Detecting Herbicide Injury on Turfgrasses

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Turfgrass managers use various pre-emergence and post-emergence herbicides to control weeds on their courses. Herbicides applied according to instructions specified by the manufacturer are intended to kill undesirable weeds, however misapplication of herbicides can cause injury to desirable plant species. Herbicide injury can happen due to a single factor or a combination of various factors like herbicide residues in soil, carry over from a previous application, spray drift during an application, tank-mix compatibility problems. The general symptoms of herbicide injury are yellowing, curling of leaves, bronzing, stunting of growth of plants etc. These symptoms vary with the different types of herbicides used, application rate, carrier volume, or adjuvants used along with the herbicide.

Biochemical diagnostic tools are being made available to superintendents and custom applicators to identify the herbicide, which caused the injury. Knowledge about the herbicide's mode of action and mechanism of action are very helpful in identifying the injury. Herbicides use different biochemical pathways to act on plants. The mode-of-action refers to the overall manner in which an herbicide affects the plant at the tissue or cellular level. Herbicides with the same mode-of- action will have the same translocation (movement) pattern and produce similar injury symptoms. Selectivity on crops and weeds, behavior in the soil and use patterns are less predictable, but are often similar for herbicides with the same mode-of-action (Ross and Childs 1996).

Plants are complex organisms with well-defined structures in which multitudes of vital (living) processes take place in well-ordered and integrated sequences. Plants are made up of organs (root, stem, leaf, and flower); organs consist of tissues (meristems) and tissues are made up of cells. Plant cells contain subunits including walls, membrane systems (golgi, plasma membrane, nuclear membrane, endoplasmic reticulum) and organelles (mitochondria, nucleus, chloroplasts), and undifferentiated cytoplasm. Some vital metabolic plant processes include photosynthesis (capture of light energy and carbohydrate synthesis), amino acid and protein synthesis, fat (lipid) synthesis, pigment synthesis, nucleic acid synthesis (RNA - DNA essential to information storage and transfer), respiration (oxidation of carbohydrate to provide CO_2 and usable energy), energy transfer (nucleic acids) and maintenance of membrane integrity. Other vital processes include growth and differentiation, mitosis (cell division) in plant meristems, meiosis (division resulting in gamete and seed formation), uptake of ions and molecules, translocation of ions and molecules, and transpiration. One or more of the vital processes must be disrupted in order for an herbicide to kill a weed (Ross and Childs 1996).

Factors Contributing to Herbicide Injury

There are various factors, which can contribute to herbicide injury.

1. **Soil Residues.** Some pre-emergence soil applied herbicides may persist in the soil for months after application. The amount of herbicide sorbed on the soil particles in governed by soil texture and organic matter content. In sandy soils the rate of application should be lower than the rates in finer texture soils (more clay content). In sandy soils the majority of the herbicide is present in the soil solution since sand is an inert material and does not adsorb any herbicide molecule. Clay and organic matter bind the herbicide molecules on their surface. Most herbicides are organic molecules and are attracted to negative sites on clay or organic matter. Strongly bound herbicides do not pose a potential threat to sensitive plants, but herbicides, which are not strongly bound may desorb into the soil solution. The amount of herbicides that are actually present in the soil solution are responsible for herbicide injury. Roots of sensitive plants when exposed to the herbicide molecule will affect them physiologically resulting in herbicide injury. The roots of younger plants are more prone to damage. Hence, often it is recommended to wait a minimum time period before planting or sodding after an herbicide application. Superintendents should be careful when they are doing a renovation job since they often use a post-emergence non-selective herbicide to kill the turf and weeds.

2. **Soil pH.** Soil pH is a very important factor influencing herbicide persistence. Herbicides often breakdown to different chemical compounds in the soil. The chemical breakdown or metabolization of a herbicide is governed by soil pH. Some actual herbicide molecules are not toxic to plants, but their metabolites are. Hence the breakdown of a particular herbicide plays a very important role. Certain herbicides are adsorbed more at lower pH, which makes them unsafe to use in soils with high pH. For eg. the herbicide Balance® (isoxafultole) is adsorbed more at lower soil pH (Mitra et al. 2000). An applicator has to be careful when they are applying Balance® in a sandy soil with low organic matter and high soil pH.

3. **Weather.** The weather conditions play an important role in herbicide injury to plants. Herbicides applied on a very warm day can injury the turf, since the turf will be growing rapidly and might take up larger amounts of herbicide. Certain plants do have the capability to detoxify herbicides by forming conjugates with other molecules in the plant (eg. Atrazine®) or they metabolize the herbicide molecule into non-phytotoxic substances. If the plants absorb a larger amount than they can handle physiologically it will lead to an injury. Cool-season turf is prone to herbicide injury on a warm day due to the heat and drought stress. Stressed plants are more susceptible to herbicide injury than non-stressed plants.

4. **Spary drift.** Spray drift is the major contributing factor to off-target injury. Herbicides applied under windy conditions may be carried over to adjoining areas and might cause damage to sensitive plants. Herbicides applied at high pressure tend to produce finer droplet size and are

more prone to spray drift. Superintendents can use drift retardants to reduce spray drift and should apply herbicides either early in the morning or in the late afternoon.

5. Rate of application. Selective herbicides are formulated by manufacturers to be safe to non-target plants only at specified rates. Hence, herbicides applied at higher than recommended rates result in injury. Over application can result due to faulty spray equipment, wrong calibration, uneven pressure along the boom, excess overlap or faulty nozzles.

6. Contamination. Spray tanks should be triple rinsed every time after applying any pesticide. Herbicide injury caused due to contamination is evident when symptoms are observed where the spraying starts and then slowly the symptoms become less noticeable. The water source is also a source of contamination. Water sources containing excess bicarbonates and other cations might react with the herbicide. Superintendents should be careful when they use Roundup® (glyphosate) with a contaminated water source.

7. Tank-mix compatibility. Turf Managers should be careful and follow label directions while mixing different pesticides. Some herbicides cannot be tank mixed with other herbicides or other pesticides. It is always advisable to do a jar shaker test before actually mixing any chemicals. The jar shaker test is a quick and easy test in which a solution is made with the chemicals in question and the water to be used. The jar should be shaken and observed for any precipitates. If any precipitate is observed then the chemicals should not be mixed.

MATERIAL AND METHODS

Experiments were conducted at the Center for Turf Irrigation and Landscape Technology (C-TILT) at California State University, Pomona.

Treatments

Different glyphosate based herbicide formulations were applied on a creeping bentgrass putting green. QuikPro® powered by Roundup®, and Roundup ProDry® were applied at three rates (5, 5.88, and 6.8 kg ae/ha). All the treatments were compared to an untreated check and a Finale® 2SC (glufosinate) treatment applied at 1.7 kg ae/ha.

Extraction of Shikimate

Shikimate was extracted using following Singh and Shaner's (1998) method. Plant material was ground in liquid nitrogen in a mortar pestle and then further ground in 0.25 N HCl in the same mortar. Leaves were ground in a 1:3 ratio of tissue weight in g/volume of 0.25 N HCl in ml. The extract was centrifuged at 15,000 *g* for 15 min. The supernatant was collected and used directly for the shikimate assay.

Determination of Shikimate

Shikimate was determined according to a modification of the method of Gaitonde and Gordon (1958). An aliquot of the test sample (50 µl) was mixed with 0.5 ml of a 1% solution of periodic acid to oxidize shikimic acid. After 3 h, the sample was mixed with 0.5 ml of 1 N NaOH, and 0.3 ml of 0.1 M glycine was added. The solution was then thoroughly mixed and the optical density at 380 nm was measured immediately. The concentration of shikimate was presented on a fresh weight basis.

RESULTS AND DISCUSSION

The mode-of-action of an herbicide offers a clue in identifying herbicide damage. Various biochemical pathways are used by herbicides to act on plants. The herbicides produce different metabolites or derivatives, which can be used to positively identify the herbicide. For eg. Roundup® (glyphosate) acts on the shikimic acid pathway in plants. Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, which produces EPSP from shikimate-3-phosphate and phosphoenolpyruvate in the shikimic acid biosynthetic pathway (Amrhein et al. 1980). EPSP inihibition leads to the depletion of the aromatic amino acids phenylalanine, tyrosine and tryptophan, all needed for protein synthesis in plants (Ahrens 1994). As a result of the herbicide application there is an accumulation of a particular chemical in plants called, shikimate. Scientists have used chemical methods to identify and quantify the amount of shikimate from Roundup® treated plants.

Problems Associated with using Biochemical Methods

Generally plant samples are collected after a long time has lapsed since the injury was first observed. Since the biochemical tests have to be conducted when the plant tissue is still alive sometimes it is too late to conduct a test. Turf managers should send samples out for biochemical analysis as soon as they observe any visual signs of herbicide damage. The symptoms of herbicide injury resemble other kinds of damage or injury. Stunting, twisted leaves or death of growing points are some of the general symptoms of herbicide damage. Herbicide residue in affected plant tissues is often quite difficult to detect and the presence of certain metabolites is not always definitive.

Shikimate Content

Within 48 hrs after treatment Roundup ProDry resulted in significantly higher amount of shikimate in the plant tissues compared to the untreated check and the QuikPro powered by Roundup treatments. After 72 hrs there was no difference in the amount of shikimate in the plant tissues between the different glyphosate formulations (Fig. 1). A spike in the amount of shikimate content in the herbicide treated plant tissues confirms that the plant was affected by a glyphosate based herbicide. There are also some differences between the different glyphosate based herbicide formulations in the amount of shikimate present in plant tissues, but the differences are observed very early (within 48 hrs after application). As the plant starts to die the tissues start to accumulate high levels of shikimate irrespective of the herbicide formulation and once the tissues die it is very difficult to extract shikimate from the plant tissues. Hence using these biochemical methods we can detect the amount of shikimate present in plant tissues and can positively identify glyphosate injury to plants.

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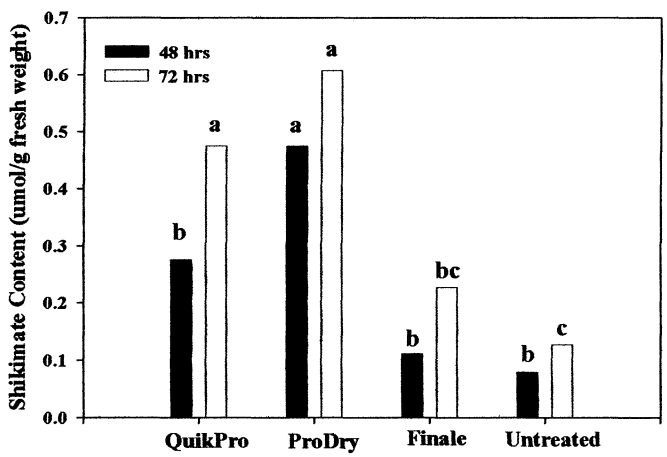

Fig. 1. Amount of shikimate in plant tissues as observed at 48 hrs and 72 hrs after treatment. Each column with a letter in common are not significantly different at p = 0.05 level. Means were separated by Duncan's New Multiple Range Test.