Influence of Clomazone Herbicide on Postharvest Quality of Processing Squash and Pumpkin†

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Certain cultivars of processing pumpkins and squash, such as Golden Delicious and Butternut squash, are more sensitive to application of clomazone (Command herbicide) than others, resulting in paler coloration of fruit tissues—an undesirable condition for food processors. The objective of this research was to evaluate the influence of clomazone on fruit color retention over time in six processing squash and pumpkin cultivars that exhibited various degrees of clomazone sensitivity. Color retention was monitored by total carotenoid content after curing. Relative levels of fatty acids and peroxidase (POD) and lipoxygenase (LOX) enzyme activities, associated with membrane and pigment integrity in higher plants, were assayed. Total carotenoid content of treated tissue varied, with Golden Delicious and Butternut squash significantly affected by herbicide treatment. Treatments of clomazone resulted in increased POD activity in Turk's Turban and Buttercup squash. No difference was observed for LOX activity in treated or control samples.

Keywords: *Herbicide; carotenoids; quality; squash*

INTRODUCTION

Clomazone (Command herbicide) is a soil-applied, preemergence herbicide currently labeled for use with bell peppers, tobacco, pumpkins, and processing squash (Warfield et al., 1985). Clomazone is an inhibitor of photosynthesis and carotenoid biosynthesis in higher plants; application to sensitive species results in the bleaching of photosynthetic tissues, chlorosis, and, eventually, death (Duke et al., 1985). Although clomazone has limited postemergence activity, under certain environmental conditions soil-applied clomazone is translocated to developing tissues and can cause significant bleaching of crop plants and fruit tissues (Scott and Weston, 1992).

Certain cultivars of processing pumpkins and squash are more sensitive to clomazone application than others, resulting in paler coloration of fruit tissues—an undesirable condition for food processors. Clomazone is most often applied commercially in combination with ethalfluralin herbicide to enhance the spectrum of weed control (University of Kentucky Cooperative Extension, 1994). Ethalfluralin (Curbit herbicide) is the industry standard which is used in cucurbit crops for preemergence weed control. Ethalfluralin acts as a mitotic inhibitor of tubulin biosynthesis, resulting in death of susceptible seedlings. It has no known effects on carotenoid production or photosynthesis in higher plant systems (Parka and Soper, 1977; Weed Science Society of America, 1989). Therefore, these experiments were initiated to evaluate the influence of various rates of clomazone, clomazone plus ethalfluralin, or ethalfluralin alone on color retention and other important postharvest quality attributes of six processing squash and pumpkin cultivars exhibiting various degrees of sensitivity to clomazone. Color was monitored by total carotenoid levels, and quality attributes including ascorbic acid content, relative levels of fatty acids, and enzymes associated with membrane and pigment integrity in higher plants (peroxidase and lipoxygenase) were assayed in treated fruit tissue of each cultivar evaluated.

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MATERIALS AND METHODS

Experimental Design. Marrow squash NK530, field pumpkin Howden 691, Buttercup squash NK 773, Golden Delicious squash NK 792, Butternut squash Waltham 825, and Turk's Turban NK 822 were direct seeded on June 23, 1992, into Maury silt loam soil (pH 6.2, OM 3%). Labeled rates for clomazone application are 0.28–0.84 kg of active ingredient (ai)/hectare (ha), while labeled rates for ethalfluralin are 0.84–1.68 kg of ai/ha. Herbicide treatments were applied on June 23 and included (1) ethalfluralin at 0.83 kg of ai/ha preemergence (control), (2) clomazone alone at 0.56 kg of ai/ha applied preplant incorporated (ppi), (3) clomazone + ethalfluralin (0.27 kg of ai/ha ppi + 0.83 kg of ai/ha applied preemergence), (4) clomazone + ethalfluralin (0.56 kg of ai/ha ppi + 0.83 kg of ai/ha applied preemergence), and (5) an untreated control and were replicated three times and arranged within a randomized complete block design.

Clomazone was applied preplant and incorporated at a depth of 2.5 cm as indicated by label specifications, whereas ethalfluralin was applied after direct seeding as a preemergence treatment without incorporation. Clomazone and ethalfluralin were applied alone and in combination to evaluate the ability of ethalfluralin to enhance broad spectrum weed control of clomazone at various rates without loss of important postharvest characteristics of processing squash and pumpkin. An untreated control was also planted in an attempt to determine the effects of either herbicide on postharvest fruit quality. However, weed populations in the untreated plots were so dense by midseason that many cucurbit plants were unable to survive the weed pressure. As a result, no marketable fruit were harvested from the untreated plots. Because of plant spacing and extensive vining, it was impossible to cultivate this treatment for weed control.

Standard cultural practices were followed throughout production, and fruit were harvested on October 19, 1992, 120 days after planting. Fruit were cured outdoors for 4 weeks

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after harvest and, on November 23, fruit were counted, graded, and placed into storage at approximately 15 °C and 65% relative humidity (RH) for 0, 2, and 4 weeks after curing.

At each storage interval, three fruit per treatment obtained from three separate plants (three replications) per sampling time were randomly selected from all fruit harvested per plot and cleaned. Fruit were peeled, and 80 g was ground using a Kitchen-Aid vegetable grinder at speed 2; the fruit was frozen at −80 °C under CO_2 until further analyses, which included moisture, total carotenoid and reduced ascorbic acid contents, and soluble solids, peroxidase (POD), lipoxygenase (LOX), and fatty acid composition. Data were subjected to analyses of variance for a factorial experiment, with treatment and time analyzed as main effects. The interaction of treatment and time factors was also examined for each parameter measured. Bartlett's test for homogeneity of variance was performed for each analysis, and variance was determined to be homogeneous for each parameter measured.

Moisture Content. Ground squash (~5 g) was evaluated for moisture content using a drying oven method (AOAC, 1992). Moisture content and percent solids were determined.

Total Carotenoid Content. Ground tissue (1 g) was extracted in 5 mL of acetone for 30 s using a mortar and pestle (Mayfield et al., 1986). The extract was centrifuged for 10 min at 2000*g* and the concentrated supernatant solution diluted with an equal volume of Milli Q purified water. Absorbance of the supernatant was recorded at 445 nm. Total carotenoid content was expressed as micrograms of total carotenoid per gram of fresh fruit tissue by using the molar absorbance coefficient (E_{mol} = 2500) specifically for the determination of total carotenoids in plant tissue (Davies, 1976). The amount of carotenoid in micrograms per gram of material was calculated by using the expression

$$\mu\text{g of carotenoid/g} = (A \times V \times 10^6/E_{mol}) \times 100G$$

where A is the A_{445}, V is the total volume (mL) containing G grams of the sample, and E_{mol} is the molar absorbance coefficient.

Reduced Ascorbic Acid (RAA). RAA was measured by titrimetric assay (Pelletier, 1985). Ground squash (5 g) was extracted with 25 mL of 3% metaphosphoric acid for 3 min using a Tekmar tissue homogenizer Model SDT 100 (Tekmar, Cincinnati, OH) at 70 rpm and filtered (Whatman No. 1). RAA content was expressed as milligrams of RAA per gram of fresh fruit tissue.

Soluble Solids. Portions (0.5 mL) of the juice present after tissue grinding were measured for percent soluble solids (°Brix) at each storage interval using a Leica temperature compensated hand-held refractometer Model 10430 (Leica, Buffalo, NY). Brix corresponds to the percent sucrose concentration in a solution (°Brix = % wt/wt of sucrose).

POD and LOX Activity Levels. POD (Hemeda and Klein, 1990) and LOX (Hildebrand and Hymowitz, 1981) activities were determined in the treated squash and pumpkin tissue. Samples (1 g) were homogenized using a microhomogenizer for 30 s and then centrifuged at 13000*g* for 15 min at 5 °C. POD activity was determined by spectrophotometric assay at 470 nm using guaiacol and H_2O_2 as the hydrogen donor and substrate, respectively. Absorbance readings were made at 470 nm. LOX activity was measured by spectrophotometry at 234 nm in the presence of linoleic acid (18:2). Enzyme activities were expressed as micromoles per minute per gram of fresh weight.

Fatty Acid Analysis. The fatty acid composition was measured by gas chromatography (Dahmer et al., 1989) after transmethylation. Ground samples (20 mg) were placed in culture tubes containing 2 mL of methanol plus 2% H_2SO_4. Samples were heated (80 °C) for 1 h resulting in 0.5 mL volume, Na_2SO_4 (~2 mg) was added, and samples were vortexed. The methylated fatty acids were extracted with 1 mL of hexane with 0.1% BHT and vortexed. The top layer was transferred into a 2 mL gas chromatograph (GC) vial. Samples (2 μL) were analyzed by gas chromatography for fatty acid composition using a Hewlett-Packard FFAP capillary column (Hewlett-Packard, Little Falls, DE).

RESULTS AND DISCUSSION

There were no significant differences in yielding ability or visual appearance of fruit in processing pumpkins and squashes at harvest maturity due to selected herbicide treatments. However, all herbicide treatments resulted in significant weed suppression throughout the growing season, such that a marketable crop was not obtained due to weed infestation. Handweeding, which was not possible due to the large plot sizes and lack of labor availability, would be required in the absence of herbicide to obtain harvestable produce. This is not a commercially acceptable practice.

Immediately after curing, fruit tissues were evaluated for postharvest quality, total carotenoids, ascorbic acid, POD, LOX, and fatty acid composition. Since untreated herbicide control plots produced no fruit, comparisons in fruit quality or enzyme activities can be made only between clomazone and ethalfluralin treatments with the ethalfluralin alone treatment (the industry standard). Since ethalfluralin has a known specific site of action involving inhibition of mitosis in expanding tissues, and no known effect on other growth parameters, especially carotenoid biosynthesis or photosynthesis, it is logical to compare the postharvest responses of fruit treated with clomazone herbicide to the control treatment of ethalfluralin alone. Following curing, fruit quality appeared to be similar among treatments and cultivars in terms of texture, firmness, and typical color. Moisture content and soluble solids did not change significantly over time or by herbicide within each cultivar (data not shown). Initial total carotenoid levels in ethalfluralin-treated (0.83 kg of ai/ha) samples at week 0 (Table 1) varied by cultivar, and the highest levels were observed in Golden Delicious (2.46 μg/g) and Buttercup squash (2.76 μg/g) followed by Marrow (1.70 μg/g) and Butternut (1.02 μg/g). Initial carotenoid levels observed in Golden Delicious and Buttercup were up to 5 times greater than in Howden and Turk's Turban. Marrow and Butternut cultivars contained up to 2 times greater carotenoid levels than Howden and Turk's Turban.

Herbicide treatment had variable effects on fruit tissue color of certain cultivars of processing squash and pumpkin. Carotenoid content was 3-fold higher in Butternut squash treated with clomazone plus ethalfluralin (0.56 + 0.83 kg of ai/ha). However, clomazone treatment (0.56 kg of ai/ha) alone resulted in lower total carotenoids in those cultivars exhibiting high initial carotenoid levels (Golden Delicious and Buttercup). No significant difference was observed in carotenoid levels among other treated cultivars. Ethalfluralin alone (0.83 kg of ai/ha) generally did not result in significant enhancement of total carotenoid levels in fruit tissues or in darker visual coloration of fruit tissue. Significant changes were observed in carotenoid levels in Howden, Buttercup, and Butternut cultivars over storage time. In Howden pumpkin, which showed the lowest levels of carotenoids throughout the study, all treatments that included clomazone showed increased total carotenoid levels with increased postharvest storage. Sensitivity of squash cultivars to clomazone has been reported by researchers at Michigan State University and FMC Corp. It appears that those cultivars with higher initial carotenoid levels might be most sensitive to clomazone, a known inhibitor of carotenoid biosynthesis in higher

Table 1. Total Carotenoid Levels in Herbicide-Treated Squash Tissue over 4 Weeks of Storage (15 °C, 65% RH)

treatment	sampling time (weeks after curing)	μg/g Howden	Marrow	Golden Delicious	Buttercup Acorn	Butternut	Turk's Turban
1. ethalfluralin (0.83 kg of ai/ha)	0	0.56	1.70	2.46	2.76	1.02	0.46
2. clomazone (0.56 kg of ai/ha)	0	0.30	1.78	1.68	2.66	0.92	0.42
3. clomazone + ethalfluralin (0.27 + 0.83 kg of ai/ha)	0	0.32	1.64	2.66	1.52	3.28	0.52
4. clomazone + ethalfluralin (0.56 + 0.83 kg of ai/ha)	0	0.26	2.78	2.78	2.26	1.10	0.54
1. ethalfluralin (0.83 kg of ai/ha)	2	0.44	2.52	2.48	4.04	2.22	0.58
2. clomazone (0.56 kg of ai/ha)	2	0.52	2.16	1.86	2.88	2.28	0.76
3. clomazone + ethalfluralin (0.27 + 0.83 kg of ai/ha)	2	1.28	1.66	2.82	3.00	2.42	2.30
4. clomazone + ethalfluralin (0.56 + 0.83 kg of ai/ha)	2	0.58	2.62	2.86	3.26	2.62	0.54
1. ethalfluralin (0.83 kg of ai/ha)	4	0.36	2.28	2.60	3.08	1.30	0.40
2. clomazone (0.56 kg of ai/ha)	4	0.56	2.22	2.34	2.42	1.44	0.44
3. clomazone + ethalfluralin (0.27 + 0.83 kg of ai/ha)	4	0.52	2.96	2.36	3.34	2.52	0.86
4. clomazone + ethalfluralin (0.56 + 0.83 kg of ai/ha)	4	0.52	3.06	3.28	2.60	2.18	0.78
least-squares difference		0.224	0.548	0.585	0.645	0.436	0.705
significance[a]							
treatment		NS	NS	*	NS	*	NS
time		*	NS	NS	*	**	NS
treatment × time		NS	NS	**	NS	NS	NS

[a] * Significance at 95% confidence level; **significance at 99% confidence level.

Table 2. Reduced Ascorbic Acid Levels in Herbicide-Treated Squash Tissue over 4 Weeks of Storage (15 °C, 65% RH)

treatment	sampling time (weeks after curing)	mg/100 g Howden	Marrow	Golden Delicious	Buttercup Acorn	Butternut	Turk's Turban
1. ethalfluralin (0.83 kg of ai/ha)	0	9.3	10.3	4.6	4.4	4.4	3.8
2. clomazone (0.56 kg of ai/ha)	0	16.7	6.8	7.2	2.7	8.6	3.9
3. clomazone + ethalfluralin (0.27 + 0.83 kg of ai/ha)	0	11.1	6.1	5.0	5.6	4.7	5.6
4. clomazone + ethalfluralin (0.56 + 0.83 kg of ai/ha)	0	7.9	5.7	4.0	4.4	3.6	3.5
1. ethalfluralin (0.83 kg of ai/ha)	2	14.8	2.8	7.2	2.8	2.6	4.8
2. clomazone (0.56 kg of ai/ha)	2	13.7	6.8	8.8	4.2	4.1	4.0
3. clomazone + ethalfluralin (0.27 + 0.83 kg of ai/ha)	2	9.7	6.5	10.3	3.0	4.3	3.6
4. clomazone + ethalfluralin (0.56 + 0.83 kg of ai/ha)	2	17.8	11.1	7.0	2.7	4.0	3.7
1. ethalfluralin (0.83 kg of ai/ha)	4	19.2	6.7	6.4	4.2	5.6	5.4
2. clomazone (0.56 kg of ai/ha)	4	19.2	6.2	7.2	5.0	4.7	3.9
3. clomazone + ethalfluralin (0.27 + 0.83 kg of ai/ha)	4	12.6	6.0	5.5	4.6	3.5	3.5
4. clomazone + ethalfluralin (0.56 + 0.83 kg of ai/ha)	4	9.3	5.5	4.7	3.7	3.8	3.0
least-squares difference		1.0	0.4	0.4	0.2	0.4	0.1
significance[a]							
treatment		NS	NS	NS	NS	NS	*
time		NS	NS	*	NS	NS	NS
treatment × time		NS	*	NS	NS	NS	*

[a] * Significance at 95% confidence level; ** significance at 99% confidence level.

plants. Increases in total carotenoid levels over time were also observed in Buttercup and Butternut cultivars, except when treated with clomazone plus ethalfluralin (0.56 + 0.83 kg of ai/ha). There was a significant treatment by time interaction observed in carotenoid levels in Golden Delicious squash. Treatment with clomazone alone (0.56 kg of ai/ha) resulted in lowest total carotenoid levels at all time intervals, whereas highest carotenoid levels were observed in clomazone plus ethalfluralin (0.56 + 0.83 kg of ai/ha) treatment after 4 weeks of storage. This consequently resulted in an increase in Golden Delicious fruit flesh coloration during postharvest storage.

Initial reduced ascorbic acid contents also varied by cultivar and were generally higher in Howden pumpkin (9.3 mg/100 g) and Marrow (10.3 mg/100 g) compared to other cultivars (Table 2). Preemergence herbicide treatment of clomazone plus ethalfluralin (0.27 + 0.83 kg of ai/ha) resulted in higher ascorbic acid levels in Turk's Turban. No other differences in initial ascorbic acid levels were observed among other treated cultivars. Increased ascorbic acid levels were observed in all treated Golden Delicious samples at 2 weeks after field harvest. However, ascorbic acid levels returned to initially reported levels by 4 weeks after harvest in Golden Delicious. No other differences in ascorbic acid levels were observed over time among other cultivars. Ascorbic acid levels in Marrow and Turk's Turban were significantly influenced by treatment and time. Clomazone (0.56 kg of ai/ha) and ethalfluralin (0.83 kg of ai/ha) resulted independently in lower ascorbic acid levels in these cultivars. Ascorbic acid levels in treatments containing clomazone were lower at 0 time and by 4 weeks of storage. Although the ascorbic acid content was greatest in the clomazone plus ethalfluralin (0.27 + 0.83 kg of ai/ha)-treated Turk's Turban at 0 time, levels were lower in all Turk's Turban samples treated with clomazone at 2 and 4 weeks of storage intervals.

LOX and POD are enzyme systems recognized as important in oxidative catalysis in plant tissue, resulting in destruction of carotenoids and subsequent color changes in stored foods. Lipoxygenase is recognized as one of the main oxidative catalysts in vegetables, and

Table 3. Peroxidase Activity in Herbicide-Treated Squash Tissue over 4 Weeks of Storage (15 °C, 65% RH)

treatment	sampling time (weeks after curing)	μmol min^{-1}/(g of fresh wt)$^{-1}$ Howden	Marrow	Golden Delicious	Buttercup Acorn	Butternut	Turk's Turban
1. ethalfluralin (0.83 kg of ai/ha)	0	21.0	4.8	27.0	55.8	108.6	251.4
2. clomazone (0.56 kg of ai/ha)	0	11.4	5.4	15.6	643.8	307.8	168.6
3. clomazone + ethalfluralin (0.27 + 0.83 kg of ai/ha)	0	10.8	4.2	12.6	133.2	270.0	74.4
4. clomazone + ethalfluralin (0.56 + 0.83 kg of ai/ha)	0	16.8	3.6	4.2	34.2	147.0	256.8
1. ethalfluralin (0.83 kg of ai/ha)	2	9.6	21.6	82.8	118.2	120.0	96.0
2. clomazone (0.56 kg of ai/ha)	2	34.8	16.8	66.6	152.4	172.2	102.0
3. clomazone + ethalfluralin (0.27 + 0.83 kg of ai/ha)	2	8.4	12.0	43.2	20.4	192.6	144.0
4. clomazone + ethalfluralin (0.56 + 0.83 kg of ai/ha)	2	122.4	30.6	55.8	98.4	184.2	110.4
1. ethalfluralin (0.83 kg of ai/ha)	4	13.8	74.4	59.4	937.2	297.6	60.6
2. clomazone (0.56 kg of ai/ha)	4	87.6	89.4	104.4	850.2	39.0	521.4
3. clomazone + ethalfluralin (0.27 + 0.83 kg of ai/ha)	4	25.2	81.0	66.0	250.8	69.0	67.2
4. clomazone + ethalfluralin (0.56 + 0.83 kg of ai/ha)	4	63.6	75.0	68.4	84.0	46.2	83.4
least-squares difference		110.4	28.8	46.2	491.4	204.0	198.0
significance[a]							
treatment		NS	NS	NS	*	NS	**
time		NS	**	**	**	NS	NS
treatment × time		NS	NS	NS	NS	NS	**

[a] * Significance at 95% confidence level; **significance at 99% confidence level.

Table 4. Relative Fatty Acid Levels in Herbicide-Treated Squash Tissue at 0 Weeks of Storage (15 °C, 65% RH)

fruit	palmitic acid 16:0	stearic acid 18:0	oleic acid 18:1	linoleic acid 18:2	linolenic acid 18:3	total polyunsaturated fatty acid
Howden	22.4	1.6	32.1	25.0	19.1	44.8
Marrow	38.4	1.0	7.8	33.5	18.8	52.3
Golden Delicious	22.7	1.7	31.1	23.9	20.7	44.6
Buttercup Acorn	20.1	2.2	41.3	22.9	14.9	37.2
Butternut	31.4	2.4	13.7	19.7	33.8	52.5
Turk's Turban	30.5	1.5	7.2	25.7	37.6	64.5

lipoxygenase-catalyzed lipid peroxidation is often coupled with carotenoid destruction. Peroxidase will also destroy carotenoids coupled to lipid peroxidation and might play an important role in oxidation reactions in color changes of stored foods (Hidako et al., 1986). In this study no significant differences were observed in LOX activity among treatments over storage time. LOX activity was low in all tissue samples (data not shown). It is possible that the higher levels of carotenoids found in squash tissue might have inhibited LOX activity.

POD activity was highly dependent upon squash or pumpkin cultivar (Table 3). Among cultivars, higher POD activity was observed in Buttercup (55.8 μmol min^{-1} g^{-1}), Butternut (108.6 μmol min^{-1} g^{-1}) and Turk's Turban (251.4 μmol min^{-1} g^{-1}) samples at 0 weeks of storage. POD activity was lowest in Marrow samples (4.8 μmol min^{-1} g^{-1}) and was 10−100 times lower than POD activity in Buttercup and 10−50 times lower than Butternut and Turk's Turban, depending upon treatment. POD activity in Howden and Golden Delicious was similar and was 2−50 times lower than Buttercup and 4−20 times lower than Butternut and Turk's Turban.

Treatments of clomazone resulted in a dramatic increase in POD activity in Buttercup squash (55.8 vs 643.8 μmol min^{-1} g^{-1}) at 0 weeks of storage. Increasing clomazone concentration from 0.27 to 0.56 kg of ai/ha in the presence of ethalfluralin (0.83 kg of ai/ha) resulted in 4 times greater POD activity in Buttercup squash. A dramatic increase in POD activity was also observed when Buttercup squash was treated with clomazone alone. POD activity observed in clomazone-treated Buttercup tissue was 5 times greater than clomazone plus ethalfluralin (0.27 + 0.83 kg of ai/ha), 10 times greater than the ethalfluralin alone-treated samples, and 20 times greater than the clomazone plus ethalfluralin (0.56 + 0.83 kg of ai/ha)-treated Buttercup samples. These results suggest ethalfluralin might reduce the effect of clomazone on POD activity in Buttercup squash. Similar results were also observed in the treated Turk's Turban samples. POD activity was 168.6 μmol min^{-1} g^{-1} in the clomazone (0.56 kg of ai/ha)-treated Turk's Turban samples at 0 time. The addition of ethalfluralin resulted in >50% decrease in POD activity in the clomazone plus ethalfluralin (0.27 + 0.83 kg of ai/ha)-treated Turk's Turban samples as compared to the clomazone alone treatment.

POD activity of fruit tissue generally increased with increased postharvest storage. After 4 weeks of storage, POD activity increased in Marrow and Golden Delicious over all herbicide treatments. In comparison to 0 weeks of storage, POD activity in Marrow was 15 times greater among all treatments after 4 weeks of storage. POD activity was also 2−10 times increased in Golden Delicious squash after 4 weeks of postharvest storage. Generally, POD activity increased in Buttercup with increased postharvest storage. Treatment and storage time also influenced POD activity in Turk's Turban samples. In the samples treated with lower clomazone (0.27 kg of ai/ha) or without clomazone, decreased POD activity was observed over time. Relative to initial POD activity in Turk's Turban, clomazone application rate (0.56 kg of ai/ha) increased POD activity over time. The association between enzyme activity and color loss in squash samples is unclear at this time. However, both clomazone treatment and postharvest storage time significantly increased POD enzyme activities in several squash cultivars.

The composition of fatty acids in squash samples varied according to species (Table 4). C_{18} was the predominant fatty acid among all cultivars. Relative polyunsaturated fatty acid levels were greatest in Turk's

Turban (64.5%) and lowest in Buttercup squash (37.2%). Linoleic acid (18:2) was the major polyunsaturated fatty acid in Howden, Marrow, Golden Delicious, and Buttercup samples. Of total fatty acids, Marrow contained approximately 33% 18:2. However, in Butternut and Turk's Turban cultivars linolenic acid (18:3) was the major polyunsaturated fatty acid (~35%). Oleic acid (18:1) was the highest unsaturated fatty acid in Howden (32.1%), Golden Delicious (31.1%), and Buttercup (41.3%) samples. The major fatty acid in Marrow squash (38.4%) was palmitic acid (16:0). Herbicide treatment had no apparent effect on C_{18} polyunsaturated fatty acids in the squash samples.

SUMMARY

Total carotenoids and reduced ascorbic acid levels, as well as fatty acid composition and enzyme activities, varied among the five squash and pumpkin cultivars evaluated. Herbicide treatments had varying effects on these parameters. As observed previously, clomazone treatment (0.56 kg of ai/ha) resulted in reduced carotenoid levels, especially in Golden Delicious and Butternut samples ($p < 0.05$). POD activity was increased, especially in Buttercup and Turk's Turban samples treated with clomazone. Ethalfluralin treatment alone did not generally result in significant enhancement of carotenoid levels or darker orange appearance of fruit or internal tissue comparison to other herbicide treatments. During storage, effects of herbicide treatment varied among all cultivars. There was a significant treatment by time interaction observed in carotenoid levels in Golden Delicious squash, with lowest levels observed in clomazone alone (0.56 kg of ai/ha) treatments immediately after harvest and highest levels observed in treatments of clomazone plus ethalfluralin after 4 weeks of storage. Limited effects of herbicides or storage times upon reduced ascorbic acid were observed. After 4 weeks of storage, increased POD activity was observed among all treatments in Marrow, Golden Delicious, and Buttercup samples. No differences were noted in LOX activity or relative fatty acid levels due to herbicide treatment. The effect of clomazone or ethalfluralin treatment upon POD in fruiting tissue is unclear. Sensitivity to clomazone appears to be cultivar-specific, as previously reported.

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