flowers with an increased amount of cyanidin-type anthocyanidins compared to the original suppressed line. Hoshino et al. (2003) found that mutants of *Ipomoea* flowers, containing the *magenta*, *pink*, and *fuchsia* alleles produce reddish flowers. Wild type flowers are normally bright blue or dark purple and accumulate cyanidin pigments. The mutants flowers contained pelargonidin derivatives, and all of them were deficient in the gene for flavonoid 3'-hydroxylase. Hence, if 2,4-D shifted the activity of flavonoid 3'-hydroxylase so that more dihydroquercetin and peonidin derivatives were produced, potato periderm color could be modified toward a purplish hue.

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