

## ***Diagnosing Long- and Short- Term Effects of Herbicides on Trees***

*For: MN Shade Tree Conference, March 18, 2015*

Hannah Mathers, PhD, Mathers Environmental Science Services

Preemergence herbicides should be recommended as the “backbone” of a landscape weed control program. Achieving satisfactory weed control generally requires repeated applications of one or more herbicides (Gilliam, 1989). Two applications, one in the spring (March) and one in the fall (August) of preemergence herbicides are recommended. Using less than two, in most situations, will result in more frequent use of postemergence herbicides and thus increased potential injury to ornamental plants. The use of postemergence herbicides versus preemergence herbicides is always a high-risk behavior!

Preemergence herbicides commonly used around deciduous trees and in established woody plant beds 15-20 years ago included Princep (simazine), Goal (oxyfluorfen) and Gallery (isoxaben). Although, all are great herbicides, Goal and Princep, were known to cause injury to certain woody plant materials. Goal is very safe around coniferous materials but should be used with caution around deciduous plants. Gallery has become one of the most widely used preemergence herbicide in landscape beds, as it is one of two active ingredients (ai) in the granular formulation, Snapshot (isoxaben + trifluralin) (Table 1).

SureGuard 51 WDG or BroadStar G both contain flumioxazin as their active ingredient. These preemergence herbicides were released 2003/ 2004 in nursery and later in the landscape industry. Both provide excellent control in woody beds. Many new preemergence herbicides were researched and released between 2008 and 2014 for the ornamental market. These new products are capable of dealing with heavy weed pressures and are generally less phytotoxic to woody plants than their older counterparts. These new products include [FreeHand 1.75 G (dimethamid-p + pendimethalin) by BASF; Biathlon 2.75 G (oxyfluorfen + prodiamine) by OHP; Marengo G (indaziflam) or Marengo SC by OHP for nurseries or Specticle (indaziflam) by Bayer for landscapes; Tower (dimethamid-p) by BASF which when combined with Pendulum Aqua Cap (BASF) provides excellent control of most broad leaf and grassy weeds (Table 1). Dow AgroScience has also come out with a newer formulation of Gallery DF vs the old SC. The Gallery 75DF is easier to pour and mix and has improved safety around landscape plants compared to the Gallery SC. Marengo as a new herbicide is of particular interest as it is the first active ingredient for ornamentals from the Group 29 mode of action (MoA). Group 29 herbicides inhibit cellulose biosynthesis. Mathers and Case (2013) found indaziflam caused no injury on a wide variety of plants, applied at normal rates including: *Buxus* ‘Green velvet’, *Rosa* ‘Knockout’, *Berberis thunbergii* ‘Crimson pygmy’, *Itea* ‘Little Henry’, *Viburnum plicatum* ‘St. Keverne’, *Viburnum* X ‘Juddi’, *Rosa* ‘Home Run Red’ or *Euonymus* ‘Compacta.’

Use rates have also declined in these new herbicides, further increasing safety around ornamental plants and in the environment (Table 1). The lowest use rate in the ornamental market today is with (indaziflam) (Table 1). OHP, Inc., Mainland, PA launched the granular Marengo in September, 2013 for nursery containers. The Marengo SC formulation had been released in 2012. Both are also registered for landscape use. The attributes of indaziflam are long-lasting, soil degradation not occurring until 150 days after treatment (DAT), non-volatile, non-mobile in soil once watered in and less required applications. Marengo also requires only 0.25” of irrigation or rainfall to activate, half that required for other products and it is effectively incorporated up to 21 DAT. In addition, the Verge™ granule used for Marengo G and Biathlon 2.75G, further reduces injury to desired plants while maintaining exceptional weed control (efficacy) and residual control.

**Table 1.** Standard use rates of common granular preemergence herbicides used in landscape beds and nurseries comparing older products to newer products released on the market in the past 5 years.

Trade name	Landscape rate lb./ac	Active ingredient (ai) names	lb. a.i./ ac
<b>Released prior to 2009</b>			
Snapshot 2.5 TG	150	Trifluralin + Isoxaben	3.75
Rout	100	Oxyfluorfen + Oryzalin	3.0
OH-2	100	Oxyfluorfen + Pendimethalin	3.0
Regal Star	100	Oxadiazon + Prodiamine	3.0
Ronstar G	200	Oxadiazon	4.0
BroadStar 0.25G	150	Flumioxazin	0.375
<b>Released since 2010</b>			
FreeHand 1.75 G	150	Dimethamid-p + Pendimethalin	2.63
Biathlon 2.75 G	100	Oxyfluorfen + Prodiamine	2.75
Marengo G	200	Indaziflam	0.0448

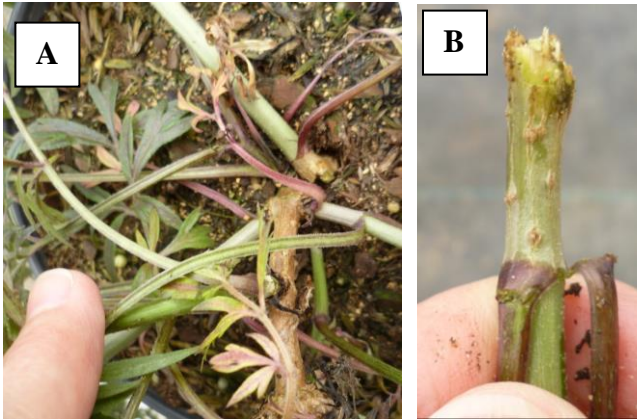
## How preemergence herbicides work?

### Group 3

Although preemergence herbicides are preferred due to their lower phytotoxicity, all herbicides are plant-killers and as such can cause plant problems. Some of the most commonly used preemergence herbicides in ornamentals are inhibitors of microtubule assembly [Weed Science Society of America (WSSA) Group 3 herbicides]. The mode of action (MoA) of Group 3 herbicides is mitosis inhibitors (Mi). These Mi herbicides include the dinitroaniline herbicide family which are root inhibitors. Barricade 65WG (Prodiamine); Pendulum 2G, Pendulum 3.3 EC, Pre-M 60DG, Pre-M 3.3 EC, or Corral (Pendimethalin); Surflan T/O (Oryzalin); Treflan 5G or Trifluralin EC (Trifluralin) are all dinitroaniline herbicides. Dimension (Dithiopyr) or Dacthal (DCPA) are also Mi herbicides in the pyridine and benzoic acid herbicide families, respectively. Mi herbicides also include shoot inhibitors such as the chloroacetanilide herbicide family [ex. Pennant (metolachlor) and Tower (dimethamid-p)] and the acetamide family [Devrinol (napropamide)]. For Mi herbicides to be effective, the germinating seed must contact the top half inch of the media surface where the herbicide has been incorporated. If the seed is not in contact with the incorporated layer germination will not be inhibited. Any seed that has already germinated will be unaffected by the Mi herbicides.

During mitosis identical copies of the genetic material are split and pulled apart so that two daughter cells arise from one mother cell. Spindle fibers, formed in the dividing cell, are the structures that pull the sister chromatids apart. Spindle fibers are composed of microtubules made from tubulin. Dinitroaniline herbicides impact the formation of tubulin. The result is root cells with multiple sets of chromosomes (polyploidy) and faulty cell walls incapable of further growth or nutrient absorption. Symptoms include clubbed or swollen root tips, yellowing or purpling of leaves and stem often associated with nutrient deficiencies. Whitening may also occur due to inability to produce chlorophyll. Weak stems (Fig. 1A) are common due to poor cell wall development. Callus proliferation is another symptom (Fig. 1B). Callus is usually formed from structural tissue, not individual cells. The disruption of mitosis leads to the proliferation of callus (Fig 1.B). Injury with Mi herbicides usually occurs when they are applied too

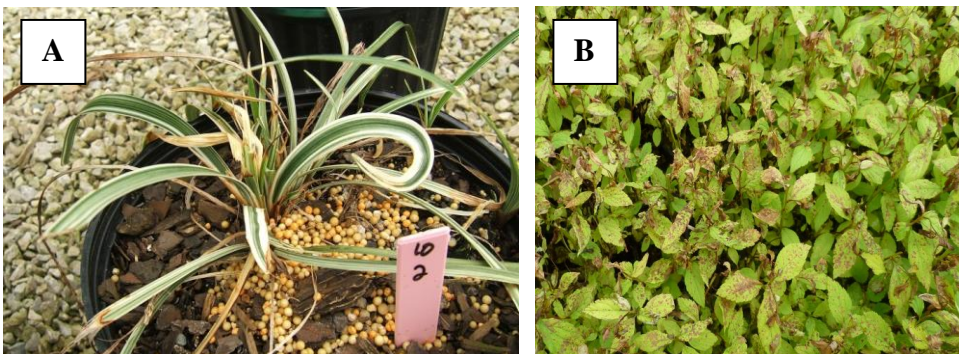
frequently or at too high a rate. Species tolerance also plays a significant role. Root damage is usually limited to the top 5 cm of the soil since the chemical has limited water solubility; therefore, long-term injury to established trees is unlikely; however, the lack of lateral production may have longer-term consequence. Shoots of callused stems wilt readily when conditions are dry and the long-term impact of this growth is unknown.



**Fig. 1A and B.** FreeHand (dimethenamid-p+pendimethalin) applied at a twice the normal rate (i.e. 300 lb./ac) to Elderberry (*Sambucus nigra* Blacklace™) spring 2012 caused **A.** significant stem brittleness and weakening plus **B.** callus proliferation at the plant crown. (Photos by H. Mathers).

#### Group 14 & 21

Some **non-Mi** preemergence herbicides used in landscaping are Goal (oxyfluorfen), SureGuard (flumioxazin) and Ronstar (Oxadiazon) which are Group 14, protoporphyrinogen oxidase (PPO) inhibitors and Gallery (isoxaben) a Group 21, cell wall synthesis inhibitor (CWSi). PPOs are found in the plant chloroplast and their inhibition leads to lack of a precursor of chlorophyll and heme; however, it is the formation of triplet oxygen that causes the characteristic rapid destruction of contacted tissue (Fig 2 A and B) of PPO inhibitors. Tolerance is determined by the plants' ability to metabolize the herbicide rapidly and thus prevent its accumulation to toxic concentrations. PPO inhibitor damage can appear as mottling, leaf crinkling (Fig. 2A), malformation and puckering from the rapid destruction of the contacted tissue (Fig. 2B). Cell walls are found in all plant cells. CWSi herbicides prevent new cells in the root and shoot apices from producing cell walls causing plant growth to cease. When absorbed by roots, Gallery moves primarily in the xylem and causes severe root and shoot stunting (Fig. 3). When absorbed by leaves, however, there can be slow basipetal or phloem translocation which results in mottling (Fig. 4A) and *random* leaf chlorosis and necrosis of contacted tissue (Fig. 4 B).





**Fig. 2 A and B.** Symptoms of injury caused by the PPO inhibitor herbicide BroadStar (flumioxazin) such as **A.** leaf crinkling and malformation on variegated *Liriope* or **B.** rapid destruction of contacted tissue *Spiraea* sp. (Photos by H. Mathers).



**Fig. 3.** Root absorbed Gallery (isoxaben) a cell wall synthesis inhibitor herbicide causing severe root and shoot stunting to *Spiraea japonica* 'Neon Flash' from left to right, control no Gallery applied, 1X (Gallery 1.3 lb./ac), 2X (Gallery 2.6 lb./ac) and 4X (5.2 lb./ac). Picture taken spring 2012 by H. Mathers.



**Fig. 4. A and B.** **A.** Damage from Gallery + Barricade at 1.0 lb. + 0.66 lb. ai/ac, respectively on *Echinacea* 'Purple Magnus' at two weeks after application showing severe mottling from leaf absorbed Gallery (isoxaben) a cell wall synthesis inhibitor herbicide two weeks after application. **B.** Mottling and random leaf chlorosis caused by the cellulose inhibitor Gallery on *Buddleia davidii* (Photos by H. Mathers).

#### Group 5 – Triazines and uracils

Healthy established plants show considerable tolerance to all the MoA's cited above; therefore, injuries occurring with these MoA's need to occur early in the life of the plant. Injuries from Photosystem II (WSSA Group 5 MoA) preemergence herbicides, however, can have longer-term consequence especially at high concentrations. Group 5 includes the triazine family of: Princep Liquid; Simazine 4L, 90 DF, 90 WDG; Atrazine 4L, Atrazine 90DF, 90 WDG (simazine). In the foliage the effects of the triazines are yellowing or leaf chlorosis, veinal, interveinal, marginal or overall chlorosis. Injury appears first in the new growth, since the chemical is translocated to the growing point (Fig. 5A). Plants may outgrow injury, which is the result of low concentrations. New leaves will eventually become greener. The whole leaf may

become chlorotic at high concentrations. Leaves may turn brown and die. Excessive root absorption of the chemical is the major cause of plant injury and long-term effects. Repeated applications over several years will cause soil buildup. Plants transplanted into soils with significant triazine accumulations may be damaged or killed by the carry-over in the soil (Fig. 5B). Another Group 5 family of herbicides are the uracils. A study conducted by McCloskey and Maurer (1998) of grapefruit trees in Arizona showed significant foliar injury from Hyvar (Bromacil) applied in 1995 when measured one year after in 1996. In 1997, yield was shown to be not impacted when a Tukey's mean comparison was used; however, comparing to the control the injury of the Hyvar reduced fruit yield significantly, 2 years after application.



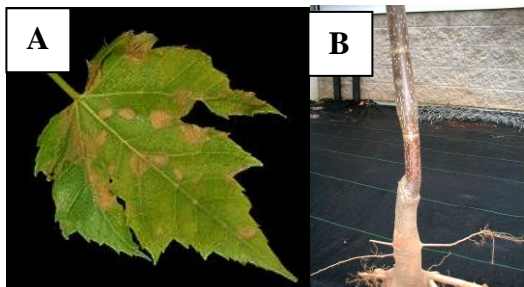
**Fig. 5 A and B.** A. Princep injury showing marginal chlorosis and B. death of *Thuja occidentalis* after transplanting into soils with triazine herbicide build-up (B. Picture by H. Mathers)

Although the preemergence herbicides listed above caused some short- and long-term problems, the incidence of herbicide injury is much higher with postemergence herbicides. Postemergence products can cause a larger variety of short-term injuries the consequences of which have long-term effects in the tree.

### Postemergence Herbicides: “The Troubles We’ve Seen”

#### Group 22

The short term injuries of postemergence herbicides are usually seen in the foliage of woody plants. Gramoxone (Paraquat) a contact postemergence herbicide causes contact burn of plant foliage. Brown leaf spots on the leaves result from spray drift (Fig. 6A). Overall leaf death will result if the entire plant is sprayed. Injury appears within several hours when sprays occur on hot, humid days. Gramoxone causes contact, not systemic injury, new growth will not be damaged. Plants may outgrow injury if only a few branches affected. However, paraquat, when sprayed on tree trunks, repeatedly, can cause injury, looking like a stem weakness or constriction, similar to a graft incompatibility (Fig. 6B). This trunk injury will cause long term, severe, problems for the tree.



**Fig. 6A and B.** A. Injury on maple leaf from spray drift occurring as browning and speckling on the foliage and the characteristic pattern of following along veins of Group 22 herbicide Paraquat. Paraquat injury

can also occur on trunks due to over application and acts as it does on foliage as a desiccant. Constriction of growth via dehydration of tissue results.

#### Group 4

The long-term impact of growth regulator herbicides such as the phenoxy family (WSSA Group 4) have been well-documented in forestry, reducing tree survival long-term. Herbicides such as 2, 4-D, MCPA and MCPB are members of this family and are severely phytotoxic to woody dicot plants. In the foliage 2, 4-D will cause tip chlorosis, tip dieback, epinasty, twisting and abnormal appearance (Fig. 7 A). This chemical causes the largest percentage of documented herbicide injury to plants in the landscape. Injury may be the result of spray drift, volatilization, or root uptake. Symptoms depend on the concentration to which the plant has been exposed. Low concentrations cause shoot tips to twist, leaves become cup-shaped with margins curling up or down (Fig. 7A). Leaf petioles may bend down giving the plant a wilted look. Cracked calluses appear on longer stems (Fig. 7B). At low concentrations leaves which develop after contact may be long, strap shaped, darker green in color with prominent veins. At higher concentrations, shoot tip and leaves turn chlorotic and die, eventually leading to the death of the entire plant. Long-term use of even low concentrations of 2, 4-D around older trees will cause significant growth deformations of stems (Fig. 7B). Phenoxy herbicides should only be used around trees with extreme caution and when all other options have been exhausted.



**Fig. 7A and B. A.** Twisting of foliage (epinasty) of Redbud (*Cercis canadensis*) and leaf curling and cupping common symptoms of phenoxy (Group 4) postemergence herbicides. **B.** Long term use of group 4 herbicides around trees at low concentrations will cause significant stem callusing and distortion, as seen on this Deodar Cedar (*Cedrus deodara*) (B. Photo by H. Mathers). At high concentration death may occur.

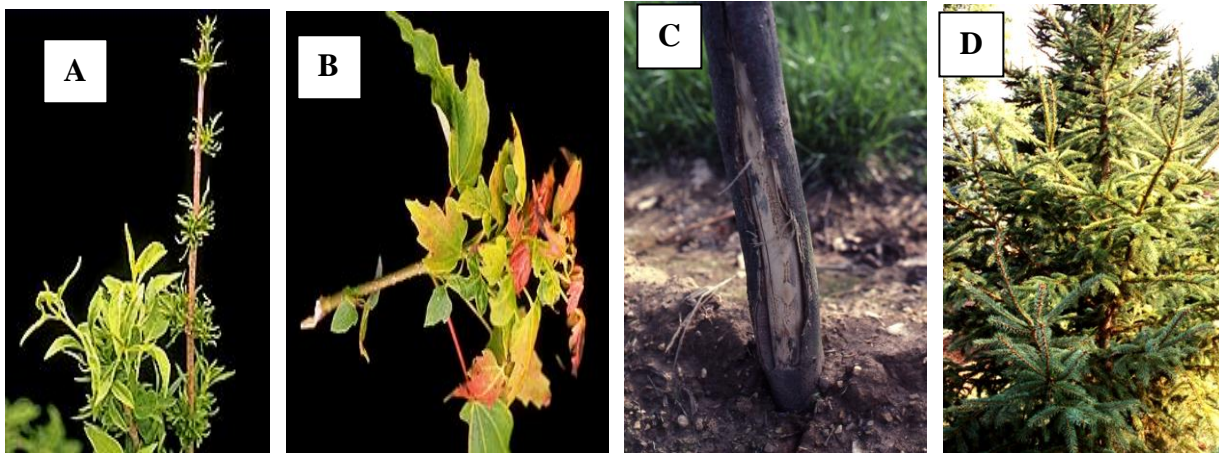
While phenoxy herbicides may cause the most *documented* injuries to trees in the landscape. I speculate glyphosate (N-(phosphonomethyl glycine) causes the most long-term, *undocumented* injuries. Glyphosate is the most widely used herbicide in the world (Duke and Powles, 2008; Santos et al., 2007; Vereecken, 2005) and results in the accumulation of shikimic acid, depletion of aromatic amino acids, and a reduction in phenolic compounds (Duke, 1988). The Monsanto glyphosate patent expired in 2000, thus opening the market to many generic brands of glyphosate (Tu et al., 2001) and a dramatic reduction in price. Manufacturers have incorporated various surfactants at various doses to hydrophilic glyphosate to break down the plant cuticle and allow passage through the plasma membrane to the site of action since 2000. Also various surfactants have been added to increase the rate of action, pleasing consumers who want to see immediate impact, 2-3 days versus 12 days with older products. Today over 750 products contain glyphosate in the US market.



## Glyphosate Injury is Difficult to Diagnose

### Group 9

Glyphosate is readily broken down within the soil, once in the phloem of a plant; however, it may take years to break down. The advent of increased and improved surfactants, also increased the potential loading of glyphosate in nursery and landscape trees receiving repeated exposure, limited breakdown and increased uptake. Non-metabolized glyphosate is rapidly translocated to the roots (Duke 1988). Presumably, it is stored along with sugars flowing to the roots in the summer and fall. In the spring when the flow of sugars is reversed, glyphosate can be translocated to meristematic regions to continue its effects (Stasiak et al., 1992). This reversal shows up as glyphosate carry-over injury which is characterized by witches broom, cupped, puckered, strap-like, stunted growth (Mathers, 2006), chlorosis (Fig. 8 A and B), and/or death (Ferrell et al. 2006) and potentially bark splitting (Kuhns, 1992) (Fig. 8C). Glyphosate carry-over injury can occur one, two or three years after application. Because of the spiral pattern of the vascular system in many conifers, glyphosate carry-over in spruce appears as a spiral in the tree (Fig. 8D). White spruce (*Picea glauca*) is particularly susceptible to glyphosate injury, even drift (Fig. 8D)



**Fig. 8 A, B, C and D.** **A.** Reversal of glyphosate stored in roots from the previous season shows up as glyphosate carry-over injury one, two or three years after and is characterized by strap-like, stunted growth (Mathers, 2006) and chlorosis or **B.** witches broom, cupped and puckered foliar growth. **C.** Roundup damage due to roundup (Courtesy of Dr. Larry Kuhns (formerly of PSU)). **D.** On white spruce glyphosate carry-over will take on a spiral pattern up the tree of chlorotic tissue (picture by H. Mathers).

Shikimic acid is frequently used as a highly sensitive, real time biomarker to determine if a plant has been exposed to glyphosate. Shikimic acid levels begin to rise within hours of glyphosate exposure and can remain elevated for several days to several years, depending on the dose and species (Anderson et al., 2001; Metallo et al., 2009; Stasiak et al., 1991). Stasiak et al. (1991) found a positive correlation between shikimic acid levels immediately following glyphosate dosing on one and two year old *Prunus pensylvanica* (Pin cherry) and *Populus tremuloides* Michx. (Trembling aspen). Stasiak et al. (1992) found that shikimic acid levels in two year old *Betula papyrifera* Marsh. (White birch) seedlings were increased 10-fold in trees treated with glyphosate, even at a rate of 2.1 kg ha<sup>-1</sup> (1.848 lb. ac<sup>-1</sup>) compared to the control. One year after application these two year birch seedlings still had significant higher shikimic acid levels in leaves compared to controls, suggesting prolonged effects of glyphosate in the plant (Stasiak et al. 1992). The accumulation of shikimate results from the inhibition of the chloroplast localized 5-enol-pyruvylshikimate-3-

phosphate synthase (EPSP synthase) in the shikimic acid pathway (Klee et al., 1987; Rubin et al., 1982; Steinrucken and Amrhein, 1980). The shikimic acid pathway converts simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway into aromatic amino acids (Herrmann and Weaver, 1999) and is vital for production of phenylalanine, a precursor of lignin (Hermann 1983), and precursors to tannins, suberin, indole-3- acetic acid (IAA), and phenolic compounds (Weaver and Herrmann, 1997). Plant phenolics are biosynthesized by several different routes; however, the shikimic acid pathway is the most common for their biosynthesis. A direct correlation exists between the accumulation of phenolic compounds and increased cold hardiness in a variety of plant species (Chalker-Scott, 1992; Chalker-Scott, 2004).

Mathers (2008) surveyed the nursery usage of glyphosate products, and identified five high risk practices such as over use of glyphosate, glyphosate exposure to green-bark, sucker removal with glyphosate, glyphosate applications immediately following sucker removal and elimination of preemergence herbicides for reliance on glyphosate. One nursery surveyed was using glyphosate as frequently as eight times per season (i.e. every 2.5 weeks) at one quart/ac. Photosynthetic actively growing tissue is required for postemergence herbicide uptake and this includes “green-bark.” The label of most product containing glyphosate will indicate “do not apply to green-bark.” There is extensive research to support that thin-bark with green pigment is photo-synthetically active and able to absorb postemergence herbicides (Borger and Kozlowski, 2006; Groh et al., 2002; Kuhns, 1992; Lange et al., 1975; Langenfeld-Heyser et al., 1996). Lange et al. (1975) found that glyphosate rates as low as 4.48 kg·ha<sup>-1</sup> (3.94 lb. ac<sup>-1</sup>) applied to young green to light-brown branches and trunks of apple and peach orchard trees caused severe burn, splitting and exudations. Lange et al. (1975) also applied glyphosate at rates of 3.36 (2.95 lb. ac<sup>-1</sup>) to 13.44 kg·ha<sup>-1</sup> (11.82 lb. ac<sup>-1</sup>) to the basal four or six inches of peach trunks or to the soil only (the trunks were shielded) and found significant stunting when glyphosate was applied to the trunks. No stunting occurred when only soil was sprayed, which suggested that the glyphosate uptake was through the bark (Lange et al., 1975). Lenticels which are rich in chlorenchyma can also take up herbicide (Langenfeld-Heyser et al., 1996). Micro-autoradiography experiments indicate that woody stem chlorenchyma of *Fraxinus excelsior* L. assimilate external <sup>14</sup>CO<sub>2</sub>. The areas bordering the lenticels showed the highest assimilation, indicating that <sup>14</sup>CO<sub>2</sub> enters the stem through the intercellular spaces of the lenticels (Langenfeld-Heyser et al., 1996). The permeability of single lenticels of *Betula potaninii* L. and *Sambucus nigra* L. stems to water and oxygen has been found to be significantly higher than that of the phellem areas lacking lenticels (Groh et al., 2002). Because of this inhibition of the shikimate acid pathway by glyphosate and subsequent reduction of aromatic amino acids, phenolics and production of lignin, suberin; it is speculated that the increasing use of glyphosate and increased surfactants in nursery and around landscape trees has increased their susceptibility to bark cracking.

### **Sucker Removal:**

The use of glyphosate to remove suckers is a non-registered use. Scythe (pelargonic acid), a contact herbicide is the only herbicide labelled for sucker removal. The use of glyphosate shortly after mechanical removal of suckers is a high-risk practice. Kochenderfer et al. (2006) described that applying postemergence systemic herbicides after sucker removal is similar to basal stump applications performed to control root and stump sprouts. Howell and Weiser (1970) noted herbicides should be used with caution around previously injured young tissue, as re-injury will occur in the breaks. Borger and Kozlowski (2006) found wound periderm formation was prevented by as little as 1000 ppm of herbicide 2, 4-D.

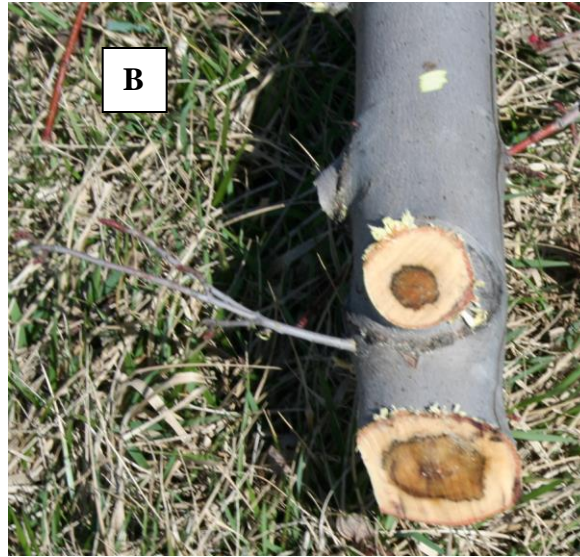


- The best way to conduct sucker control is with a sprout inhibitor such as Tre-Hold® Sprout Inhibitor A-112, Tre-Hold® RTU or other formulations containing naphthaleneacetic acid (NAA) to inhibit the development of adventitious shoots. These products replace the apical dominance of the tree, which is why the tree is suckering, i.e. due to disruption of the normal apical dominance of the plant. Mechanical removal of suckers, which is another common practice, has been shown to increase sprouting, as this creates a wound response in the plant and initiation of more adventitious shoots. Paint or spray the NAA products onto pruning cuts after sprouts are removed. A 1% solution applied to run off, to the lower 30 inches of trunk in March or April when growth has just begun and all leaves are removed in the area to be sprayed is recommended. [http://www.amvac-chemical.com/trehold\\_labels.html](http://www.amvac-chemical.com/trehold_labels.html).

Studies were conducted at Ohio State University, Waterman Farm, Columbus, OH to determine the role of other suspected contributing factors to bark cracking using *Acer x freemanii* 'Jeffersred' (Autumn Blaze™ maple), *Malus* 'Prairifire' (Prairifire crabapple), *Cercis canadensis* (Eastern redbud) and *Quercus rubra* (red oak). Adventitious shoots at the root shank (shank suckers) were removed mechanically on June 2007 and 2008. Trees that lacked shank suckers had razor blade incisions made near the tree crown. Immediately following wounding each year, four postemergence herbicides were targeted to six inches from the crown and two controls (wound/no herbicide; no wound/no herbicide) were imposed. Wounding was conducted on the north (N) and the south (S) sides of the tree trunk. The frequency of bark cracks was increased in all species the spring of 2009; however, only significantly so for *Acer x freemanii* 'Jeffersred' (Fig. 9A). No correlation was found to wounds made on the N or S side. Over the four species, Roundup Original Max® (48.7% potassium salt of glyphosate), followed by Roundup Pro® (41% isopropylamine salt of glyphosate) caused significantly more cracking. The no wound/no herbicide had the least frequency of cracks and the wound/no herbicide control, KleenUp Pro® (41% isopropylamine salt of glyphosate) and Scythe® (57% pelargonic acid) which were not statistically different from one another, had significantly more cracks than the no wound/no herbicide control. Bark cracks on maple were correlated with increased woody decay three years after application (Fig. 9B). Although not significant for increased cracking, Red bud also showed considerable wood decay three years after application (Fig. 9C). Wood decay fungi are the primary and most common cause of failure in standing trees and illustrates the long-term impact of glyphosate applied and cracked trees. Trees with decay are structural unsound and are more prone to becoming hollow and hazard trees, long-term.

Detached current year shoots were subjected to freeze tests and evaluated in January, 2009. Significantly decreased cold hardiness, over species, occurred in shoots with basal applications (BA) of Roundup Original Max® followed by Roundup Pro®. The no wound/no herbicide control had the hardest shoots, followed by the wound/no herbicide and KleenUp Pro® which were not statistically different. On November 2, 2011 (fall) and June 25, 2012 (summer), AquaMaster® (53.8% isopropylamine salt of glyphosate) and Roundup Pro® were sprayed on *Syringa reticulata* (Japanese tree lilac) in 7 gallon containers at OSU, Columbus, OH as over-the-top (OTT) or BA applications directed 2.5 in from crown/media union. Shikimic acid (SA) levels (as a real time biomarker for determination of plant exposure to glyphosate), were quantified by extraction from leaf tissues. Extractions followed by isocratic reversed phase HPLC-UV were not sufficient to separate co-eluting peaks from shikimic acid, resulting in artificially inflated shikimic acid data. A new shikimic acid detection system utilizing LC/MS/MS was developed for more accurate quantification. Leaf samples were collected 0, 3, 7, 14 and 30 days after treatment (DAT) for June 25, 2012 applications only. SA levels following the summer and fall + plus summer Roundup Pro® OTT increased 17.5 fold and 10.2 fold versus the control at 14 DAT, respectively.

Summer Roundup Pro® OTT applications at 30 DAT were still elevated 6.0 fold versus the control and fall + summer 6.3 fold. No increase in SA levels could be detected in leaf samples following AquaMaster® OTT or BA applications in summer or fall + summer or with BA applications of Roundup Pro®. Our research supports certain glyphosate formulations are disrupting the SA pathway, reducing cold hardiness, and increasing frequency of bark cracking in susceptible species; wounding (of any kind) does increase cracking; and, the influence of glyphosate applied near basal bark wounds is experienced in current year's shoot growth. High risk practices with glyphosate, exposure to green-bark, sucker removal, applications immediately following sucker removal and elimination of preemergence herbicides for reliance on glyphosate are considered contributory to stock losses from bark cracking and mortality in landscapes.



**Fig. 9 A and B.** Maple with bark cracking following application of glyphosate and **B.** internal wood decay. **C.** Shows considerable wood decay found in Red Bud associated with cracking. Pictures taken 2010 three years following applications at OSU Waterman Farm, Columbus, OH. (Pictures by: H. Mathers).

