Glyphosate Exposure Contributes to Internal Browning of Apples during Long-Term Storage

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> **"Empire apple trees exposed to glyphosate during late July or early August produced fruit that developed more internal browning during eight months of controlled atmosphere storage than did fruit from control trees in the same orchards. Results need to be verified by repeating the trials, but apple growers should take precautions to avoid exposure of apple trees to glyphosate, especially in Empire orchards where fruit is designated for long-term storage."**

Glyphosate (Round-Up and generics) is an important tool for managing ground cover beneath apple trees. According to the last available data from the USDA's National Agricultural Statistics Service, 54% of New York apple acreage was treated with glyphosate at least once in 2007, and 10 percent of that acreage received two applications (Anonymous, 2008). However, there are increasing concerns that glyphosate may sometimes damage fruit trees in subtle ways that may be largely unrecognized.

Glyphosate kills plants by blocking a critical enzyme pathway known as the shikimic acid pathway. The blocked enzyme is essential for respiration in plants, so plants that receive a full dose of glyphosate cannot survive unless they are bioengineered to be glyphosate-resistant or have evolved to be resistant as is the case with some weed species in the Midwest. At lower concentrations, however, glyphosate can adversely affect plants without producing any immediately visible effects.

In other crops, glyphosate exposure has been shown to reduce root growth and seed production and affect seed quality and plant nutrient balances. In soils, the affinity of glyphosate for cations can reduce availability of calcium, magnesium, manganese, copper, iron, nickel, and zinc either by direct chemical interactions or by negative effects on soil microbes involved in making these minerals available to plants. Glyphosate taken up by roots can also interfere with movement and availability of some of these minerals inside plant tissue (Cakmak et al., 2009). Glyphosate tends to accumulate in meristematic tissue and storage organs. No one knows how much of the glyphosate taken up by apple trees is transported to apple fruit, but it seems possible that glyphosate may influence apple fruit physiology either by partially blocking the shikimic acid pathway or by affecting nutrient balances within fruit.

Internal browning is a disorder that can appear during controlled atmosphere (CA) storage. Even mild cases of internal browning are unacceptable for fruit used in fresh-cut slices. Incidence and severity of internal browning vary from orchard to orchard, but the underlying factors that contribute to differences among blocks have not yet been determined. In a recent article in *New York Fruit Quarterly*, James *et al.* (2010) reported that the risk of browning in Empire apples could be minimized by harvesting fruit during the early part of the harvest window. Other factors that have been evaluated for their effects on browning have not produced consistent results.

In late summer of 2009, we initiated a trial to determine if exposure of Empire apple trees to glyphosate might influence the incidence and severity of internal browning that develops in apples that are held in long-term CA storage. A brief summary of this work was previously published in *Scaffolds Fruit Journal* (Rosenberger *et al.*, 2010).

Experimental Design

The trial was replicated on three farms in western New York in 'Empire' blocks known to produce fruit that developed internal browning during CA storage. On each farm, three treatments were replicated four times using single trees as the experimental unit. Thus, the experiment encompassed 12 trees on each farm. Details for each farm are outlined in Table 1. Treatments included an untreated control, glyphosate applied to one or several terminal shoots on lower fruiting limbs, and glyphosate applied to fruit and leaves on one or several lower fruiting limbs on each tree. Because we wanted to collect 25 fruit for each sample, limbs that were treated were selected to ensure that we could collect at least 25 fruit from the treated limbs at harvest. Treatments never involved more than 30% of the leaf area on lower scaffold limbs, and no glyphosate was applied to leaves or fruit above the lowest tier of scaffold limbs.

The glyphosate formulation used in this trial was Monsanto's Roundup PowerMax®, which contains 5.5 pounds per gallon of the active ingredient (glyphosate) in the form of its potassium salt. This product is labeled for use on tree fruits at rates of 11 fl oz to 3.3 qt/acre per application with a maximum yearly rate of 5.3 quarts/acre and a 1-day preharvest interval. A rate of 44 fl

Table 1. Descriptions of Empire apple orchards used for glyphosate-browning trials in 2009.

Farm	Root-stock	Year planted	Limbs treated with glyphosate	Harvest date
A*	M.26	1991	12 Aug 2009	6 Oct 2009
B**	M.9	1990	28 July 2009	7 Oct 2009
C***	M.9	1993	22 July 2009	8 Oct 2009

* Grower sprayed glyphosate (Makaze) on the block in May 2009.
** No glyphosate sprayed by grower for the past two years (2008 and 2009).
*** Glyphosate applied twice per year, once in spring and once in summer, at 2 qt/treated acre.

Table 2. Treatments from which fruit samples were collected on each of the three farms

Treatment 1: Control tree with no glyphosate exposure other than via grower applications to the block
1-a. Fruit from lower limbs
1-b. Fruit from upper limbs
Treatment 2: Glyphosate applied to lower terminal shoot(s)
2-a. Fruit from lower limbs on which the terminal shoots received glyphosate
2-b. Fruit from upper limbs not directly exposed to glyphosate
Treatment 3: Glyphosate applied to fruit and leaves on lower limb(s)
3-a. Fruit from lower limbs that where both leaves and fruit had been directly sprayed with glyphosate
3-b. Fruit from upper limbs not directly exposed to glyphosate

Table 3. Results from statistical analyses of four different parameters used to evaluate effects of glyphosate treatments and farm of origin on incidence and severity of firm flesh browning and core browning that developed after eight months of controlled atmosphere storage on Empire apples harvested from trees exposed to glyphosate during July-August.

		P-values from statistical analyses*			
	degrees	flesh browning		core browning	
Main effects	of freedom	incidence	severity	incidence	severity
Rep (tree)	3	0.991	0.920	0.663	0.204
Treatment (A)	2	0.004**	0.002**	0.006**	0.021**
Farm (B)	2	<0.001**	<0.001**	0.078	0.029**
Interaction: (A*B)	4	0.066	0.044**	0.024**	0.008**

*A separate two-way analysis was performed for each of the four browning parameters shown in the table. Each analysis included three treatments, three different farms, and four replicates with two sub-samples of 25 fruit from each replicate.

** Double asterisks indicate effects where significant differences existed among the grand means ($P \leq 0.05$). Glyphosate exposure had a significant effect on all four measures of internal browning. Differences in the incidence of flesh and core browning for the three glyphosate treatments are shown in Figure 2.

Figure 1. Core browning as assessed on June 16, 2010. Top: Apples sliced and spread out for browning evaluations. Bottom: Severe core browning (left) and slight core browning (right).

oz/A is recommended for controlling weeds greater that 12 inches high or vines greater than 6 inches long. At 44 fl oz/A, a grower would be mixing approximately 1.5 fl oz of Roundup Powermax per gallon of water in an herbicide sprayer calibrated to deliver 30 gallons of spray per sprayed acre.

To simulate what might occur if glyphosate sprays drift into the lower limbs of treated trees, Roundup Powermax was mixed with water at the rate of only 0.034 fl oz/gal (i.e., 1 ml/gal) for this experiment. This solution was applied to treated limbs using a hand-pumped "Solo" type of sprayer, and the solution was misted over the marked limbs. Applications were done on dry days with no rainfall predicted for the next 24 hours.

In early October when fruit were mature, 25 fruit were collected from the treated lower limb(s) and another 25 were collected from the upper canopy on each of the 12 test trees in each orchard. Thus, samples collected from each farm represented the treatments shown in Table 2.

At Farm C, we were unable to get harvest samples from all of the trees because some trees had been harvested before samples could be collected. For this farm, we recovered fruit samples for only two of four replicates for treatments 1a, 1b, and 2a, and for only three of the four replicates for treatments 2b, 3a, and 3b.

Fruit were harvested in the morning, and then transported to Ithaca for storage later that same day. In Ithaca, fruit were cooled overnight and were then placed into a CA storage that was held at 36° F with 2% oxygen and 2% carbon dioxide for 8 months. Fruit were not treated with 1-MCP (Smartfresth). On 9 June 2010, fruit were removed from storage and held at 68° F for 7 days before they were evaluated for internal browning.

Core browning was assessed by cutting through the equator of the apple to hit the center of the core. Browning around the core was rated as slight (meaning just barely noticeable), moderate, or severe (Fig. 1). Flesh browning was assessed by cross-sectioning the apple with the first cut targeted to the plane where the stem is attached to the apple, the second through the core as for assessing core browning, and then a third slightly above the calyx. On many fruit, additional cuts were made between these three main cross-sections if no browning was noted in the first few cuts. If there was evidence of flesh browning by the stem but not as far down as the core, it was rated as slight and given a rating of 1. If the browning was also evident at the core but not near the calyx, it was rated as moderate (rating = 2). When the flesh browning extended from the stem area, through the core area, and all the way down to the calyx, it was rated severe (rating = 3). The firm flesh browning in Empire always starts up in the stem area. Mean severity for each treatment was calculated by taking the average severity rating for all fruit in the treatment, including fruit that were rated as having no internal browning.

Data was analyzed using the SuperAnova Statistical package for Macintosh computers. We used a two-way or factorial analysis for effects of the 3 treatments across 3 different farms. For each variable, we analyzed the trees as a whole using fruit from the upper and lower limbs as subsamples, and we then conducted separate analyses to evaluate effects of treatments on fruit from the lower limbs and from fruit on the upper limbs. Where samples were missing for the Farm C, we entered the data as missing and allowed the software package to adjust for the missing data.

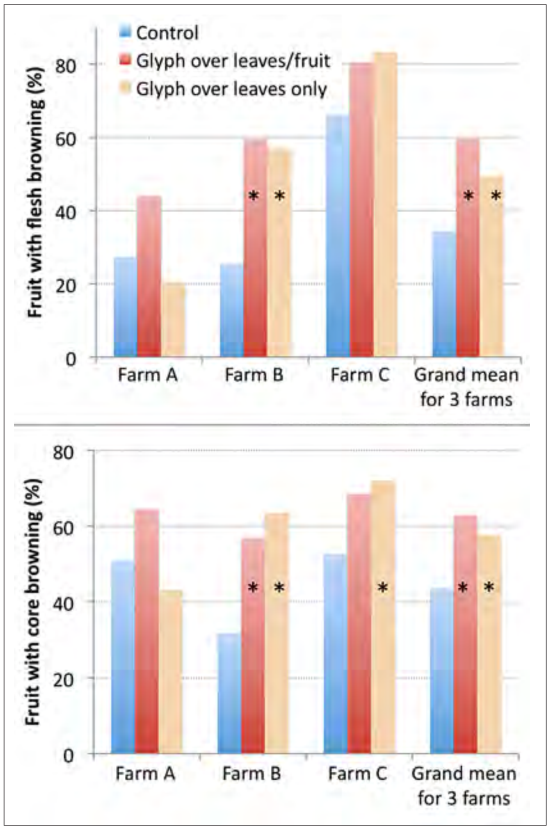

Figure 2. Effects of glyphosate on the incidence of firm flesh browning (upper graph) and on core browning (lower graph) on three different farms. Asterisks indicate comparisons where glyphosate limb treatments resulted in statistically significant increases in browning compared to control fruit.

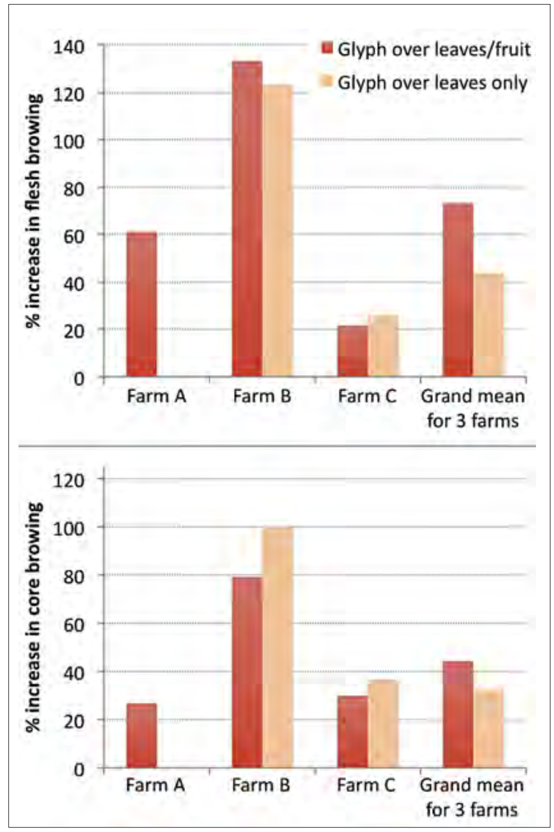

Figure 3. Percentage increases (as compared to non-treated controls) in the incidence of firm flesh browning (top) and core browning (bottom) caused by glyphosate treatments applied to Empire trees on three different farms.

Results

Glyphosate treatments applied to trees increased the incidence and severity of both flesh browning and core browning (Table 3) when grand means for incidence and severity were compared across all three farms including apple samples from both upper and lower limbs on each tree. When treatments were compared within farms, the fruit from Farm B showed the same pattern and statistical effects as noted for the grand means. However, treatments produced more variable results on the other two farms, and effects of treatments usually were not statistically significant when means within each of those farms were compared (Fig. 2). The percentage increase in browning incidence for glyphosate-treated compared to control fruit varied from 0 to more than 130% (Fig. 2).

When we analyzed only data for fruit harvested from the tops of trees and looked at the grand means across all three farms, glyphosate treatments caused significant increases in both the incidence and severity of flesh browning and in the incidence but not the severity of core browning (data not shown). The trends for treatment effects within farms, based on data from only upper branches, were similar to those noted in Figure 2 and Table 3, but there were fewer significant effects due to the reduced sample size. Surprisingly, comparisons of grand means for fruit harvested from the bottom limbs of trees showed no significant differences due to glyphosate treatment, and that was true both for fruit sprayed directly and for fruit from lower limbs where only terminal leaves were sprayed. However, the data trends were similar to those noted for fruit from the tops of the trees, and we might have detected treatment effects if we had used a larger sample size.

Discussion

Results from this trial provide initial evidence (from only one year of data) that glyphosate uptake by Empire trees can affect the incidence and severity of internal browning during long-term CA storage. For the samples from Farm C, fruit from glyphosate-exposed trees always had more browning than fruit from non-exposed trees even though the numerical differences often were not statistically significant. The same was true at Farm A for the treatment where glyphosate was sprayed over fruit, but not where it was applied only to terminal shoots. In the latter case, the leaf surface area exposed to glyphosate may have been too low to allow uptake of enough glyphosate to affect the whole-tree physiology. Presumably more leaf area was exposed to glyphosate on trees where fruit and leaves were treated than on trees where only terminal shoots were treated.

Treatment effects were more evident for fruit in the tops of exposed trees than for fruit taken directly from the lower limbs subjected to glyphosate exposure. This result may reflect the way glyphosate is transported within trees after uptake, or it may relate to other differences in fruit physiology between fruit growing on lower limbs and fruit growing on upper limbs.

For all three farms, there was a significant "background" level of internal browning in the control trees. It is not clear whether this "background" of internal browning represents fruit that develop internal browning for reasons unrelated to glyphosate exposure, or whether the browning in control trees is attributable to glyphosate exposure that occurred when the cooperating growers applied glyphosate beneath trees in each of these orchards. The latter possibility is supported by the fact that, on Farm B where we found the largest and most consistent glyphosate-stimulated increases in browning (Fig. 3), the grower had not applied glyphosate in the test orchard in either 2008 or 2009. The other two growers routinely applied glyphosate in early summer, and trees on Farm C, which had the highest level of browning in the controls (Fig. 1), usually received a second application in early August. Other factors that presumably affect how much glyphosate is absorbed into apple trees from herbicide applications include the type of equipment, the number of root suckers that are exposed to glyphosate, the weather conditions at the time of application, and whether or not the growers use drift-inhibitors to minimize the number of small droplets produced by their herbicide sprayers. Thus, there is no way to account for all of the variables that may have affected glyphosate exposure from grower-applied sprays in these orchards.

The glyphosate exposure that we introduced in this trial might be considered extreme because we directly sprayed branches in a way that commercial growers would never do intentionally. However, we used a very low rate of glyphosate and the limbs that were sprayed showed no evidence of glyphosate injury in spring or summer of 2010. Thus, it is conceivable that trees in orchards where glyphosate is used as an herbicide could have glyphosate exposures equal to what we introduced in this trial without showing any foliar evidence of glyphosate damage.

The results reported here were derived from only one year of data, one application timing, and one cultivar, so it would be premature to conclude that glyphosate exposure is the major contributing factor for internal browning problems in apples. Nevertheless, the data from our 2009-10 trial suggests that glyphosate may exacerbate this storage problem. Growers may wish to minimize glyphosate exposure at least for Empire apples that will be placed into long-term CA storage. Rosenberger *et al.* (2010) have listed various approaches for minimizing exposure of trees to glyphosate where this herbicide is being used.

Results from this trial raise numerous questions that can be resolved only via additional research. Factors such as timing of glyphosate exposure (spring versus summer), exposure route (via spray drift, through root suckers, or through bark), and glyphosate formulation may all impact the glyphosate-browning interaction. We don't know if storage life of cultivars other than Empire can be affected by glyphosate exposure. Many additional experiments will be required to discern all of the potential interactions that may be occurring when apple trees are exposed to glyphosate.

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Acknowledgments

Funding for the work reported here came from USDA Smith-Lever and from USDA funding for the Multi-State Project NE-1036 titled "Postharvest Biology of Fruit". The authors thank Frederick Meyer for assistance with statistical analyses. Special thanks to Bud Beckens of GMB Farms LLC in Sodus, Eric Brown of Orchard Dale Fruit Farm in Waterport, and J.D. Fowler of Fowler Fruit Farms in Wolcott for permission to use trees on their farms for the work that is reported here.

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