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Greenhouse study to identify glyphosate-resistant weeds based on canopy temperature

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Abstract. Development of herbicide-resistant crops has resulted in significant positive changes to agronomic practices, while repeated and intensive use of herbicides with the same mechanisms of action has caused the development of herbicide-resistant weeds. As of 2015, 35 weed species are reported to be resistant to glyphosate worldwide. A greenhouse study was conducted to identify characteristics which can be helpful in field mapping of glyphosate resistant weeds by using UAV imagery. The experiment included three species of susceptible and resistant weeds: waterhemp (Amaranthus rudis), kochia (Kochia scoparia) and common ragweed (Ambrosia artemisiifolia). Thermal images of these weeds were acquired before and soon after glyphosate application with a thermal infrared camera. The results indicate that there is significant difference between the canopy temperature of glyphosate resistant and susceptible weeds. This research is continuing to develop a methodology for identification and mapping of herbicide resistant weeds and weed species in commercial fields using UAS-based high-resolution imagery.

Keywords. Glyphosate resistant weed, Thermal signature, Canopy temperature, Weed identification.

Introduction

Weed management is an important aspect of production agriculture as the economic cost of not managing weeds with herbicide is estimated as $21 B or more (PSU Extension, 2003). Recently, several species of glyphosate resistant weed are spreading across the US and around the world, raising concerns about their potential impact on agriculture. The result of one survey in the US indicated that farmers and neighbors who successfully managed glyphosate resistant weeds in corn and soybean crop improved their profits by more than 20% (USDA Economic Research Service, 2015).

Development of herbicide-resistant crops has resulted in significant positive changes to agronomic practices, while repeated and intensive use of herbicides with the same mechanisms of action has caused the evolution of herbicide-resistant weeds. Glyphosate or N-(phosphonomethyl) glycine is the aminophosphonic acid analog of the natural amino acid glycine. It is a widely used herbicides in agriculture and forestry for industrial weed control, and in outdoor residential applications in the United States. Glyphosate is a non-selective broad-spectrum, systemic, post-herbicide that prevents the plants from making certain proteins that are vital for plant growth. This herbicide comes in a number of chemical forms but most of the formulated products contain the isopropylamine salt (Jayasumana et al., 2014). On foliar application, the herbicide is absorbed by the foliage and translocated throughout the stems, leaves and roots of the entire plant, finally accumulating preferentially in young growing tissues. Glyphosate translocate in plants via the phloem, following absorption through the leaf tissues, and moves to actively growing parts of the plant such as meristems and roots. Glyphosate acts on enolpyruvyl-5-shikimate-3-phosphate-synthase (EPSPS), the sixth enzyme in the plant shikimic acid pathway. It converts shikimate-3-phosphate (S-3-P) together with phosphoenolpyruvate (PEP) into enolpyruval shikimate-3-phosphate (EPSP), aprecursor in the formation of aromatic amino acids and many secondary aromatic plant metabolites. Due to a missing feedback inhibition at this step, the S-3-P concentration in plants increases continuously after glyphosate treatment. With its high-energy bonds, the phosphate group of S-3-P is unstable in plant cells, and degrades into shikimic acid, which can be used as an early indicator of the inhibitory activity of glyphosate in plants. EPSPS is most active in young, actively growing plant tissue; therefore, the highest concentration of shikimic acid can be measured in these tissues, and particularly in the meristems, after glyphosate treatment. Both target and non-target-site-based resistance mechanisms are responsible for glyphosate (Vijay K. Nandula - Science – 2010)

Two glyphosate resistance mechanisms have been identified in naturally occurring glyphosate resistant (GlyphosateR) weeds namely, reduced target-site affinity for glyphosate and reduced translocation of glyphosate to meristematic regions (Pollegioni 2011**).** Alterations in the EPSPS gene sequence, specifically at the amino acid P106, have been reported in several glyphosate-resistant weeds.

Weed scouting is a key component of integrated weed management programs, particularly if there are resistant weeds involved. If resistant weeds can be mapped without having to scout the fields, it would contribute to the effectiveness of weed management. Many of the proposed automatic weed identification methods use a variety of visual characteristics of the plants, and sensing technologies (Lin 2009). Three general characteristics used for plant species identification are biological morphology, spectral characteristics, and visual texture (Hong 2012).There are several methods to identify weed species in crop field based on processing of remotely sensed image data but there is no reliable method to identify glyphosate resistant weeds in the field. Therefore, this study was conducted with the objective developing and validating a method in greenhouse to identify resistant weeds from susceptible ones in selected weed species, using canopy temperature. We introduce the concept of thermal signature in this study for detecting resistant weeds in greenhouse conditions.

Materials and methods

Greenhouse study

A greenhouse experiment was conducted on three species of weeds to test the hypothesis that canopy temperature after herbicide application could be a good indicator of herbicide resistance in weeds. The three weed species selected were waterhemp (Amaranthus rudis), kochia (Kochia scoparia) and ragweed (Ambrosia artemisiifolia). Seeds collected from susceptible and resistant plants by weed scientists were used ensure that there will be approximately same number of resistant and susceptible weeds in the experiment. Bulked weed seeds (100 seeds for each species) were planted in labelled cones, and arranged in a trays randomly.

Thermal images of the individual weeds were acquired with a Model 9640 P-Series USB Thermal Infrared Camera (Infrared Camera Inc. Texas, US) mounted above the plants on a cross-beam (Figure 1) before and periodically after spraying glyphosate on them. The camera was recorded long-wave infrared radiation in the 7-14 μm range, and converted it to grey level images of 640 by 480 pixel size. The camera weighed 74.5 g, and was designed for operation from an Unmanned Aerial System (UAS) platform.

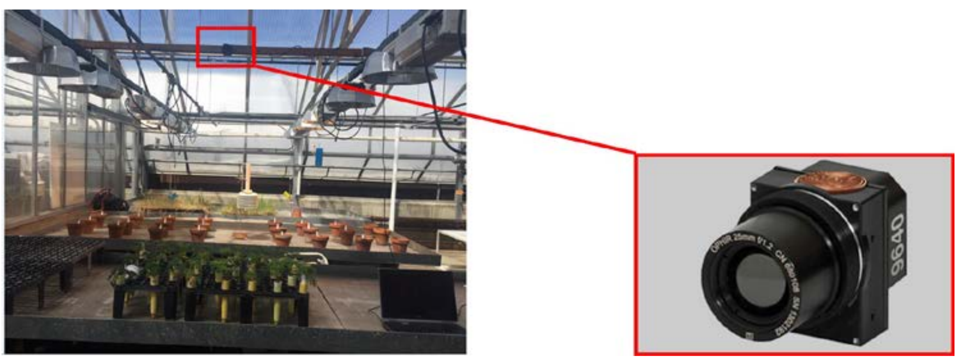


Figure-1. Thermal images in NDSU greenhouse, with a close-up of the thermal camera.

The weeds were sprayed with recommended full rate (1.7%) of glyphosate with a cabinet sprayer right before the plants height reached 10 cm (Figure 2). Thermal images of both susceptible and resistant weeds were acquired on an hourly basis in the first 72 hours after herbicide application. The air temperature in the greenhouse was also recorded by a HOBO pro series sensor (Onset MA, US).

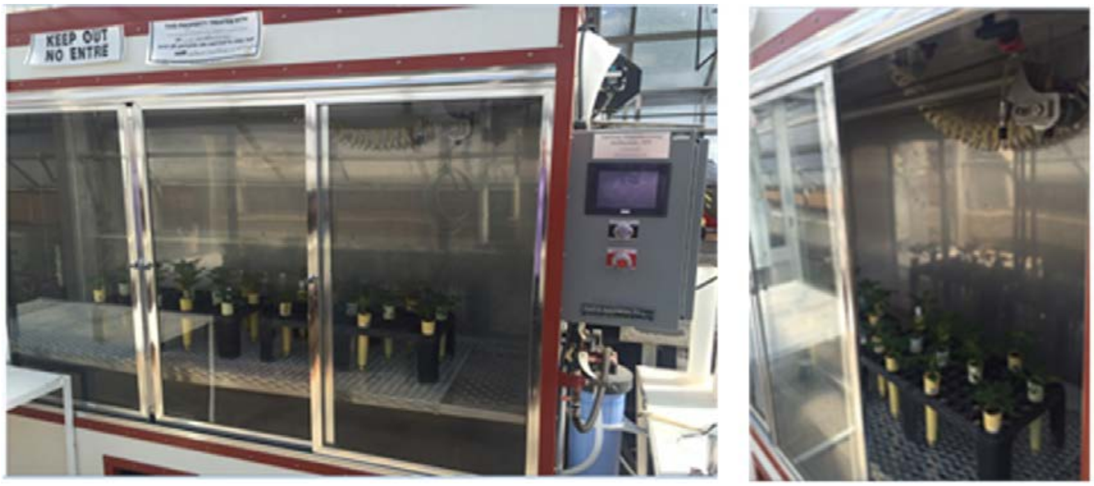


Figure-2. Plants being sprayed with Glyphosate in a cabinet sprayer

In grey level values of the thermal images were converted to temperature values using a built-in calibration function in the IR Flash software (Infrared Camera Inc. Texas, US) (Figure 4).Thermal signatures of the weeds were developed based on individual plant canopy temperature changes during 72 hours after spraying. Weeds were classified as resistant and susceptible 15 days after glyphosate application based on its survival condition. Dried plants were labeled as susceptible while green and alive plants were labeled as glyphosate resistant weeds.

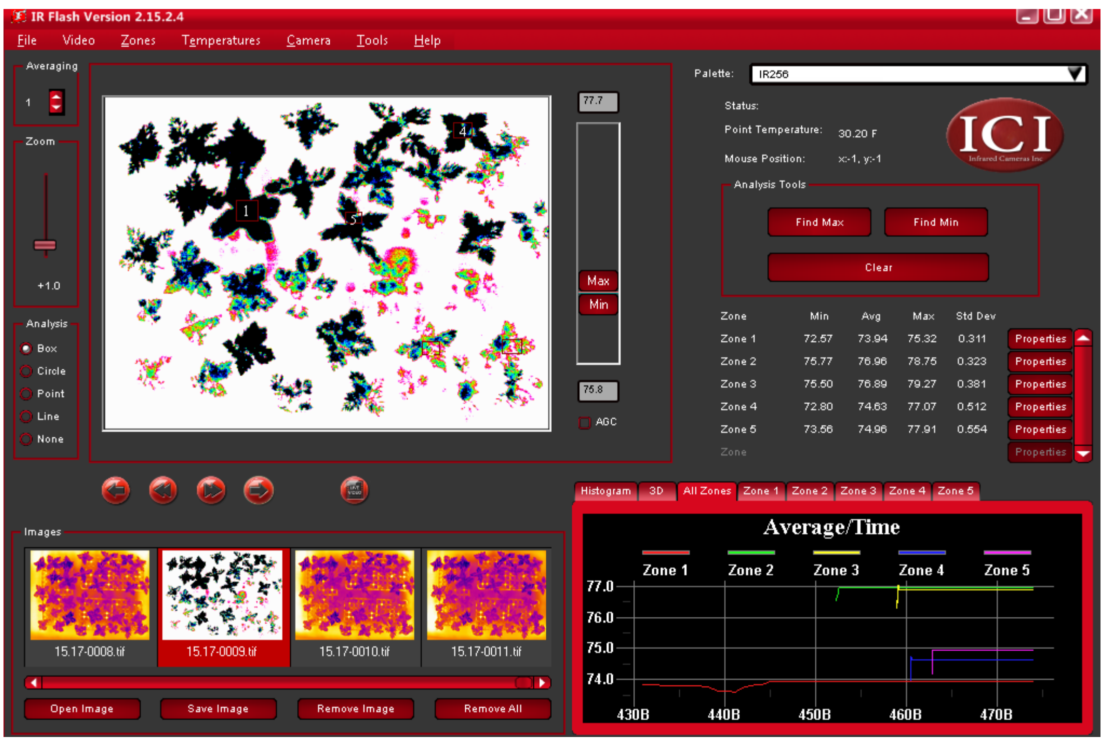
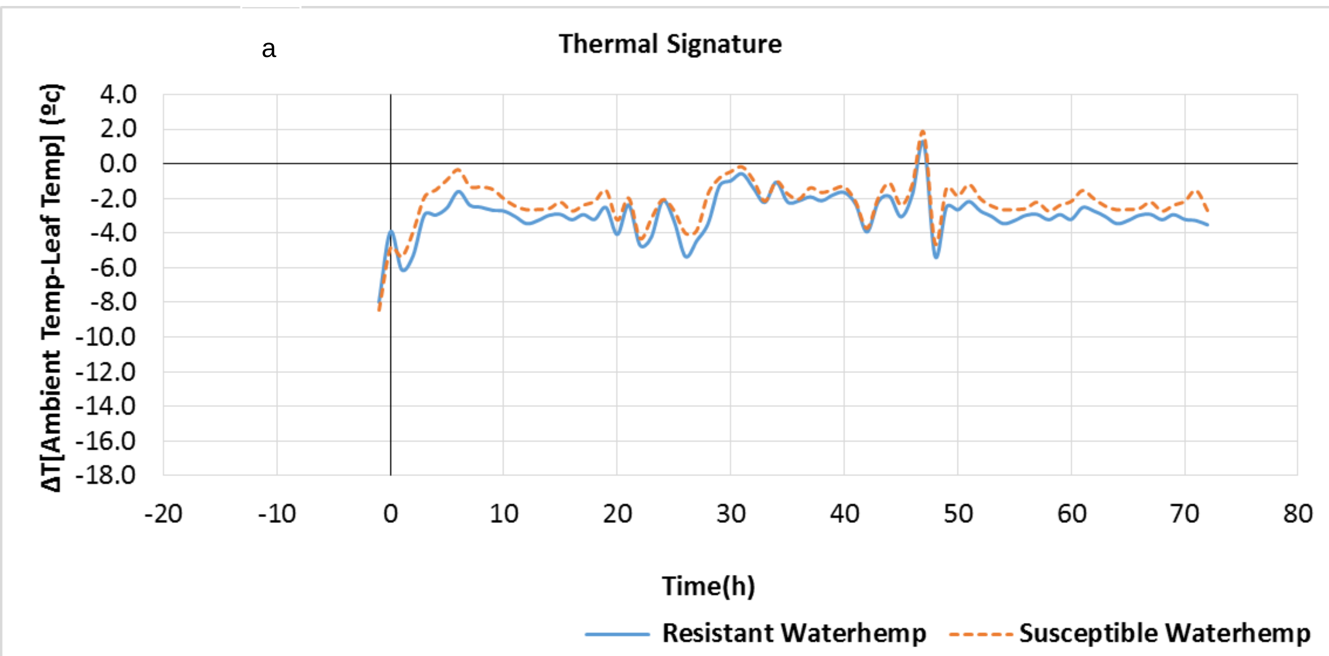


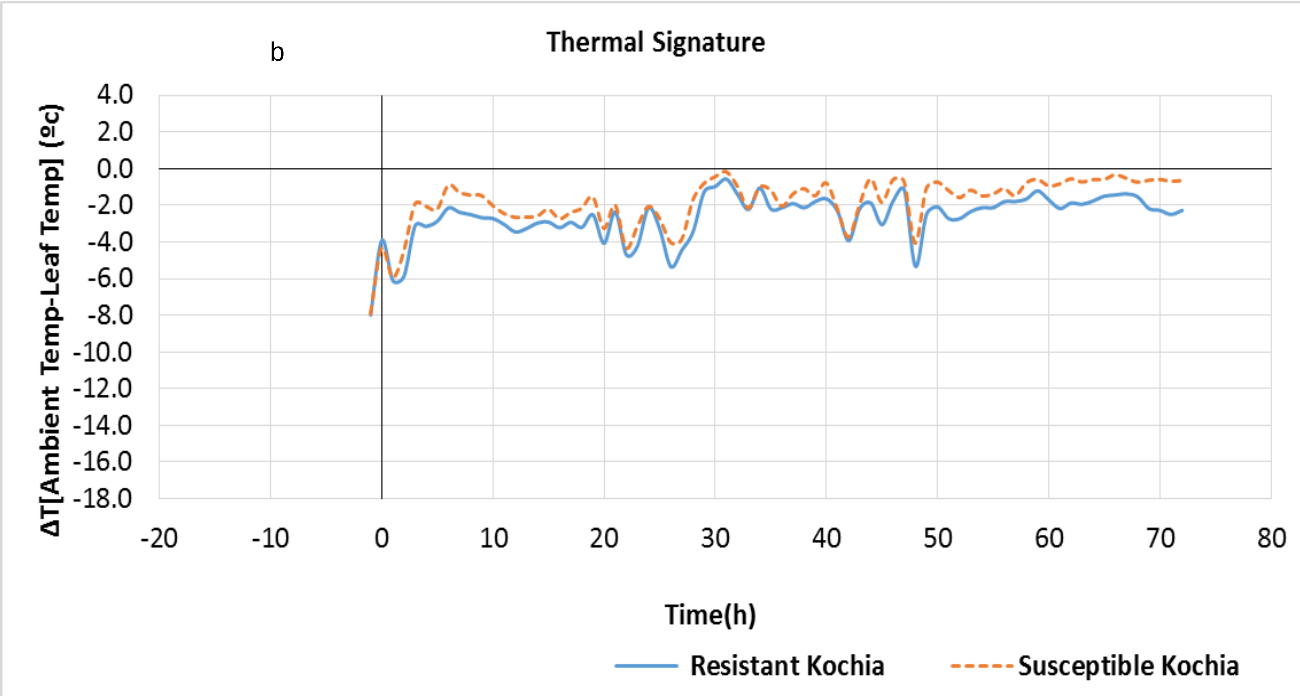
Figure-4. An example thermal of the weed plants viewed in the IR Flash software. The average temperature for the various zones can be seen on the right hand panel.

RESULTS AND DISCUSSION

Although equal number of susceptible and resistant seeds were planted, there were more susceptible weeds than resistant. Among the 100 plants in each species, only 40, 32 and 36 of the plants turned out to be resistant in waterhemp, ragweed and kochia respectively. This is because of these plants are cross-pollinating species. Almost all the susceptible plants died within 12 days of herbicide application. The resistant weeds exhibited different degrees of resistance, some indicating some stress soon after herbicide application, and then recovering into active vegetative growth.

The plant canopy temperature extracted from the thermal images indicated that the temperature of susceptible weeds increased within 1-2 hours of spraying Glyphosate (Figure 5).The resistant weeds exhibited much lower canopy temperature than the susceptible weeds for all three weed species. The temperature difference between the susceptible and resistant weeds of the same species fluctuated throughout the 72 hours after herbicide application. This indicate that there perhaps is an optimal window where the temperature difference may be the highest.





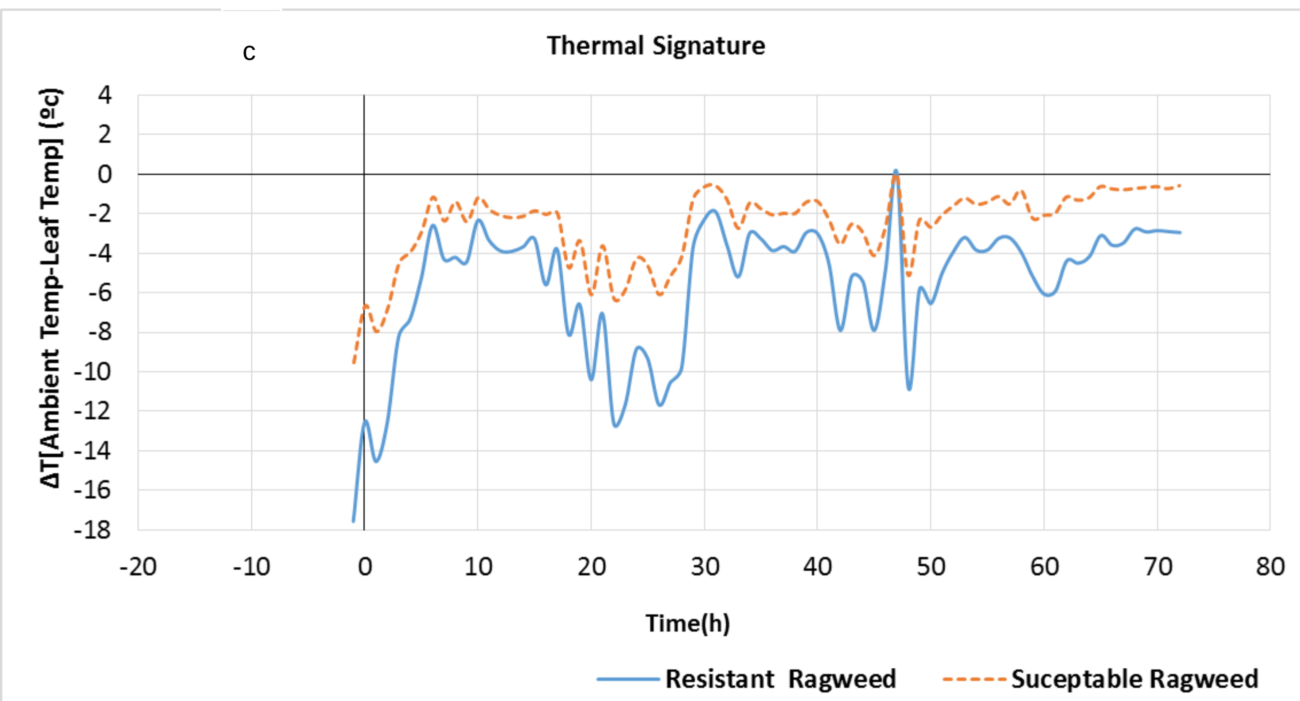


Figure-5. Thermal signature of three weed species used in the study. a) Waterhemp, b) ragweed, c) Kochia

The canopy temperatures of susceptible weeds increase after Glyphosate application due to the damages caused by the blocking of amino acid synthesis which is important for photosynthesis. When photosynthetic mechanism is less efficient or not working properly, more light energy become lost as heat. On the other hand, the temperature of resistant weeds remains same because the more efficient photosynthesis.

Shikimate accumulation following glyphosate application has been used to identify glyphosate-resistance plants. Quantifying shikimate accumulation in suspected glyphosate-R and known susceptible (S) plants can provide a quantitative measure of resistance in the plant. If the resistance mechanism in a mutated target site with a lower affinity for glyphosate, the shikimate accumulation will be lower in the R plants than in the known S plants because of lower pathway blockage. If both R and S plants accumulate shikimate at the same rate, then pathway blockage is similar, and the target site is inhibited.

The absorption pattern of 14C-glyphosate in the waterhemp and ragweed populations was similar in R and S population up to 24 hours after herbicide application. After this time, resistant weeds absorption is more than susceptible weeds (Berwer et al 2009, Whitaker et al 2013).

Extraction and quantification of accumulated shikimate indicated that both R and S populations accumulate shikimate. The accumulation of shikimate following glyphosate application indicates that EPSPase is inhibited in both R and S common ragweed and waterhemp. Accumulation slowed between 2 and 3 days after treatment for the R populations, while continuing to rise in the S population (Berwer et al 2009, Whitaker et al 2013).

Conclusion

The research results show clear difference in canopy temperature between susceptible and resistant weeds for the three species tested, as we hypothesized. However, further data analysis and testing are necessary to develop criteria for discriminating resistant weeds from susceptible weeds.

This project started in May 2014, and results shown here are preliminary. Future work will include field validation of the methodology we developed by sensor data collected from a UAV platform.

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