Effects of Glyphosate on Melatonin in Wild Strawberries under Three Different Environmental Conditions

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1. INTRODUCTION

- Glyphosate is a post emergent, nonselective, broad-spectrum, systemic herbicide (Duke, 2018; Lévesque & Rahe,1992) used to control competitive vegetation in forests where wild strawberries grow in British Columbia (Govindarajulu,2008).
- Melatonin is a plant hormone present in strawberries that acts as an antioxidant, growth promoter, cell protector and helps to combat biotic and abiotic stress conditions (Arnao & Hernández-Ruiz, 2015; Hernández-Ruiz et al., 2004).
- Glyphosate disrupts the precursors of melatonin (tryptamine and tryptophan) by blocking an enzyme, 5- enolpyruvyl shikimate -3- phosphate synthase (EPSPS), required for the biosynthetic shikimic acid pathway that produces the amino acids tyrosine and tryptophan (Duke et al., 2012).

Fig 1: Spraying of herbicide to control vegetation

2. OBJECTIVES

- To determine the changes in melatonin production after glyphosate treatment in wild strawberries.
- To determine the effects of three different temperatures and photoperiods on melatonin production in glyphosate treated wild strawberries.
- To determine the stress response in wild strawberries after glyphosate treatment.
- To determine the effect of temperature and light treatments on glyphosate to AMPA residues in tissues.

3. EXPERIMENTAL DESIGN

- Strawberry plants will be planted in three growth chambers (24 plants/chamber) under different temperatures and photoperiod conditions.
- Half the plants (12 plants) will receive an application of 50% operational dose of glyphosate (Vision Max®) and the other half (12 plants) will serve as a control.

Fig 2: An example of how plants will be divided between chambers for the experiment. 24 plants will be grown in each growth chamber with half of the plants (12 plants) treated with glyphosate and the remaining 12 plants acting as controls.

Trial 1

Temperature
Chamber 1 : 8°C/4°C
Chamber 2 :12°C/6°C
Chamber 3 : 20°C/14°C
Photoperiod
12 hrs/day (constant)

Trial 2

Temperature
12°C/6°C (constant)
Photoperiod
Chamber 1 : 6 hrs
Chamber 2 :12 hrs
Chamber 3 : 18 hrs

Trial 3

The photoperiod and temperatures of the growth chambers will vary, using the variations from Trials 1 and 2.

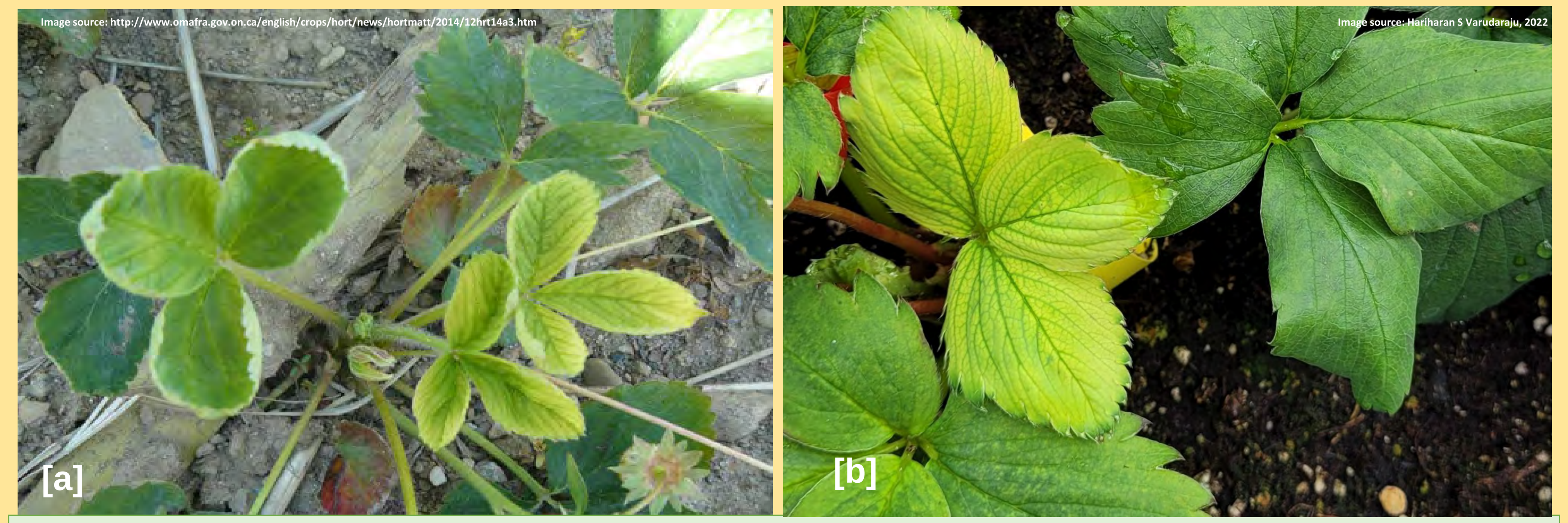

Fig 3a: Yellow colouring and cupped leaves are a common symptom of glyphosate injury in strawberries. **Fig 3b:** Appearance of chlorotic leaves when 50% of glyphosate were sprayed to actively growing strawberry plants at 20°C.

- Strawberry leaves will be collected from the growth chambers weekly for melatonin estimation. Plant leaf area, fluorescence and new leaves will be measured weekly as indicators of growth stress.
- Petiole length, root length, fresh and dry weight of shoot and root will be measured at the end of each trail.

4. MELATONIN ESTIMATION

The weekly collected leaf samples will be ground using liquid nitrogen and stored at -20°C. The samples will be analyzed using enzyme-linked immunoassay (ELISA) (analytical technique).

5. MEASUREMENT

- Leaf area will be measured using NIS-Elements (image analysis software).
- Fluorescence will be measured using chlorophyll fluorometer.
- The leaves that are unfurled are counted as new leaves
- Fresh and dry weight of shoot and root will be measured at the end of each trial.

6. EXPECTED OUTCOMES

1. Melatonin levels will be affected by glyphosate application as it disrupts the precursors (tryptophan and tryptamine) of melatonin.
2. Change in the duration of light exposure will alter the level of melatonin production as it is a light response molecule.
3. Stress factors such as low and high temperature will increase melatonin levels as it helps plants to overcome cold and heat stress conditions.
4. Leaf area and number of new leaves will be reduced due to glyphosate exposure and different environmental conditions.

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