SIMULATED GLYPHOSATE DRIFT EFFECTS ON 'RED NORLAND' COMMERCIAL AND SEED POTATO INDUSTRIES

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MASTER OF SCIENCE

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ABSTRACT

Field, greenhouse, and laboratory experiments were conducted in 2014 and 2015 to evaluate effects of glyphosate drift on ‘Red Norland’ potato (*Solanum tuberosum* L.) grown for commercial and seed industries. Glyphosate drift was simulated by applying sub-lethal doses during the growth stages of tuber initiation (TI), early tuber bulking (EB), and late tuber bulking (LB). Sub-lethal glyphosate doses applied to mother plants had the greatest effect on daughter tubers during TI, resulting in high tuber sets and increased unmarketable tubers. Glyphosate applied to mother plants resulted in residue in the daughter tubers. When these tubers were used as seed, glyphosate doses at EB and LB resulted in decreased progeny emergence and vigor. The presence of glyphosate residue in tubers used for seed was confirmed through an enzyme-linked immunosorbent assay. Precautions should be taken to avoid glyphosate contamination of ‘Red Norland’ mother plants to prevent unmarketable tubers and poor quality seed.

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DEDICATION

To my Grandfather, Joseph Senko

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CHAPTER 1. INTRODUCTION

Since the introduction of glyphosate in 1971 (Kafarski et al. 1988), and the commercial release of glyphosate-tolerant crops in 1996 (Green and Owen 2011), glyphosate has slowly gained momentum and today has become the most widely used pesticide in the United States (Fernandez-Cornejo and Osteen 2015). In 2015, glyphosate-tolerant soybean (*Glycine max* L.) and corn (*Zea mays* L.) represented 94 % and 89 % of total hectares, respectively, across the nation.

However, along with the production of glyphosate-tolerant crops, such as corn, soybean, canola (*Brassica napus* L.), and sugar beet (*Beta vulgaris* L.), the Red River Valley that follows the North Dakota-Minnesota border, is also an area conducive for successful potato (*Solanum tuberosum* L.) production. North Dakota is the third largest potato production state within the U.S. and on average produces 25 % of the red-skinned potato cultivars (National Potato Council 2015). The unique soil characteristics in the RRV are attributed to its creation and evolution after the retreat of the glacial ice. The combination of high organic matter, dark silty-clay-loam soils, diurnal temperature fluctuations, and adequate seasonal precipitation allows for successful, non-irrigated red-skinned potato production. Although there is a series of popular cultivars grown in the RRV, the Red Norland cultivar has the highest number of hectares planted. 'Red Norland' is grown for both the fresh market and certified seed production. Production of a high-valued potato crop in the same region as multiple glyphosate-tolerant crops introduces the threat of glyphosate drift onto susceptible potato plants.

This research evaluated the effect of glyphosate drift on Red Norland produced for both fresh market and certified seed. Many objectives were evaluated throughout this study by addressing the following questions. What plant growth stage is most sensitive to glyphosate?

What dose of glyphosate is needed to cause unmarketable tuber symptoms or make seed tubers unproductive? How does seed age affect glyphosate sensitivity? Characteristics measured for the fresh-market daughter-tubers included: tuber number per plant, unmarketable tubers, total yield, marketable yield, and foliar fresh height and weight. An enzyme-linked immunosorbent assay (ELISA) was used to detect glyphosate residue that accumulated in the daughter tubers from the field. Characteristics measured for certified seed plants and granddaughter tubers included: emergence, vigor, height, tuber number per plant, unmarketable tubers, total yield, and marketable yield.

Through this research, both fresh-market and certified seed growers of the Red Norland cultivar will start to understand how a glyphosate drift event on their field may impact their production.

CHAPTER 2. LITERATURE REVIEW

2.1. Glyphosate

The establishment of herbicide-tolerant crops in the 1990's caused a shift in herbicide use (Fernandez-Cornejo et al. 2014). Today, glyphosate has become the most widely used herbicide with the increase of glyphosate tolerant (GT) crops grown in the United States (Fernandez-Cornejo and Osteen). Glyphosate is a non-selective herbicide used to control annual and perennial weeds in minimum tillage cropping systems and GT crops. In 2000, Monsanto's patent on the formulation of glyphosate expired, and it became available for other pesticide companies to begin marketing generic glyphosate products (USDA 2012). This made the product more readily available to the consumer. New compounds, formulations and suggested application rates of glyphosate developed at this time also increased the consumer's support of glyphosate products.

2.1.1. Uses

Glyphosate is a versatile herbicide product. It is registered for summer fallow, pre-crop emergence and in-season weed control in GT crops such as soybean (*Glycine max* L.), canola (*Brassica napa* L.), cotton (*Gossypuim hirsutum* L.) and corn (*Zea mays* L.) (Anonymous, 2007). In addition, glyphosate is registered for desiccant use on glyphosate-susceptible crops such as barley (*Hordeum vulgare* L.), flax (*Linum usitatissimum* L.), lentils (*Lens culinaris* Medic.), peas (*Pisum arvense* L.), safflower (*Carthamus tinctorius* L.), sunflower (*Helianthus annuus* L.) and wheat (*Triticum aestivum* L.).

Adoption of GT crops in the United States altered herbicide use. The active ingredient glyphosate was applied more frequently, while the use of other, less effective and more toxic formulations were in decline. (Fernandez-Cornejo et al. 2014). The volume of glyphosate applied

in soybeans, corn, and cotton hectares increased in almost every year since 1996. In 2008, 43 million and 30 million kilograms of glyphosate were applied to soybeans, and corn acres, respectively. Glyphosate captured 50 % of herbicide use in the United States in 2008.

North Dakota farmers that raise GT crops apply glyphosate at least once per season, and many small grain crops are desiccated pre-harvest with glyphosate. As a result of the numerous application timings for glyphosate, the potential for drift can occur many times throughout the growing season. In 2012, 33 % of all herbicide-treated acres received an application of glyphosate in North Dakota (Zollinger et al. 2012).

2.1.2. Action and Fate

Glyphosate, N-(phosphonomethyl) glycine, is the active ingredient of Monsanto's herbicide Roundup WeatherMAX® (Pawel et al. 1988). The mode of action is inhibition of 5-enolpyruvyl shikimate 3-phosphate synthase (EPSP synthase), a key enzyme of the shikimate pathway (Steinrücken and Amrhein 1980). Glyphosate inhibits three essential aromatic amino acids within the plant tissue, phenylalanine, tryptophan, and tyrosine and results in the accumulation of shikimic acid.

Glyphosate is a foliar applied, systemic herbicide. Glyphosate is not metabolized inside the plant but is symplastically translocated within the plant and moves bi-directionally (Ross and Childs 1996). The movement of glyphosate in the plant follows the same pathway as nutrients, this movement is often referred to as a "source-to-sink" pattern. Once inside the plant tissue, glyphosate moves from the leaves, which is a source of sugar, to the sites of metabolic activity, which is the sink of sugar. The sites of metabolic activity are root meristems, shoot meristems or storage organisms such as tubers or seeds (Smid and Hiller 1981). Glyphosate primarily accumulates in the eyes of the tubers, which is the area of increased meristematic activity.

Glyphosate has a rapid absorption and translocation rate within the plant due to its relatively low molecular weight and high water solubility (Pawel et al. 1988). The amount of glyphosate translocated within the plant may vary based on the amount coming in contact with the plant, as well as the environmental conditions (Masiunas and Weller 1988). Absorption of glyphosate is greater at increased ambient temperature (Masiunas and Weller 1988), and is most rapid during the first two days after contact with glyphosate, accumulating to maximum levels after four to eight days (Smid and Hiller 1981).

Felix et al. (2011) were able to quantify its accumulation by using a shikimic acid assay test to confirm glyphosate levels for each treatment. Shikimic acid accumulation in potato plants increased with increasing glyphosate dosage. Plants sprayed at the hooking growth stage had the greatest amount of shikimic acid accumulation, triggered by the lowest glyphosate dose. Significantly greater amounts were needed to trigger the same response in the treatment timings of tuber initiation and tuber bulking. Results also suggested that less glyphosate was needed to reduce tuber yield compared to the amount needed to elicit visible injury to the crop. Tuber injury severity and shikimic acid accumulation increased together with increased glyphosate dose. They found that the greatest amount of injury to potato plants receiving a glyphosate dose of 54 g/ha or greater was during the early tuber development stages of hooking and tuber initiation.

Glyphosate binds tightly to the soil particles and is not available for uptake by roots, which provides minimum exposure or risk of damage to rotational crops (Duke et al. 2012) Aminomethylphosphonic acid (AMPA) is the initial degraded product of glyphosate within the soil, further degrading to natural occurring substances such as carbon dioxide and phosphate. Glyphosate is registered for both ground and aerial application in North Dakota.

2.1.3. Herbicide Drift

Herbicide drift is a risk for any non-target susceptible crop as it can cause plant damage, plant death, yield loss and financial burden. This concern is amplified when growing certified seed. Seed growers are responsible for supplying the industry with high quality seed to produce the crop volume required from year to year. Due to the non-selective qualities of glyphosate, most cultivated crops in North Dakota are not naturally tolerant to the herbicide, with the exception of GT crops. Potatoes are sensitive to glyphosate, and may be exposed to it in a number of different ways. Particle spray drift, tank contamination, misapplication, and inversions are events that could potentially cause glyphosate damage to the susceptible potato crop (Robinson and Hatterman-Valenti 2013).

Herbicide spray drift is the aerial movement of herbicide from the target area to areas where herbicide application is not intended (Dexter 1995). Herbicide drift can accumulate on the downwind side of a field, at the edge of a field, or in an adjacent field. Spray particle size plays a large role in the drift of herbicides. Spray drift can be reduced by increasing the size of the droplet. Droplet size can be adjusted by reducing spray pressure, increasing nozzle orifice size, using drift reduction nozzles, adding additives that increase spray viscosity, or adjusting to rearward nozzle orientation. A spray droplet with the diameter size of 5 microns produces a fog-like droplet that will take 66 minutes to fall 3 meters and has the capability to travel laterally up to 4.8 kilometers in a 4.8 km hr^{-1} wind speed. Comparatively a larger droplet diameter size of 1,000 microns, where a fine rain-like droplet will be produced, falls 3 meters in 1 second and only moves 1.43 meters in a 4.8 km hr^{-1} wind speed.

Glyphosate has low volatility, making the product less likely to evaporate from the target surface to move through the air to non-target plants after application, which means it is most

susceptible to particle drift (Dexter 1995). Environmental conditions other than wind can have an effect on drift. Low relative humidity and/or high temperature will cause rapid evaporation which in turn creates smaller spray droplets and increases the chance of drift to occur. Bird et al. (1996) reported that the only climatic parameters shown to affect drift are atmospheric stability and wind speed. Increasing wind speed from 2.5 to 5.0 m/s doubled the drift potential to off-site targets.

With any form of pesticide application, a small fraction of the product is destined to drift with the potential of depositing on off-target susceptible surfaces (Reddy, et al. 1996). When pesticides are applied aerially, due to convenience and adaption to environmental conditions, drift is more likely to occur. If an aerial drift event would occur, it would cause a rapid decline in surface deposition when the distance from the target site is increased. Approximately one-third of the drift loss occurred in the first 10-20 m downwind (Kirk 2000).

2.1.4. Injury Symptoms

Glyphosate damage on the plants' foliage first appears in the newest plant tissue (Penn State Extension 2013). The systemic properties of glyphosate enable the chemical to be translocated from the leaf surface to the growing points. The growing points will begin to show chlorosis before becoming necrotic and dying within a week or so after exposure. During the development of daughter tubers in a potato plant, the tubers become the strongest pull of assimilates absorbed into the plant, which causes an accumulation of glyphosate within the daughter tubers (Robinson and Hatterman-Valenti 2013). The cultivar Red Norland exhibits a determinate growth habit. Once the mother plant starts to initiate daughter tubers, foliar growth decreases, making the tuber the actively growing meristem tissue that accumulates the glyphosate. A glyphosate drift event occurring after tuber initiation will be near impossible to

visually detect by foliar damage, as typical foliar symptoms will not be expressed.   Injury can also be found on the daughter tubers of affected plants. Symptoms on the tubers may include superficial cracking of the skin, deep cracks into the potato flesh, malformed tubers, and necrotic tissue that could be followed by secondary pathogens. Daughter tubers produced for the fresh table-market may become unmarketable due to visual defects with daughter tubers being produced for certified seed, a glyphosate drift will potentially cause emergence and growth problems the following season.

2.2. Red Norland

The Norland potato cultivar was developed by North Dakota State University and released in 1957 (NDSU 2012). The parentage of the hybrid cross was ND626 x ‘Redkote’. It was developed for table stock utilization. In 1965, Nebraska selected the more popular sport called ‘Red Norland’ (Crop Watch 2014). Red Norland was an improved cultivar due to the retention of the dark red skin color throughout maturity and storage.

2.2.1. Growth Characteristics

Red Norland is an early maturing cultivar that is adapted to the cooler northern climates of the U.S. and Canada, grown for the fresh table-stock market (Anonymous 2013). It is a short season cultivar, maturing in 70-90 days from planting, combined with a short dormancy period making it ideal for the fresh market (Crop Watch 2014). Plant characteristics are rapid emergence, determinate growth habit, and variable tuber set.

Growth of a potato plant occurs in many different stages (Dwelle and Love 2003). If ideal conditions are present, the first stage that occurs after planting is sprout development. This is the stage when the eyes on the seed piece begin to develop into sprouts that will emerge from the soil to become the stems. Eyes on the tuber develop in a spiral phyllotaxis arrangement, with a

concentration at the apical end of the tuber (Spooner 2010). The eyes that express apical dominance will be the first to sprout. This tendency is reduced when a larger seed piece is cut into smaller seed pieces. There is potential for a difference in emergence rate between an apical end and a stem end seed piece. Roots will also begin to form at the base of the emerging sprouts (Dwelle and Love 2003). Once the sprout has emerged, photosynthesis and vegetative growth commences, leaves and branches develop from above-ground nodes, while roots and stolons develop from the below-ground nodes. Tuber initiation begins when tubers begin to form at the ends of the developed stolons, and this stage may be influenced by plant stress. The tubers are present but are not yet enlarging. This stage often is coupled with early flowering in most cultivars but the relationship is not related. After tuber formation, tubers begin to bulk in size by an expansion of cells with the accumulation of water, nutrients, and carbohydrates. The tubers become the dominant site of nutrient deposit. Nearing the end of the season, the mature plant will begin to naturally senesce. The dry matter content within the tuber reaches the maximum quantity and the skin sets.

Dormancy length for most common potato cultivars is a period of 60 to 130 days, depending on holding temperature in storage (Kleinkopf 2010). Dormancy is the regulation of bud development on the tubers, and if the dormancy is broken, the buds begin to sprout (Bussan and Olsen 2010). Due to the short-term dormancy of the Red Norland cultivar, it may be susceptible to storage problems that physiologically age the seed. Physiological age is different than chronological age, in that it is influenced by growing conditions, handling of the tubers, storage and cutting procedures of the tubers (Bohl et al. 2000). When seed is physiologically aged, it alters the potential growth characteristics such that the seed piece exhibits rapid

emergence, increases in stem number and tuber number and can develop a smaller, ununiformed tuber profile.

2.2.2. Production Management

Growers typically manage growth disorders in 'Red Norland' production by planting at a smaller within row spacing to influence uniformity in tubers and optimize yield with the short season (Crop Watch 2014). 'Red Norland' resists most internal and external defects under ideal environmental conditions; however, under stressful environmental conditions or poor management factors, growth cracks may occur (Thornton 2010). Growth cracks can be influenced by seed and row spacing. Producing excessively large tubers increases the chance of growth cracks developing. Water volume and distribution also plays a large role. This disorder can be managed in irrigated fields, but if grown under dry land conditions, rainfall cannot be controlled. The fluctuation of water availability can cause growth cracks and other undesirable market qualities.

Poor water management in the form of timing, volume and distribution, can directly influence the crop quality and yield (Shock 2010). Although the plant requires adequate water throughout the entire season, tuber initiation and tuber bulking are the most important plant growth stages. Monitoring the evapotranspiration rate based on temperature and weather conditions is necessary to properly manage crop water requirements. When natural rainfall is the only source of water, field selection must be taken into consideration. Soil characteristics, field topography, and cultivation practices must be selected appropriately.

A feature of 'Red Norland' is the ability to adapt well to different soil types and different climates (Anonymous 2013). A field location that has a silty clay loam soil profile has specific water capacities (Tomasiewicz et al. 2003). At the Northern Plains Potato Growers Association

research farm site south of Grand Forks, ND, the volumetric water content for field capacity is between 30-35 %, with a permanent wilting point approximately 15 %, which provides 20 % available water for the crop. Due to the location's heavier soil profile, the water may stay available for the plants for several days due to the field capacity limits. The field has the water holding capacity of 120-180 mm m^{-1}.

2.2.3. Commercial Production

'Red Norland' potatoes grown for the fresh market have different marketability and quality specifications compared to potato cultivars grown for the frozen or chip market. The consumer purchases fresh market potatoes based on physical appearance. This is why the USDA has standards in place to ensure the consumer receives the highest quality products. The USDA fresh products branch released an Official Visual Aids for Potatoes in May of 1998 that is still used to determine marketable quality (USDA 1998). Basic requirement categories include firmness, cleanness, shape, skinning, and freedom from defects. This basic list is followed by an extensive index of disease, insect damage, and physiological disorders that the tubers may express. Sixty-nine factors are taken into consideration when grading for the fresh market, which can lead to many circumstances that would reduce the grade or reject the sample. An additional USDA document entitled United States Standards for Grades of Potatoes, supports the three different grade types, U.S. No. 1, U.S. Commercial, and U.S. No. 2 (USDA 2011). U.S. No. 1 is the most desirable grade, and tubers in this grade would exhibit normal varietal characteristics, be well-shaped, firm, and clean.

2.2.4. Seed Production

Uniform commercial potato production is achieved through vegetative-propagated plant material. This process has a unique set of problems in maintaining varietal purity and the

management of seed-borne diseases (Sieczka et al. 2003). In 1914, seed potato certification was first discussed at the Potato Association of America's annual meeting; and in 1920, 12 states and all Canadian provinces were engaged in the program. Seed lots were given a limited number of generations permitted due to the plants' constant exposure to the possibility of contamination by disease-causing pathogens. The number of years the seed lots were limited to varied from five to nine years depending on the seed production area. Each state is self-regulated and the specifications of the certified seed program may differ. This practice is implemented to protect the potato industry, seed and commercial, from disease epidemics. The North Dakota State Seed Department was established in 1931 and works in conjunction with a variety of affiliates within the state (NDSSD 2008).

North Dakota has the second largest seed potato production program in the United States, inspecting and certifying an average of 15,000 to 20,000 acres of an assortment of varieties each year. A seed potato grower is subjected to numerous inspections, testing and strict certification tolerances prior to being an approved certified seed grower. State seed department inspectors inspect fields a minimum of three times during the growing season. The growers are responsible for the control of insects and weeds, and are also encouraged to rouge in an effort to remove diseased or virus-infected plants to inhibit the spread of infection. A small number of diseases are inspected at each generation and many have zero tolerance standards. Other factors involved with the field condition can lead to disqualification of certification such as excess weeds, hail, and chemical damage that can interfere with the inspections.

Today there are no specific regulations when it comes to glyphosate damage, nor any seed testing requirements, however some research on the effects of glyphosate on certified seed potatoes has been completed in North Dakota as well as the western potato producing states.

Hatterman-Valenti (2014) found that the red-skinned cultivar Red LaSoda expressed sprout inhibition when exposed to sub-lethal drift amounts of 71 g ae ha^{-1} or higher during the late bulking growth stage. Even the smallest dose of 18 g ae ha^{-1} showed a difference when compared to the untreated control. The seed tubers were described as having numerous sprouts emerging from a single eye in a cauliflower-curd fashion. Seed tubers that had received the highest amount of glyphosate did not have any sprout emergence throughout the growing season. Other symptoms noted were delayed emergence with distorted leaflets; however, the symptoms varied widely and did not appear to be affiliated with a specific glyphosate dose. A reduction in yield was associated with fewer and smaller tubers. The low dose of 71 and high dose of 282 g ae ha^{-1} had a yield decrease of 48 and 58 %, respectively, when compared to the untreated control. When administering the same treatments on a Russet Burbank the glyphosate dose of 141 g ae ha^{-1} expressed the highest sprout inhibition, while the lowest dose of 18 g ae ha^{-1} was similar to the untreated control. Weather played a large role in the yearly differences of yield ratings of the daughter tuber plants, where even the lowest glyphosate dose showed significant differences to the untreated.

Hatterman-Valenti (2014) stated, "differences in observed plant responses were attributed to differences in the amount of glyphosate entering a daughter tuber due to source-sink differences for tubers within an individual mother plant treated with glyphosate". This statement implied that each individual tuber forming from the same mother plant may accumulate different levels of glyphosate due to the location on the stem and that not every daughter tuber would react similarly when used as seed.

A similar study that evaluated sub-lethal glyphosate doses was completed in the western states of ID, OR, and WA on Ranger Russet (Hutchinson et al. 2014). Plants exposed to a

glyphosate drift occurring early in the season had a better chance to recover even at a higher dose. A grower would be encouraged to maintain the crop if a glyphosate drift event was suspected prior to tuber initiation. Plants exposed to a glyphosate drift event occurring once tubers began to initiate, may not be able to recover, and would potentially result in a decrease in yield up to 40 %, accompanied with lower crop quality.

2.3. Glyphosate Residue Analysis

Correctly identifying a foreign substance present in potatoes, as a producer or processor, assists in making the best decision for the operation and the consumer. Potatoes are not a heavily processed food, in that the tubers harvested from the field are the same tubers that end up on the consumer's plate. Being able to successfully obtain the right information regarding the product's quality will insure that we can protect our food industry. The EPA specifies a tolerance of 0.2 ppm (0.0002 mg/mL) of glyphosate within potato tubers (U.S. EPA 2012), because the function of glyphosate is the inhibition of a set of essential aromatic amino acids within a plant, mammals are considered not to be at immediate or direct risk of the herbicide because they do not generate these specific amino acids (Borggard and Gimging 2008). The popularity of glyphosate in the United States is not only within the agricultural sector, but also used extensively for home and garden, industrial, commercial and government weed control applications, totaling over 113 million kg used in all sectors in 2013 (U.S. GS 2013). With the high amount of pesticides being used, a reliable analysis for detecting residue within plant tissue should be readily available for producers at a cost-effective price.

Robinson and Hatterman-Valenti (2013) discussed the effects of glyphosate residue in seed potato pieces. Since a systemic herbicide such as glyphosate that is extensively translocated symplastically, one would expect herbicide residues to accumulate within the daughter tuber of

an exposed mother plant. These daughter tubers that are used as seed tubers may not display physical damage, yet still exhibit germination problems when planted. The issue may become even more complicated when the mother plant is exposed to low glyphosate concentrations post tuber initiation, at the bulking or senescing plant growth stages as mother plants may not exhibit any obvious signs of injury on the plant foliage. It was found that a high level of glyphosate residue in the seed can inhibit sprout growth and cause a 'cauliflower' formation of shoots at the eyes. Moderate levels may cause erratic and slow emergence, enlarged shoots, and multiple shoots coming from a single eye. Low levels may cause a weakening in the plant, swelling of shoots, and abnormal root growth.

Utilizing the appropriate tool for detection of glyphosate residue is important now that the effect of glyphosate on the performance of the seed is understood. Potato tubers have high water content, approximately 80 % (Bastin and Henken 1997), which differs from a soybean or wheat seed that is less than 15 % at harvest and needs to be taken into consideration when selecting the appropriate method of analysis. The analysis of glyphosate in plant substances is problematic due to its small molecular size and structure (Rubio et al. 2014). In addition, the glyphosate molecule is similar to many other naturally occurring plant compounds, such as amino acids and secondary plant metabolites. The extraction of glyphosate with solvents is difficult due to its high solubility in water. Finding the appropriate method to isolate and quantify glyphosate has been a challenge to the analytical chemist. Gas (GC) and liquid (HPLC) chromatographic techniques as well as enzyme-linked immunosorbent assay (ELISA) require derivatization of the product for separation (Stalikas and Konidari 2001). In order to achieve successful and reproducible results, the most critical step is for a proper derivatization method to be executed.

A variety of methods have been utilized over the last 20 years to detect different pesticide residues (Stalikas and Konidari 2001). Most laboratories are equipped with the tools and instruments required for chromatographic methods. The new approach of utilizing the ELISA technique has been recognized as a valuable tool in residue analysis. ELISA is efficient, less expensive and less time-consuming than HPLC or GC methods. Results of glyphosate detection using ELISA are equivalent when compared to HPLC. According to Stalikas and Konidari (2001) the limiting factors of ELISA for the detection of glyphosate residue is the tedious polyclonal antisera production as well as the cross-reactivity to AMPA and glyphosphine. A study done in Ontario, Canada, provided evidence that ELISA was more accurate than LC-MS based on the average recovery percentage and the correlation coefficient, when testing for glyphosate contamination in drinking water (Parmar and Lo 2016).

The ELISA technique has been used to successfully detect glyphosate in a number of substances; water, honey, corn, soy products, human and animal excrement (Abraxis 2015). The Pennsylvania based company Abraxis LLC. has provided lab-based testing solutions for pesticides and other substrates. They modified the application of ELISA for a water sample to unique food-based applications such as potato. With the ability to detect limits from 0.075 – 4.0 ppb and an average recovery of 123 %.

2.4. Red River Valley

Two field locations were used throughout this project. One location was 5 miles south of Grand Forks, ND at the Northern Plains Potato Growers Association (NPPGA) Research Farm and the second was at the North Dakota State University (NDSU) Research Farm located in Fargo, ND. Both locations are uniquely situated in the Red River Valley (RRV). The RRV is known historically as a remnant of where the historical Glacial Lake Agassiz once covered

440,000 km^2 (Schwert 2003). The NPPGA research farm is non-irrigated with silty clay loam soil. The location soil conditions are typical growing conditions for red-skinned potato cultivars in the RRV. The NDSU research site is non-irrigated with poor drainage qualities and soil mainly composed of silty clay.

2.4.1. History

The RRV is the youngest major land surface in the contiguous United States (Schwert 2003). It was first exposed 9,200 years ago after the drainage of the Glacial Lake Agassiz, making the Red River of the North only a few thousand years old compared to most river systems in the United States. Prior to the RRV being exposed, a vast lake stretched across the heart of North America (Hoffman 1979). Shorelines and a nearly flat valley that followed the Minnesota-North Dakota border north into Manitoba was what remained when the ancient Lake Agassiz drained. The RRV's fertile and productive soil is attributed to the sediments that were dispersed by the Glacial Lake Agassiz. It has been deemed as one of the most fertile and productive valleys on earth.

2.4.2. Soil Composition

Most of the soils in the RRV are Aquolls, which is a suborder of the class Mollisols (CEI 1998; USDA 2016). Mollisols are across the Great Plains and as far as the Western States (USDA 2016). Most of the Mollisols soil have supported grass vegetation or been forested at some time. Aquolls are classified as a wet Mollisols, and are broadly distributed around the glaciated areas of the Midwestern States. As the Red River of the North narrowed in width it deposited heavy layers of fine silt (Hoffman 1979). These alluvium deposits range in depth from 1.5- 18 m and are layered over a striation of silt, sand, clay and gravel known as glacial till.

2.4.3. Land Use

The valley is 515 kilometers long and approximately 563 kilometers wide. The elevation is nearly level from the south starting at 300 m above sea level and decreases gradually to 200 m above sea level towards the north (CEI 1998). The flat plain combined with the rich soil makes the RRV prime land for cultivating crops like potato, sugarbeets, onions, sunflowers, corn, grains and grasses (Hoffman 1979). Nearly all of the RRV is in farm land or ranches, with 80 % of cropland being dry-land farmed (CEI 1998). The soil is somewhat poorly drained to poorly drained, therefore in some places drainage systems are implemented.

The potato crop was the first vegetable crop to be grown in the RRV in 1801 (Kenney 1995). The commercial potato industry began over 100 years later near the small town of Hoople, ND. In 1946 an organization was created to unite the RRV's potato industry, named The Northern Plains Potato Growers Association (NPPGA 2016). The priorities of the association were to develop research and promotion opportunities to the members involved. After the inception of the NPPGA, the success of the potato industry grew in the RRV, not only raising russet-type cultivars for processing, but also cultivars for the fresh market and the chip market as well.

2.4.4. Climate

The growing season in the RRV is estimated by averaging the number of frost-free days (USDA 1997) and can range between 105 to 135 days depending on the year (CEI 1998). The short season requirements of Red Norland make it a very successful cultivar in the RRV. Weather data retrieved from the North Dakota Agriculture Weather Network (NDAWN) website provides precipitation information dating back to 1981 and air temperature from 1990 (NDSU 2016). The precipitation on the eastern side of North Dakota has historically been higher than the

western side of the state. Historical average precipitation in Grand Forks during the potato growing season of May to September registered at 68, 88, 80, 73, and 52 mm, respectively, with Fargo recording 71, 99, 71, 65, and 65 mm. Rain tapering off into September helps the crop naturally senesce. Both field research locations benefit from the adequate annual precipitation without the excess financial cost of irrigation requirements.

Temperature requirements for a potato plant are very unique in that they are classified as a short day, cool season crop (Rosen 2010). Temperature is a factor that can shift the balance between vine and tuber growth (Dwelle and Love 2003). For the standard processing cultivar, Russet Burbank, the optimum soil temperature for tuber growth is approximately 16° C, while the ideal air temperature for vine growth is 25 °C. High soil temperatures combined with high air temperature can inhibit or delay tuber growth. Heat units for potatoes are measured by Physiological-Days (P-days) and are calculated based on minimum and maximum temperatures for the specific crop (MAFRI 2016). The minimum and maximum temperatures for potato growth and development are 7 and 30 °C, respectively. The potato plant is most productive when exposed to warm day temperatures combined with cool night temperatures (Bains et al. 2003). Cool night temperatures are important for tuber production because air temperature influences the accumulation of carbohydrates and dry matter in the tubers. When night temperatures are low, respiration of the plant is decreased, which enhances the assimilation of starch into the tuber.

The northern location offers conducive climatic conditions for successful potato production in such a short season when compared to other potato production regions in the U.S. The warm day-time temperatures with the cooler night time temperatures meet the physiological requirements of the plant. The monthly average day temperatures from May to September in

Grand Forks are 20, 24, 27, 26, and 21 °C, while the average night time temperatures are 5.5, 11, 13, 12, and 6.6 °C, similar to Fargo day temperatures at 21, 25, 28, 27, and 22° C coupled with night temperature at 7, 13, 15, 14, and 9 °C (NDSU 2016).

2.4.5. Weather 2014-2015

The weather between the two field experiment years in Grand Forks, ND was not consistent. (NDSU 2016). Precipitation was heavier in 2014, with a total of 347.5 mm over the months of May to September, with June having the majority of the accumulation, compared to 308 mm in 2015, with precipitation evenly distributed throughout the months. There were no major differences between the maximum or minimum air temperatures between both years, however there was a difference in the p-days accumulated. In 2015, 824 p-days accumulated, while 2014 accumulated less, registering 707 p-days.

The Fargo field experiment was only conducted during the 2015 growing season. Precipitation from May to September recorded a total of 429.8 mm, 58 mm above the historical average. The heaviest month of precipitation was May, which delayed the planting of the Fargo field plots into mid-June. Accumulated p-days totaled 631, which was 178 less than the total season (809) due to the late planting date.

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CHAPTER 3. SIMULATED GLYPHOSATE DRIFT EFFECTS ON 'RED NORLAND' MARKETABILITY: FIELD EXPERIMENT

3.1. Abstract

The fresh market potato (Solanum tuberosum L.) is at the mercy of the critical consumer, being judged for any imperfection. This means producers must attempt to produce a flawless product. For 'Red Norland', a smooth, red-skin cultivar, glyphosate drift from a neighboring field could result in unmarketable tuber characteristics. Field experiments were conducted near Grand Forks, North Dakota in 2014 and 2015 to evaluate the effects of glyphosate drift on 'Red Norland' potato grown for the fresh market. Sub-lethal glyphosate doses of 1/4, 1/8, 1/16, and 1/32 the recommended rate (840 g a.e. ha^{-1}) were applied at the growth stages of tuber initiation (TI), early tuber bulking (EB), and late tuber bulking (LB).

Sub-lethal glyphosate doses applied to plants at TI caused chlorosis at the growing point and decreased foliar fresh weight and height but had no physical plant effect when applied to plants at EB or LB due to the determinate growth habit of 'Red Norland'. Sub-lethal glyphosate doses applied to plants at TI caused an increase of tuber number per plant and a decrease of yield compared to applications to plants at EB and LB stages. Unmarketable tubers increased five-fold when sub-lethal glyphosate doses were applied at the TI stage. Glyphosate doses applied to plants at EB caused a 19 % yield reduction when compared to applications to plants at LB. Late tuber bulking expressed no sensitivity to the sub-lethal glyphosate doses. A poor correlation between foliar and tuber effects from the sub-lethal glyphosate doses suggested that above-ground symptoms can't be relied upon to positively diagnose glyphosate drift damage.

3.2. Introduction

Potato (*Solanum tuberosum* L.) grown for the fresh market is required to meet certain quality specification levels governed by the USDA. Any defect, internal or external, will decrease the grade and marketability of the product for the end consumer. Potato is a nutritional vegetable option for the consumer, as they possess more potassium than a banana, are an excellent source of vitamin C as well as B6 and iron. A medium-sized baked potato has only 110 calories and is fat, sodium, and cholesterol free (USPB 2016). In 2014, 15.6 kg of potato classified for the fresh market was consumed per capita. Although this number has declined over the past 10 years by 4 kg, the potato remains the most commonly consumed vegetable in the U.S (National Potato Council 2015; USDA 2015). A number of parameters are considered by a consumer before a fresh potato purchase is made, including: convenience, preparation, nutrition, packaging, sensory appeal, price, familiarity, habit, sustainability, and ethics (Fernqvist et al. 2015). Appearance attributes were mainly related to discoloration or blemishes on the potato skin.

The objective of this study was to determine the effects of exposing ‘Red Norland’, grown for the fresh market, to sub-lethal glyphosate doses. Characteristics such as in-season plant response, yield results and potato marketability traits were evaluated throughout the growing season. Combinations of sub-lethal glyphosate doses and different growth stages crucial to the production of daughter tubers were used to examine potential injury throughout the growing season.

3.3. Materials and Methods

3.3.1. Experimental design and preparation

Field experiments were conducted in 2014-2015 at the Northern Plains Potato Growers Association (NPPGA) Research Farm, located five miles south of Grand Forks, North Dakota. The site is non-irrigated, with silty clay loam soil designated by the USDA Web Soil Survey as having poor drainage qualities. The experiment was set up as a randomized complete block design with a two-factor arrangement and three replicates in both years. Glyphosate treatments were 210, 105, 52.5, 26.5, and 0 g a.e. ha^{-1}. The doses are fractions (1/4, 1/8, 1/16, 1/32) of the standard field use rate of 840 g a.e. ha^{-1}. Plant growth stage (PGS) treatments were selected on the importance to the development of daughter-tubers, tuber initiation (TI), early tuber bulking (EB), and late tuber bulking (LB). Certified Red Norland seed potatoes were cut into 70 g ± 5 g seed pieces, and stored for two weeks at 12.7 ^{0}C with approximately 90 % relative humidity to induce suberization and seed conditioning prior to planting.

Individual experimental units (EU) were 6.1 m long by 3.7 m wide. Seed were planted in four rows at 30 cm spacing and 90 cm between rows with a seed depth of 10 cm, using approximately 80 seed pieces per EU. Each EU was separated by 1.5 m of a russet variety for easy identification. Planting was completed on June 9 and 12, in 2014, and 2015, respectively. Standard North Dakota potato production practices was applied throughout the growing season. Prior to planting, the experimental site was fertilized and cultivated. In-furrow insecticide and liquid starter fertilizer were applied during the planting procedure. Granular nitrogen was broadcasted prior to sprout emergence, and incorporated with the hilling procedure. Post-emergence herbicide was applied for the second flush of broadleaf and grassy weed pressure.

Applications of fungicide and insecticide, based on pest presence, were made throughout the growing season.

3.3.2. Application of treatments

Treatments were applied in progression from lowest to highest dose to the two middle rows to mitigate cross contamination of EU's. Application of glyphosate was completed with a CO_2 backpack sprayer equipped with a 1.8 m boom and either four 8002 or 11002, flat fan nozzles 45 cm apart at 275 kPa and an output of 187 L ha^{-1}. Environmental conditions during treatment application were regulated by standards of < 16 km hr^{-1} wind speed, no foliar dew present, and no precipitation expected for 24 hours after the last application. Glyphosate doses applied in 2014 were 43, 64, and 72 days after planting (DAP), and in 2015 were 49, 60, and 67 DAP, for TI, EB, and LB, respectively.

3.3.3. Evaluation of treatments

Two plants were randomly selected out of each EU, manually harvested and evaluated five times throughout the season, at 1, 3, 7, and 14 days after application (DAA), as well as at the end of the growing season, 93 DAP in 2014, and 90 DAP in 2015. To reduce variance of plant-to-plant interaction, plants were never selected from the first or last position in the EU, nor next to an already harvested plant. Data collected within the season included: foliar damage observations, number of stems, and number of tubers, unmarketable tubers, and yield. Data collected at the end of the season included the above-mentioned variables, along with foliar fresh weight and plant height above soil surface. An unmarketable tuber was classified as a tuber expressing any of the following symptoms: cracks deep into the tuber flesh, malformation, discoloration or heavy russeting of the skin and tissue death (Robinson and Hatterman-Valenti 2013).

3.3.4. Data analysis

Data from 2014 and 2015 were combined after testing homogeneity of variance confirmed variance ratio differed by less than 10, using the ten-fold f-test method, which proportions the largest variance of the treatment groups to the smallest and compares it to a critical value table (Tabachnik and Fidell 2001). The data collected were analyzed using PC SAS 9.3 (Statistical Analysis Software, version 9.3. SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513). Regression coefficients were estimated for tuber number, yield and unmarketable tuber number as a function of harvest time. Environments were considered random and treatments fixed. Differences were interrupted at $P < 0.05$ for all characteristics measured.

Due to the unbalanced evaluation times within each PGS and across each PGS, tuber number, yield, and unmarketable tubers per plant across harvest times were not analyzed using linear regression. To be able to analyze the data across harvest times throughout the growing season, five even intervals would have had to occur to avoid erroneous linear regression results.

3.4. Results

3.4.1. Foliar Measurements

3.4.1.1. Foliar fresh weight

Foliar fresh weight was affected ($P < 0.05$) by sub-lethal glyphosate doses. During field observations, plants that received sub-lethal glyphosate doses earlier in the season expressed stunting of growth, and the chlorosis/necrosis of the foliar plant parts increased with the increase of glyphosate. The linear relationship of total foliar fresh weight and sub-lethal glyphosate dose had an R^2 value of 0.711 (Figure 3.1.).

The untreated control and glyphosate doses of 26.5, 52.5, 105, 210 g a.e. ha^{-1} measured a mean fresh weight of 0.63, 0.55, 0.53, 0.51, and 0.49 kg, respectively. As glyphosate dose

increased at 50 g a.e. ha^{-1} increments, the fresh weight of the foliar plant parts decreased by 0.03 kg.

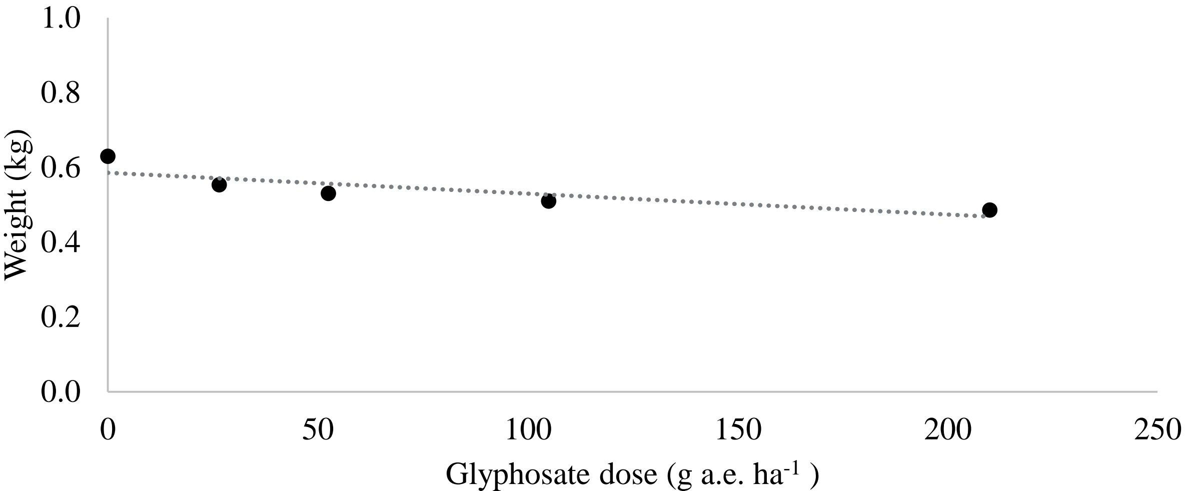

Figure 3.1. Foliar fresh weight as a result of sub-lethal glyphosate doses, Grand Forks, ND, combined over PGS (Tuber initiation, early tuber bulking, and late tuber bulking) and years (2014 and 2015).
Logistic regression for glyphosate dose: Y = -0.0006x + 0.5856 (R2 = 0.711).

3.4.1.2. Foliar plant height

Above-ground plant height was affected ($P < 0.05$) by simulated glyphosate drift applied at different plant growth stages (Figure 3.2.). Plants had a 1.48 cm height increase when the sub-lethal glyphosate dose was applied at the subsequent growth stage.

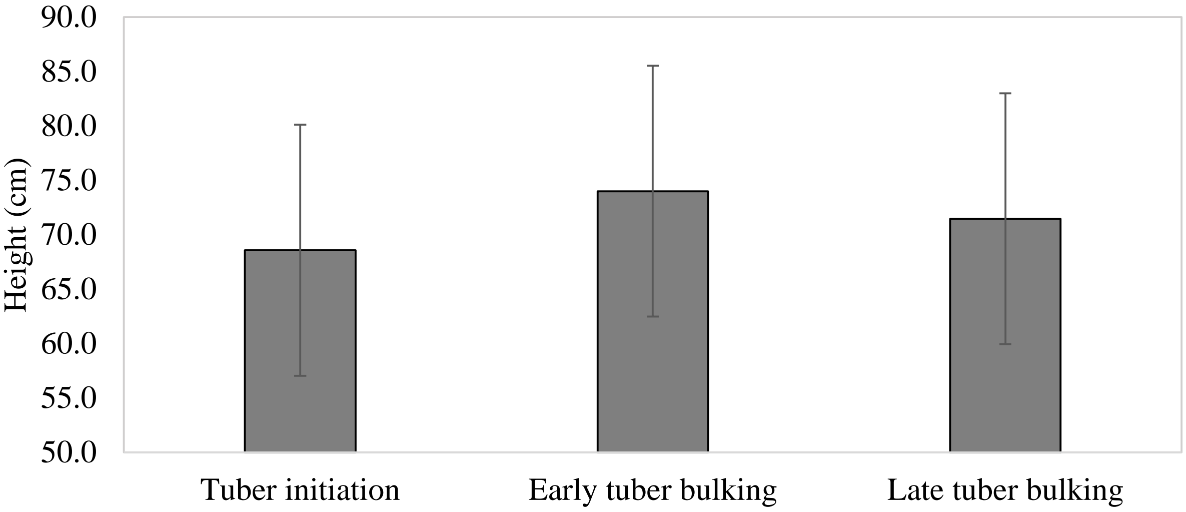

Figure 3.2. Foliar plant height as a result of glyphosate drift occurring at three different growth stages crucial to tuber production, combined over years (2014 and 2015), and glyphosate dose (0, 26.5, 52.5, 105, and 210 g a.e. ha^{-1}).

3.4.1.3. Stem number

Average stem number was affected ($P < 0.05$) by simulated glyphosate drift applied at different plant growth stages (Figure 3.3.). The differences in stem number at the growth stages didn't follow an anticipated trend and were most likely due to the establishment of all mature stems prior to the first glyphosate application during TI. Stems initiated from the seed piece are influenced by the physiological age and the apical dominance of the seed piece, therefore to impact the stem number; the stressor would most likely need to occur before the seed piece was planted.

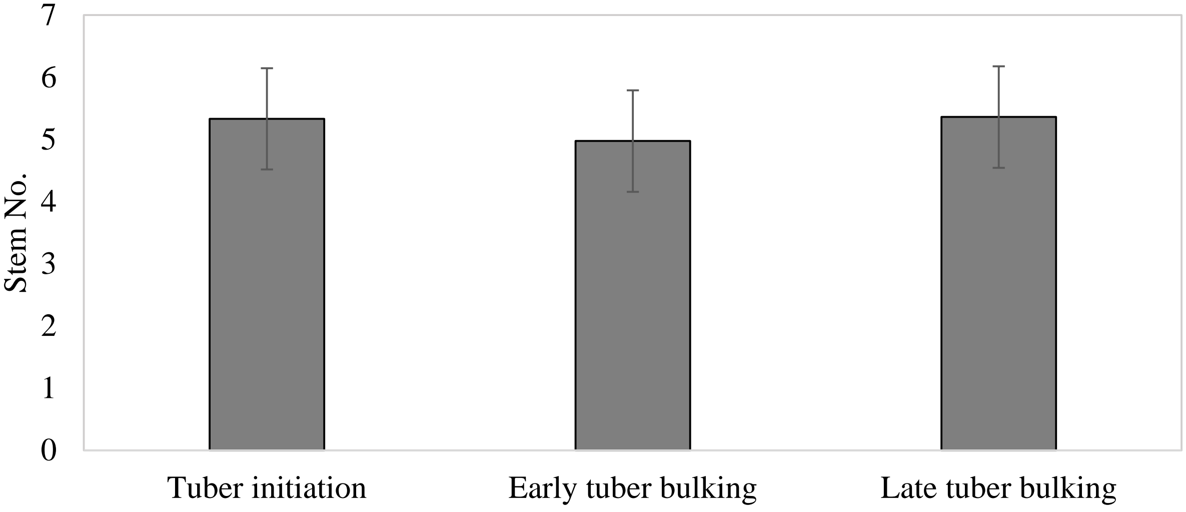

Figure 3.3. Stem number as a result of glyphosate drift occurring at three different growth stages crucial to tuber production, combined over years (2014 and 2015), harvest times (1, 3, 7, 14 days after application, and end of season) and glyphosate dose (0, 26.5, 52.5, 105, and 210 g a.e. ha^{-1}).

3.4.2. Tuber Measurements

3.4.2.1. Tuber number

Knowles and Knowles (2006) showed that tuber number was directly related to stem number produced by the seed piece, and as stem number increased, the potential number of tubers also increased. 'Red Norland' has been reported to produce a variable tuber set from six to 12 tubers (Crop Watch 2014), however 'Red Norland' in the Red River Valley often has an average tuber set of five to eight per plant. Tuber number per plant was significant ($P < 0.05$) for interactions: PGS x Glyphosate dose, PGS x Time, Glyphosate dose x Time, and the three-way interaction PGS x Glyphosate dose x Time.

Average tuber number per plant was affected by sub-lethal glyphosate doses applied at specific plant growth stages across five harvest times. This three-way interaction showed that glyphosate doses applied at TI increased tuber number per plant at each harvest time throughout the season, but have no effect on plant growth stages EB and LB (data not shown).

Average tuber number per plant was affected by sub-lethal glyphosate doses applied at specific plant growth stages. Tuber initiation was the most sensitive stage for average tuber number per plant (Figure 3.4.). The linear relationship of glyphosate dose applied at TI had an R^2 value of 0.362. As glyphosate dose increased by 50 g a.e. ha^{-1}, the number of tubers also increased by 0.5, this increase may be due to the infancy of the TI stage. Different plant stressors at this specific plant growth stage can easily manipulate the number of tubers initiated. For example, water stress near tuber initiation will cause a reduction of tubers initiated (MacKerron and Jefferies 1986).

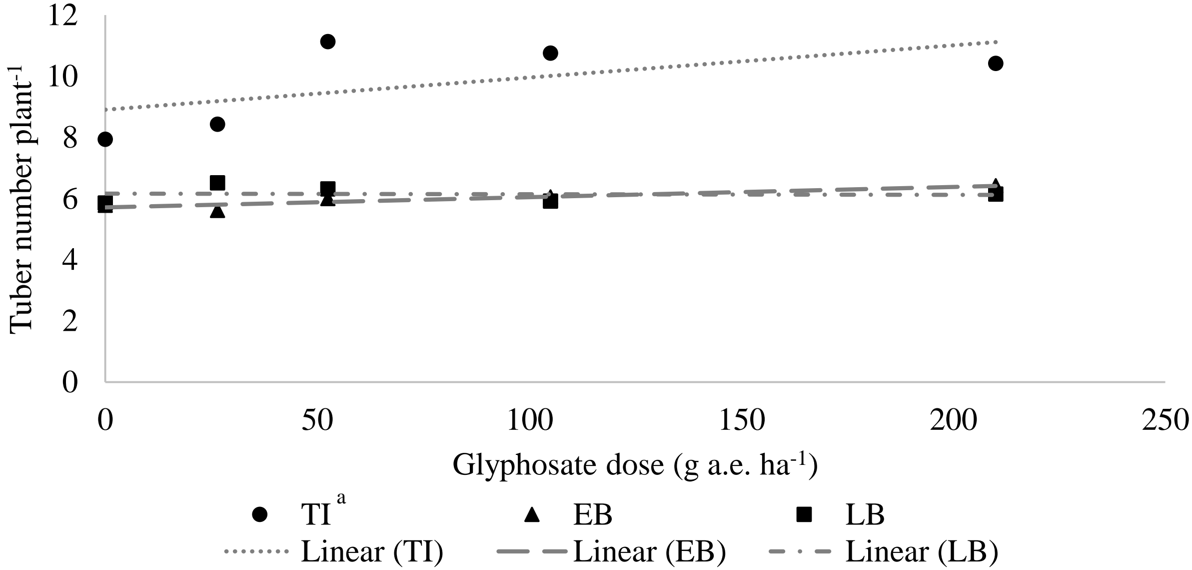

Figure 3.4. Average tuber number per plant as a result of sub-lethal glyphosate dose and plant growth stage, Grand Forks, ND, combined over years (2014 and 2015) and harvest times (1, 3, 7, 14 days after application, and end of season).
Logistic regression for TI: $Y = 0.0105x + 8.9054$ ($R^2 = 0.3623$). Logistic regression for EB: $Y = 0.0034x + 5.7069$ ($R^2 = 0.859$). Logistic regression for LB: $Y = -0.0002x + 6.1673$ ($R^2 = 0.0043$).
[a] TI, EB, LB = tuber initiation, early tuber bulking, and late tuber bulking, respectively.

Pooled across plant growth stages, the untreated control and glyphosate doses of 26.5, 52.5, 105, 210 g a.e. ha^{-1} applied to 'Red Norland' had tubers per plant of 6.5, 6.9, 7.8, 7.6, and 7.7, respectively. The increase of tuber number compared to the untreated plateaued at 52.5 g a.e. ha^{-1} glyphosate with similar tuber numbers when plants received 105 or 210 g a.e. ha^{-1}

glyphosate. Observations suggest that tubers that existed prior to the sub-lethal glyphosate doses > 52.5 g a.e. ha^{-1} to plants at TI, ceased growth, which may have caused a second set of tubers to initiate resulting in the higher number of tubers set per plant. Average tuber number was not affected to the same degree at the two later plant growth stages. Glyphosate doses applied to plants at the EB stage had a R^2 value of 0.859, which is a strong relationship between tuber number and the sub-lethal glyphosate doses. There was a slight gradual increase in tuber number, when glyphosate doses were applied during EB, as the glyphosate dose increased; however, the number never exceeded the cultivar average for the untreated. Late tuber bulking growth stage had a R^2 value of 0.004, which represents a poor relationship with the sub-lethal glyphosate dose. There was not a consistent increase of tuber number as dose increased, instead a variable pattern between 5 and 7 tubers per plant occurred. When the number of tubers per plant was increased, it caused the plant to produce a smaller tuber profile, which may influence market class.

When tuber number was evaluated across the sub-lethal glyphosate doses and the five different harvest timings, tuber number increase did not occur until 14 DAA (Figure 3.5.). In general, the slope of the line (tuber number by glyphosate dose) increased as the interval between the glyphosate application and evaluations increased. The final harvest R^2 value of 0.695, represents a moderately strong relationship between tuber number per plant and the simulated sub-lethal glyphosate dose. The R^2 value at 14 DAA was comparable to the last harvest, suggesting that plant response to sub-lethal glyphosate dose in terms of tuber number occurred by 14 DAA.

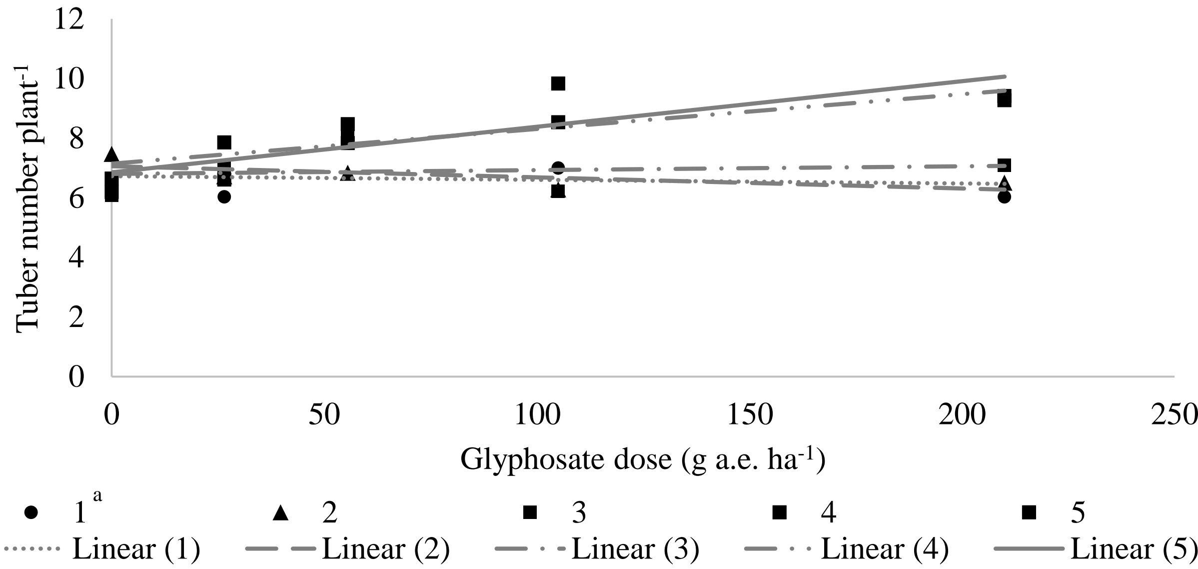

Figure 3.5. Average tuber number per plant as a result of sub-lethal glyphosate doses at different harvest times, Grand Forks, ND, combined over plant growth stages (tuber initiation, early tuber bulking, late tuber bulking) and years (2014 and 2015).
Logistic regression for 1: Y = -0.0013x + 6.7274 (R^2 = 0.0169). Logistic regression for 2: Y = -0.0037x + 7.0586 (R^2 = 0.4722). Logistic regression for 3: Y = 0.0013x + 6.8043 (R^2 = 0.0172). Logistic regression for 4: Y = 0.0117x + 7.1394 (R^2 = 0.6927). Logistic regression for 5: Y = 0.0152x + 6.8562 (R^2 = 0.6952).
[a] Variable 1, 2, 3, 4, and 5 = 1, 3, 7, 14 days after application and, end of season, respectively.

When tuber number was evaluated across the plant growth stage that the sub-lethal glyphosate doses were applied and the five different harvest timings, the TI stage appeared to be the only stage influenced by glyphosate doses (Figure 3.6.). As time progressed after application, the number of tubers generally increased only when glyphosate doses were applied to plants at the TI stage. In contrast, a glyphosate drift event that occurred during the two latter plant growth stages would have no effect on the number of tubers the plant sets.

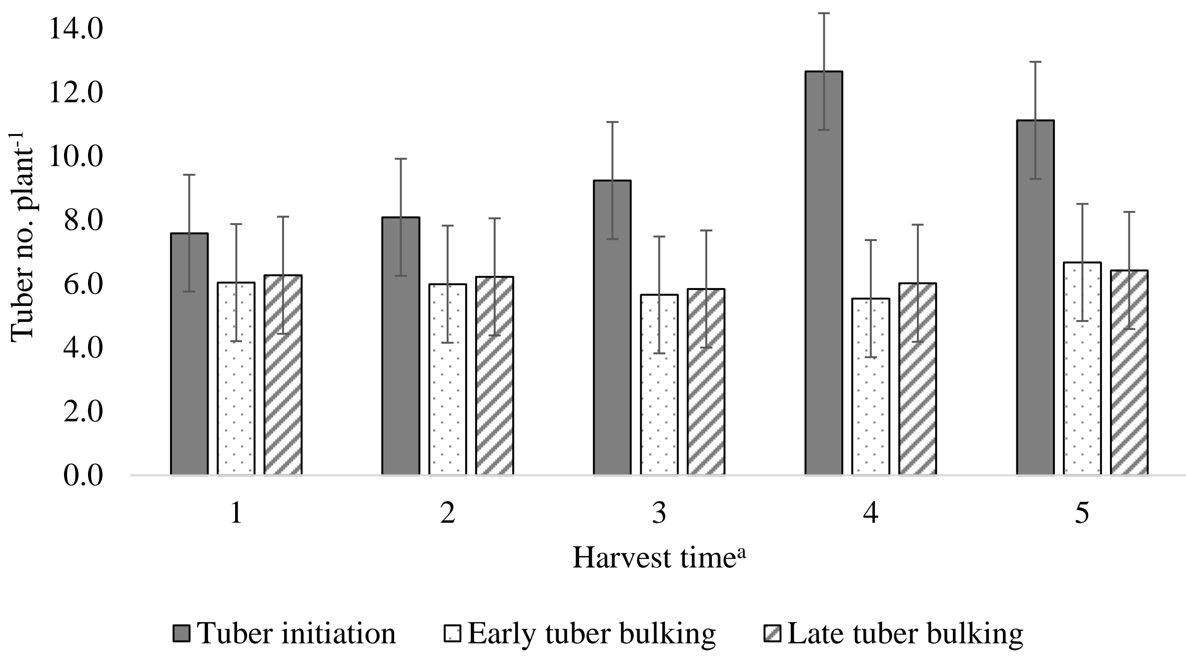

Figure 3.6. Average tuber number per plant for the interaction of harvest time and plant growth stage when the glyphosate drift occurred, Grand Forks, ND, combined over two years (2014 and 2015) and glyphosate dose (0, 26.5, 52.5, 105, and 210 g a.e. ha^{-1}).
[a] Harvest time 1, 2, 3, 4, and 5 = 1, 3, 7, 14 day after application and end of season, respectively.

The increase of tuber number per plant, for glyphosate doses applied at TI stage, was not a result of stem number increasing, like Knowles and Knowles (2006) had described, but instead due to the effects of simulated glyphosate drift. Tuber bulking has the potential to be compromised with the increase of tubers per plant, causing a decrease in yield.

3.4.2.2. Tuber yield

Yield per plant was significant ($P < 0.05$) at the interactions: PGS x Time, Glyphosate dose x Time, and the three-way interaction PGS x Glyphosate dose x Time. Collecting harvest samples throughout the season after each simulated sub-lethal glyphosate dose application allowed us to see the progression of the effects over time on tuber production. Application at TI had more days between the sub-lethal glyphosate doses to the final harvest compared to EB or LB, so it isn’t appropriate to compare the plant growth stages between themselves at each harvest

(data not shown). The three-way interaction exists based on the application time differences for each plant growth stage (TI, EB, LB application at 46, 62, 70 DAP, respectively), and therefore will not be discussed.

Evaluating plant yield by sub-lethal glyphosate doses pooled across each plant growth stage and evaluated five times after application, demonstrated the effects of the glyphosate drift evolving throughout the growing season. The untreated control yielded higher than all sub-lethal glyphosate doses except at harvest time 4 at the lowest glyphosate dose (Figure 3.7.). The first 3 harvest times (1, 3, and 7 DAA) have minimal yield changes across sub-lethal glyphosate doses, at the most a -0.0006 slope. Harvest time 4 (14 DAA) also isn't affected across sub-lethal glyphosate doses, and results in a minimal negative slope of -0.0009. It isn't until harvest 5, (end of season) when the sub-lethal glyphosate doses show more of an effect in yield. Glyphosate applied at the sub-lethal doses of 105 and 210 g a.e. ha^{-1} evaluated at the end of the season and pooled across plant growth stages, resulted in a 24 % decrease when compared to the untreated control. As glyphosate increased by 50 g a.e. ha^{-1}, yield was reduced by 0.12 kg per plant at harvest 5 (end of season).

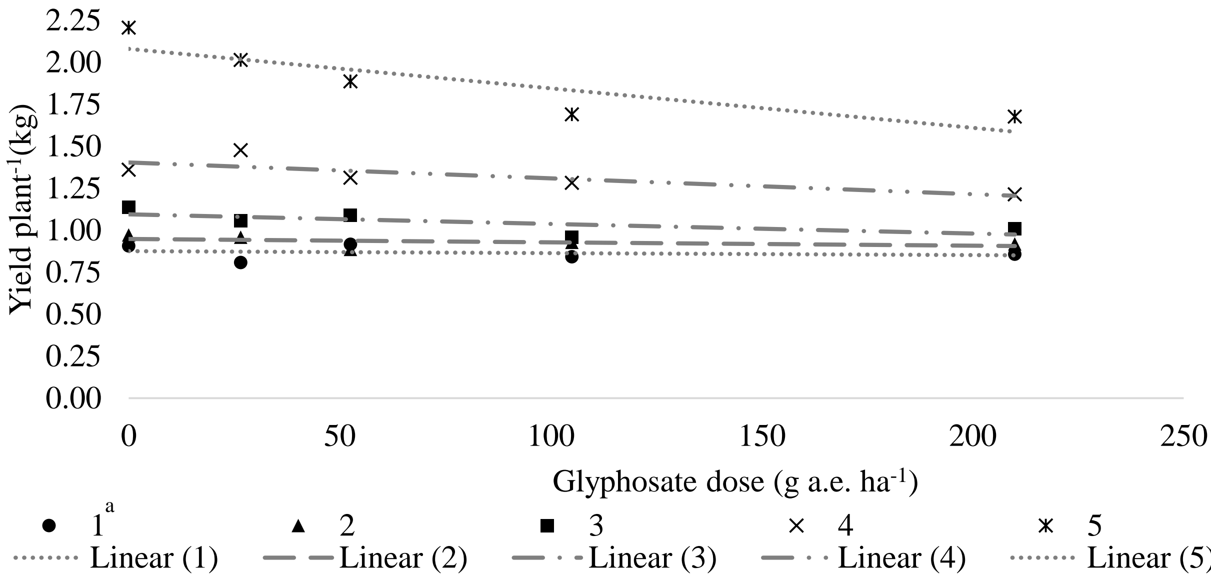

Figure 3.7. Average yield per plant as a result of sub-lethal glyphosate doses applied at different plant growth stages with data collected across different harvest timings, Grand Forks, ND, combined over PGS (Tuber initiation, early tuber bulking, and late tuber bulking) and years (2014 and 2015).
Logistic regression for 1: Y = -0.0001 + 0.8761 (R^2 = 0.0504). Logistic regression for 2: Y = -0.0002x + 0.9482 (R^2 = 0.2219). Logistic regression for 3: Y = -0.0006x + 1.0953 (R^2 = 0.4723). Logistic regression for 4: Y = -0.0009x + 1.4044 (R^2 = 0.6454). Logistic regression for 5: Y = -0.0023x + 2.08 (R^2 = 0.7539).
[a] Variable 1, 2, 3, 4, and 5 = 1, 3, 7, 14 days after application and end of season, respectively.

3.4.2.3. Unmarketable tubers

Tubers grown for the fresh market can be classified as unmarketable based on external and internal defects (USDA 2011). Potatoes grown for the fresh market have to meet strict demands of quality based on consumer expectations, therefore the evaluation of unmarketable tubers was executed as a result of the simulated glyphosate drift. Unmarketable tuber number per plant was significant ($P < 0.05$) at interactions PGS x Glyphosate dose, PGS x Time, Glyphosate dose x Time, and PGS x Glyphosate dose x Time.

The interaction between PGS and sub-lethal glyphosate dose displayed TI as the most affected stage when glyphosate doses were applied, which produced up to five times the amount of unmarketable tubers when compared to glyphosate doses applied to plants at the EB and LB

growth stages (Figure 3.8.). Glyphosate doses applied to plants at growth stages TI, EB, and LB had resulted in an average of 9.5, 3.2, and 2.9 unmarketable tubers per plant, respectively.

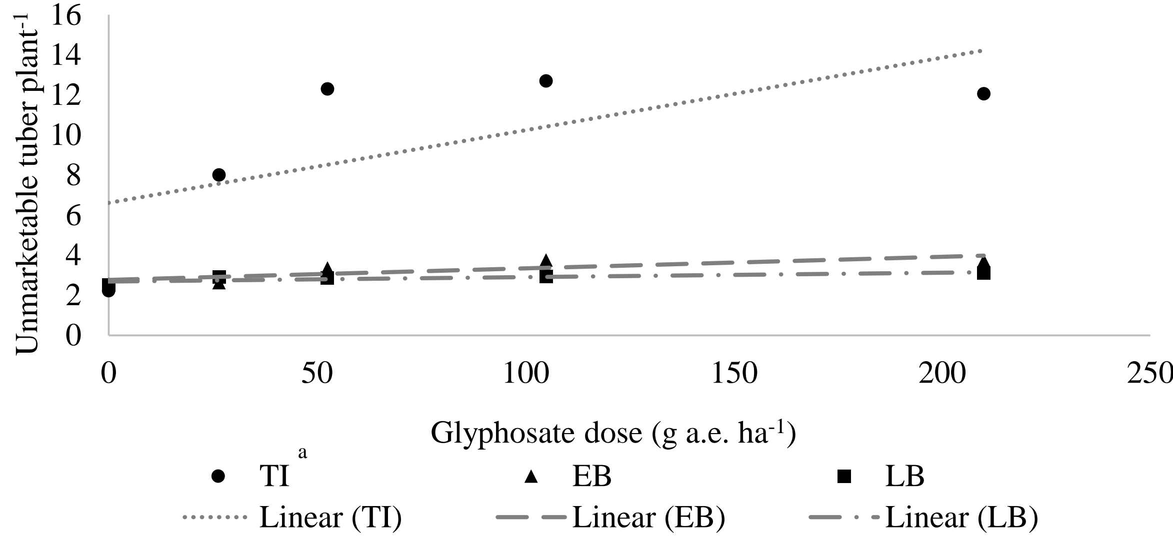

Figure 3.8. Average number of unmarketable tubers per plant as a result of simulated sub-lethal glyphosate doses applied at different growth stages, Grand Forks, ND, combined over years (2014 and 2015) and harvest times (1, 3, 7, 14 days after application, and end of season). Logistic regression for TI: $Y = 0.0362x + 6.604$ ($R^2 = 0.4538$). Logistic regression for EB: $Y = 0.0058x + 2.7555$ ($R^2 = 0.6861$). Logistic regression for LB: $Y = 0.0022x + 2.6897$ ($R^2 = 0.6622$).
[a] TI, EB, LB = tuber initiation, early tuber bulking, late tuber bulking, respectively.

Across all plant growth stages, as the glyphosate dose increased so did the number of unmarketable tubers. Untreated control, and the glyphosate doses of 0, 26.5, 52.5, 105, and 210 g a.e. ha^{-1} had 2.4, 4.5, 6.2, 6.5, and 6.3 unmarketable tubers per plant, respectively. The R^2 value of the linear regression line for glyphosate doses applied at TI was 0.454, showing some variability in linear response. When glyphosate doses were applied to the plant growth stage TI, the number of unmarketable tubers per plant increased by 1.8 as glyphosate dose was increased by 50 g a.e. ha^{-1}. The number of unmarketable tubers plateaued after the glyphosate dose reached 52.5 g a.e. ha^{-1}. The variation of unmarketable tubers per plant when glyphosate doses were applied at TI ranged between 2 and 12. The variation of glyphosate doses applied at both the EB and LB was much lower, ranging between 2 and 4. The linear slopes for unmarketable tubers

when glyphosate doses were applied at the plant growth stage EB or LB were almost flat and both at most had an increase of 0.3 unmarketable tubers per plant as the glyphosate dose increased by 50 g a.e. ha^{-1}.

A similar pattern was expressed when evaluating the interaction between PGS and harvest time (Figure 3.9.). Glyphosate doses applied at TI remained the most sensitive PGS throughout the season, and reached its maximum level of unmarketable tubers per plant at the 4th harvest time, 14 DAA. For every advancement in harvest time the number of unmarketable tubers for glyphosate doses applied at TI increased by 4.3. Unmarketable tuber numbers remained similar throughout the harvest periods when glyphosate doses were applied to plants at either EB or LB plant growth stages.

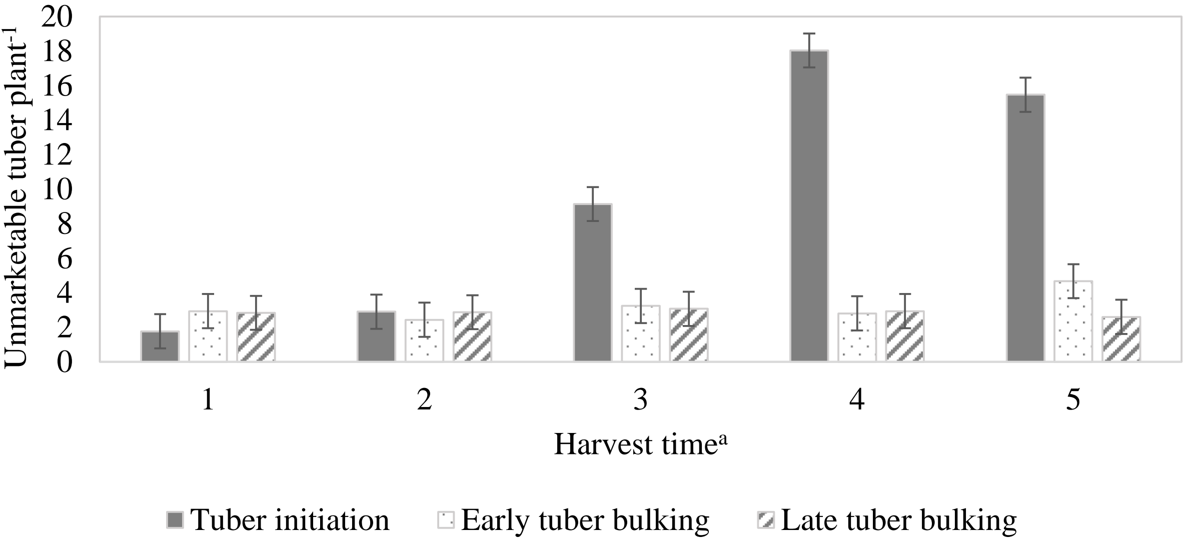

Figure 3.9. Average number of unmarketable tubers per plant as a result of simulated glyphosate drift applied at different growth stages and harvested at five separate times, Grand Forks, ND, combined over glyphosate dose (0, 26.5, 52.5, 105, and 210 g a.e. ha^{-1}) and years (2014 and 2015).

[a] Harvest time 1, 2, 3, 4, and 5 = 1, 3, 7, 14 day after application and end of season, respectively.

The interaction between glyphosate dose and harvest time displayed how the higher doses of the glyphosate affected the development of unmarketable tubers throughout the growing

season (Figure 3.10.). As the harvest time progresses further away from the application, the slope of the linear regression line became larger. Harvest 1 (1 DAA) did not vary in the number of unmarketable tubers regardless of the glyphosate dose. Harvest 5 (end of season), on the other hand, had an increase of two unmarketable tubers per plant as the glyphosate dose increased by 50 g a.e. ha^{-1}.

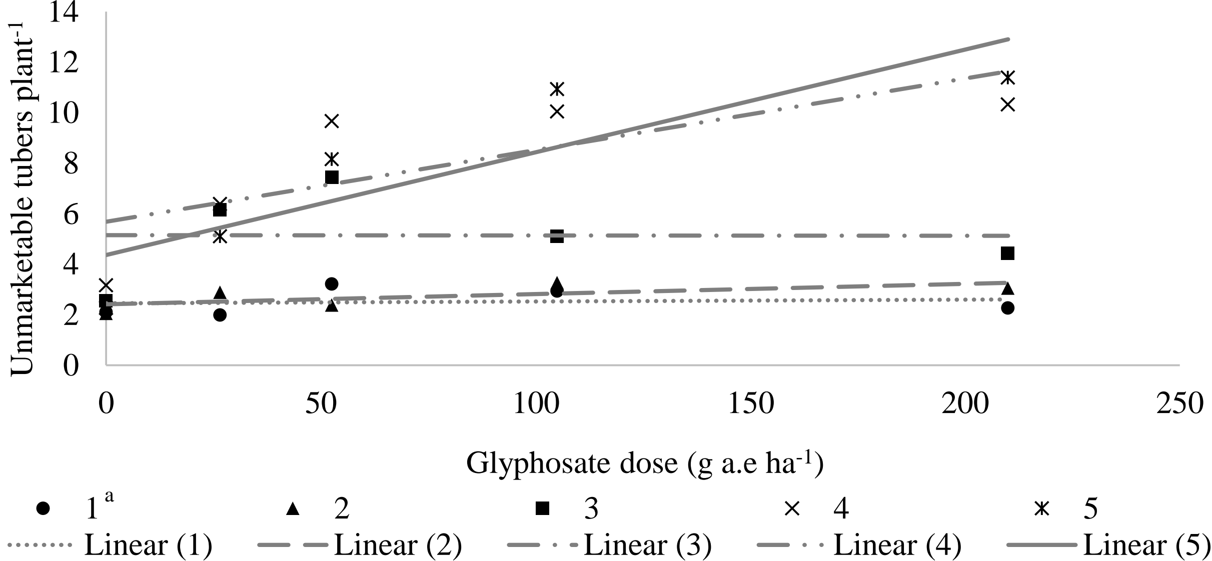

Figure 3.10. Average number of unmarketable tubers per plant as a result of sub-lethal glyphosate doses, Grand Forks, ND, combined over PGS (TI, EB, and LB) and years (2014 and 2015).
Logistic regression for 1: $Y = 0.0007 + 2.453$ ($R^2 = 0.0128$). Logistic regression for 2: $Y = 0.004x + 2.4161$ ($R^2 = 0.4453$). Logistic regression for 3: $Y = -1E-04x + 5.1521$ ($R^2 = 2E-05$). Logistic regression for 4: $Y = 0.0284x + 5.6865$ ($R^2 = 0.5777$). Logistic regression for 5: $Y = 0.0407x + 4.3745$ ($R^2 = 0.7536$).
[a] 1, 2, 3, 4, and 5 = harvest at 1, 3, 7, 14 day after application and end of season, respectively.

3.5. Discussion

Glyphosate drift on 'Red Norland' potato produced for the fresh market caused a decrease in foliar plant growth, a decrease of yield and an increase of unmarketable tubers. Tuber initiation was the most sensitive plant growth stage across all evaluated characteristics.

Despite significant foliar plant measurements, there are many other stressors that could cause similar response symptoms, and therefore foliar plant measurements are not reliable for

glyphosate drift diagnosis. A study completed in Oregon reported variable foliar injury and growth reduction from glyphosate doses (Pfleeger et al. 2008), reinforcing conclusions that environmental conditions may play a large role in the foliar response of the plants after glyphosate drift. Foliar injury such as chlorosis or necrosis at the growing points may be a more accurate symptom for positive diagnosis of glyphosate drift. Chlorosis at the growing point was identified at every sub-lethal dose applied at TI, but not when glyphosate doses were applied at plant growth stages EB or LB. This was attributed to the specific growing habit of 'Red Norland', which differs between cultivars. 'Red Norland' exhibits a determinate growth habit in contrast to 'Russet Burbank' that exhibits an indeterminate growth habit. Once stolons begin to swell and tubers initiate, determinate cultivars sharply decrease foliar growth, which alters the actively growing point from the foliage to the new tuber growth. If glyphosate drift would occur after the TI stage, typical foliar symptoms should no longer be expressed, and diagnosing a glyphosate drift based on chlorosis at the growing point will become impossible. There are many other factors that could also reduce average foliar growth, such as biotic and abiotic stressors, available soil nutrients, water management, environmental events, etc. Stem number was not affected by the glyphosate treatments. However, reduction in tuber yield and quality still occurred despite mild foliar injury across all plant growth stages. Eberlein and Guttieri (1994) found similar poor correlation between foliar injury and tuber damage with simulated drift of imazethapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyethyl)-5-oxo-1 H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) and imazamethabenz ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylthyl)-5-oxo-1H-imidazol-2-yl]-4(and 5)-methylbenzoic acid (3:2)). A study conducted by Wall (1994) evaluated the effects of dicamba (3,6-dichloror-2-methoxybenzoic acid), clopyralid, and tribenuron drift on 'Norland' potato produced under dry-land conditions in Manitoba. Results

suggested that visual foliar injury did not offer useful value in prediction of yield loss, which was recorded between 53 to 75 % of the untreated yields. Felix et al. (2011) concluded that less glyphosate was needed to reduce tuber yield compared to the amount needed to elicit visual injury, which supports the results of the current research.

Tuber number per plant was affected by glyphosate. The plant growth stage TI was the most affected by the sub-lethal glyphosate doses, resulting in an increase of tubers per plant, which consequently resulted in smaller tuber size. A plateau in tuber number after 52.5 g a.e. ha^{-1} showed that a low volume of glyphosate exposure was able to affect tuber number. Similarly, tuber number per plant was increased which resulted in a smaller tuber size profile when simulated dicamba drift was applied to mother plants (Colquhoun et al. 2014).

Felix et al. (2011) found that a higher amount of glyphosate was required to produce the same response for plants sprayed at tuber initiation and bulking than at the hooking stage. This confirmed that as the potato plant progresses through the growth stages, it requires a higher dose of glyphosate to cause the same amount of damage or yield loss. Yield per plant was affected more when glyphosate doses were applied in the TI stage than the EB or LB stages. Average yield per plant had a linear decrease as glyphosate dose increased, similar to results found by Hutchinson et al. (2014), regardless of application timing. Likewise, a dicamba drift study in Wisconsin by Colquhoun et al. (2014), found that the potato plant can compensate in total yield by increasing the number of “B” sized potatoes, (3.8 to 5.7 cm in diameter) (USDA 2011).

Unmarketable tubers, or cull tubers, cause a loss of potential profit to the producer. Glyphosate damage on tubers were characterized by growth cracks, bud end folds, malformation, and decrease in tuber size (Robinson and Hatterman-Valenti 2013; Hutchinson et al. 2014; Felix et al. 2011). Similar damage was found with this study, with the inclusion of skin discoloration

of the smooth red skin of the cultivar. Glyphosate doses applied at TI was the most sensitive growth stage for unmarketable tuber number, increasing 295 and 330 % from EB and LB, respectively. An increase of unmarketable tubers was 187, 254, 260 and 270 % above the untreated control for doses, 26.5, 52.5, 105, and 210 g a.e ha^{-1}, respectively. Hutchinson et al. (2014) found 43 and 28 % culls, as a % of total tuber yields, when glyphosate was applied at TI or hooking, respectively.

3.6. Conclusion

Our study shows that when 'Red Norland' grown for the fresh-market is exposed to glyphosate drift early in the season at tuber initiation, the most damage is caused and this results in a high number of unmarketable tubers, causing profit loss for the producer. Glyphosate drift occurring post tuber initiation, during the tuber bulking stages, had little to no visual defects and can potentially be difficult to diagnose. If the tubers cannot be identified as contaminated with glyphosate, how can they be tested with assurance for the consumer? The United States Environmental Protection Act specifies a tolerance of 0.2 ppm (0.0002 mg/mL) of glyphosate within potato tubers (U.S. EPA 2012). A residue analysis test would need to be completed on the tuber tissue to quantify the amount of glyphosate stored in the tuber.

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CHAPTER 4. SIMULATED GLYPHOSATE DRIFT EFFECTS ON 'RED NORLAND' MARKETABILITY: GREENHOUSE EXPERIMENT

4.1. Abstract

North Dakota can often provide unpredictable environmental conditions in the spring season, which can necessitate storing cut potato seed pieces in non-ideal conditions for longer than normal. This could result in physiologically aging of the seed, causing rapid emergence, an increase in stem and tuber number per plant, and a decrease in growing potential. Greenhouse experiments were conducted using physiologically aged seed in 2015 to evaluate the effect of simulated glyphosate drift applied at different growth stages of tuber development. Sub-lethal glyphosate doses of 1/4, 1/8, 1/16, and 1/32 the recommended rate (840 g a.e. ha^{-1}) were applied at the growth stages of tuber initiation (TI), early tuber bulking (EB), and late tuber bulking (LB).

An experiment completed in the greenhouse has advantages consisting of cost savings, increased consistency, and flexibility when compared to a field experiment. However, the environment in the greenhouse is not always realistically comparable to real field issues that can occur. Foliar and tuber characteristics measured were not significantly affected by either sub-lethal glyphosate dose or the stage of development the plant was in when the application was made. These results differed from those found when the same experimental treatments were administered in field conditions with non-physiologically aged seed. The lack of significance suggests effect variability from glyphosate drifting onto a 'Red Norland' potato crop.

4.2. Introduction

A general concept is that results gained from greenhouse studies may not be comparable to the same study conducted under field conditions (Pfleeger et al. 2010). The advantages to greenhouse testing is cost saving, less variability and can be completed any time during the year. Executing the same treatments as describe in chapter 1 on plants in a greenhouse location may provide insight on how the potato plant reacts and overcomes the sub-lethal glyphosate dose under ideal environmental conditions. These conditions may also increase glyphosate uptake and translocation within the mother plant.

The northern climate of North Dakota can be unpredictable, especially in the spring months, which can result in planting delays. Planting dates can be compromised, such as in the 2014 season, when extended winter and the set back of spring arrival caused a delay in potato planting in North Dakota (Robinson and Secor 2014). This situation often leads to cut potato seed pieces being stored for a longer period of time in non-ideal storage conditions that causes physiologically aging of the seed. Characteristics of physiologically aged, also called old seed, causes rapid emergence, increase in stem and tuber numbers per hill, and a decrease in growing potential (Bohl et al. 2000).

The object of this study was to determine the effects of exposing ‘Red Norland’ potato, grown for the fresh market, to sub-lethal glyphosate doses at different plant growth stages crucial to tuber production. The seed utilized in this study had become physiologically aged as a result of poor storage conditions. Characteristics such as in-season plant response, yield results and potato marketability traits were evaluated at the end of the growing season. A combination of sub-lethal glyphosate doses and different growth stages crucial to the plant’s production were used to simulate potential injury.

4.3. Materials and Methods

4.3.1. Experimental design and preparation

Greenhouse experiments were conducted during the winter in 2014/2015 at the Waldron Greenhouse facility located on the North Dakota State University campus. Two greenhouse rooms were used simultaneously to represent repetition of the study. The experiment was set up as a randomized complete block design with a two factor arrangement, replicated four times at both locations. Glyphosate treatments were 210, 105, 52.5, 26.5, and 0 g a.e. ha^{-1}. The sub lethal glyphosate doses are fractions (1/4, 1/8, 1/16, 1/32) of the standard field use rate of 840 g a.e. ha^{-1}. Plant growth stage treatments were selected on their importance to the development of daughter-tubers, tuber initiation (TI), early tuber bulking (EB), and late tuber bulking (LB). The physiologically aged tubers were certified Red Norland seed that was not properly stored. Whole tubers were cut into 70 g ± 5 g seed pieces, and stored for two weeks at 12.7 ^{0}C with approximately 90 % relative humidity to induce suberization and seed conditioning prior to planting.

A single 12 L pot with one seed piece was considered an experimental unit (EU). A total of 60 seed pieces per trial were planted at 10 cm depth in soil consisting of 7 parts peat moss and 3 parts silty, clay loam field soil. The pots were placed 0.3 m apart within the rows and 0.4 m between rows due to available bench space. Pots were rotated weekly to minimize variation. The room temperature was maintained at approximately 24 ^{0}C with 15 hours of continuous light. Plants were watered on alternating days. Slow release fertilizer was added at planting, and liquid fertilizer was applied with water after tuber initiation and mid-bulking to maintain available plant soil nutrition. Integrated pest management practices were scheduled by the greenhouse manager.

4.3.2. Application of treatments

Plants were sprayed using an Automatic Research Track Sprayer located in the Waldron greenhouse, equipped with a single flat fan nozzle at 275 kPa with a sprayer output of 187 L ha^{-1}. Treatments were applied in progression from lowest to highest dose to mitigate cross contamination of the application equipment and treatments. Four plants were placed inside the spray chamber and treated under controlled environmental conditions. Sub-lethal glyphosate doses were applied 31, 54, and 81 days after planting (DAP), for TI, EB, and LB, respectively.

4.3.3. Evaluation of treatments

All plants were harvested and treatments evaluated after 96 DAP. Data measured included: foliar fresh weight, plant height from soil surface, number of stems, and number of tubers, yield and number of unmarketable tubers. An unmarketable tuber was classified as a tuber expressing any of the following symptoms: cracks deep into the tuber flesh; predominately at the stem end, malformation, and discoloration or heavy russeting of the skin and tissue death (Robinson and Hatterman-Valenti 2013).

4.3.4. Data analysis

Data from the north and south greenhouse were combined after testing homogeneity of variance confirmed variance ratio differed by less than 10, using the 10-fold f-test method, which proportions the largest variance of the treatment groups to the smallest and compares it to a critical value table (Tabachnik and Fidell 2001). Homogeneity was confirmed for all variables with the exception of yield; however due to the ability to combine the majority, yield was also combined across locations. Data was analyzed using PC SAS 9.3 (Statistical Analysis Software, version 9.3. SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513.). Locations were considered random and treatments fixed. Differences were interrupted at $P < 0.05$ for all

characteristics measured. Treatment means were separated at the 5 % level of significance using Fisher's protected least significant difference test where appropriate.

4.4. Results

4.4.1. Foliar Measurements

4.4.1.1. Foliar fresh weight

Foliar fresh weight was not significantly affected by either PGS, glyphosate dose or the interaction between PGS and glyphosate dose. There were numerical differences between the treatments in that plants receiving glyphosate doses at TI measured the lowest fresh weight in comparison to plants receiving glyphosate doses at either EB or LB growth stages (Table 4.1.). At the TI plant growth stage, the higher the glyphosate dose, the lower the foliar fresh weight measured at the end of the growing season. This was expected since 'Red Norland' plants have a determinate growth habit and stops foliar growth once tuber initiation occurs.

Table 4.1. Average foliar fresh weight per plant as a result of sub-lethal simulated glyphosate doses applied at different plant growth stages crucial to tuber production. Fargo, ND, 2015, combined across two locations (greenhouse north and greenhouse south).

Glyphosate dose	Plant growth stage		
	Tuber initiation	Early tuber bulking	Late tuber bulking
-----g a.e. ha^{-1}-----		-----g-----	
210.0	143.6	269.0	337.2
105.0	216.3	294.3	347.3
52.5	218.3	277.1	319.8
26.5	219.5	256.1	336.7
Untreated	257.8	241.9	314.8
LSD ($P < 0.05$)	NS	NS	NS

4.4.1.2. Foliar plant height

Foliar plant height was not significantly affected by PGS, glyphosate dose or the interaction between PGS and glyphosate dose. Minor numerical differences were measured in

plant height based on the plant growth stage the sub-lethal glyphosate drift was applied (Table 4.2.). Plants receiving glyphosate doses at TI generally were shorter compared to plants receiving glyphosate doses at either EB or LB. As previously mentioned, this was expected since 'Red Norland' plants have a determinate growth habit and stop growing once tuber initiation occurs.

Table 4.2. Average foliar height per plant as a result of sub-lethal simulated glyphosate doses applied at different plant growth stages crucial to tuber production. Fargo, ND, 2015. Combined across two locations (greenhouse north and greenhouse south).

Glyphosate dose	Plant growth stage		
	Tuber initiation	Early tuber bulking	Late tuber bulking
-----g a.e. ha^{-1}-----	-----------------------------------cm-----------------------------------		
210.0	60.0	83.5	98.1
105.0	87.8	93.3	92.2
52.5	91.4	104.9	88.4
26.5	76.4	99.0	94.1
Untreated	90.8	103.1	103.9
LSD ($P < 0.05$)	NS	NS	NS

4.4.1.3. Stem number

Stem number per plant was not significantly affected by PGS or glyphosate dose or the interaction between PGS and glyphosate dose. Due to the advanced physiological age of the certified seed used in this study, the stem number was higher than less physiologically aged seed (Bohl et al. 2000). The minimum stem number was 6, the maximum was 15 and the mean was 11.1 stems per plant. Similar to the results found in chapter 1, the stems were not affected, possibly due to the plant stem number already established prior to the first plant growth stage when glyphosate was applied.

4.4.2. Tuber Measurements

4.4.2.1. Tuber number

Tuber number per plant was not significantly affected by PGS or glyphosate dose or the interaction between PGS and glyphosate dose. Results did show numerical differences in the interaction of plant growth stage and sub-lethal glyphosate doses (Table 4.3.). Tuber number per plant was increased 1.4 and 1.7 times by the highest glyphosate dose for TI and EB, respectively, compared to the untreated. When tuber number per plant increased, the tuber profile became smaller and more uneven when compared to plants with less tubers per plant.

Table 4.3. Average tuber number per plant as a result of sub-lethal simulated glyphosate doses applied at different plant growth stages crucial to tuber production. Fargo, ND, 2015. Combined across locations (greenhouse north and greenhouse south).

Glyphosate dose	Plant growth stage		
	Tuber initiation	Early tuber bulking	Late tuber bulking
-----g a.e. ha^{-1}-----	----------	No. Plant^{-1}	----------
210.0	14.1	15.8	7.5
105.0	13.7	10.6	7.4
52.5	8.1	9.5	7.1
26.5	9.6	9.0	7.4
Untreated	9.8	9.3	7.8
LSD ($P < 0.05$)	NS	NS	NS

4.4.2.2. Tuber yield

Tuber yield per plant was not significantly affected by PGS or glyphosate dose or the interaction between PGS and glyphosate dose. Yield results were surprising, as the highest average plant yield occurred when plants at tuber initiation received a glyphosate dose of 26.5 g a.e. ha^{-1} (Table 4.4.). As glyphosate dose increased for plants treated at TI, yield decreased even though the number of tubers increased. Plants treated at EB generally yielded lower than TI and LB, regardless of the glyphosate dose. These results differed from the field experiment using the same treatments, which found the lowest yields from plants treated at TI.

Table 4.4. Average yield per plant as a result of sub-lethal simulated glyphosate doses applied at different plant growth stages crucial to tuber production. Fargo, ND, 2015. Combined across locations (greenhouse north and greenhouse south).

Glyphosate dose	Plant growth stage		
	Tuber initiation	Early tuber bulking	Late tuber bulking
-----g a.e. ha^{-1}-----	----------g----------		
210.0	298.8	297.8	322.5
105.0	378.5	257.5	285.6
52.5	375.4	284.8	304.7
26.5	445.6	271.9	311.5
Untreated	374.4	333.1	324.5
LSD ($P < 0.05$)	NS	NS	NS

4.4.2.3. Unmarketable tubers

Unmarketable tubers per plant was not significantly affected by PGS or glyphosate dose or the interaction between PGS and glyphosate dose. Application of sub-lethal glyphosate doses at LB resulted in no unmarketable tubers (Table 4.5.). However, glyphosate doses of 105 and 210 g a.e. ha^{-1} to plants at the TI and EB stages had at least 3 unmarketable tubers.

Table 4.5. Average number of unmarketable tubers per plant as a result of sub-lethal simulated glyphosate doses applied at different plant growth stages crucial to tuber production. Fargo, ND, 2015. Combined across locations (greenhouse north and greenhouse south).

Glyphosate dose	Plant growth stage		
	Tuber initiation	Early tuber bulking	Late tuber bulking
-----g a.e. ha^{-1}-----	----------g----------		
210.0	4.8	7.1	0.1
105.0	5.5	3.1	0.0
52.5	0.3	0.1	0.0
26.5	0.0	0.5	0.0
Untreated	0.0	0.0	0.0
LSD ($P < 0.05$)	NS	NS	NS

4.5. Discussion

Applying sub-lethal glyphosate doses to potato plants from physiologically aged seed at three different plant growth stages that are crucial to the development of tubers had unpredictable yet non-significant effects in the greenhouse. The lack of significance for all measured variables were different than results from the field experiment. The average plant height for TI, EB, and LB in the greenhouse were 81, 95, and 96 cm compared to the field experiment, of 68.6, 74, and 71.5, respectively, a 19-32 % increase in plant height. The plant height did not correlate with the plant weight, as the plant weight in the field experiment was much higher than the plant weight recorded from the greenhouse experiment. Thus, the increase in plant height in the greenhouse may have contributed to the non-significant response from the sub-lethal glyphosate doses. Similar results were reported by Kegode and Fronning (2005) when trying to control biennial wormwood in soybean production with glyphosate, and Mellendorf et al. (2013) evaluating horseweed control at different plant heights, where herbicide efficacy decreased as the size of the target plant increased.

Another possibility for non-significance may be due to the high number of stems that were initiated from the physiologically aged seed pieces. This would increase the surface area of the plants and disperse glyphosate throughout more plant tissue, which would cause further dilution of the glyphosate, potentially having less of an effect on the foliar tissue as well as the tubers.

An additional possibility may be related to the ideal environmental conditions the greenhouse supplied to the potato plants. The artificial light extended the photosynthetic period for the plant, and the ambient temperature of 24 °C provided the plant an atmosphere within the ideal range for potato growth and development (Bains et al. 2003). The ambient temperature

within the greenhouse was closer to the ideal temperature for plant growth (25 °C) than tuber growth at (16 °C) which may have contributed to the extra height in the plants, without the added foliar fresh weight gain (Dwelle and Love 2003). There were no major temperature or moisture fluctuations that the plants had to overcome. The plants had minimal stressors to combat in combination to the application of the sub-lethal glyphosate doses.

The lack of significance may also be due to reduced glyphosate efficacy based on the environment within the greenhouse. Numerous publications identified by Satchivi et al. (2000) stated that a number of environmental factors may affect glyphosate effectiveness such as light, relative humidity, soil moisture, and air temperature.

Unmarketable tubers were still produced from the sub-lethal glyphosate dose applied at TI and EB, therefore there was still glyphosate absorption and translocation from the foliar tissue towards the developing tubers at these plant growth stages. Absorption and translocation varies between plant species (Satchivi et al. 2000) and would need to be tested with ^{14}C-glyphosate to confirm the value within the potato plants at different stages.

4.6. Conclusion

This study produced non-significant results when sub-lethal glyphosate doses were applied to ‘Red Norland’ mother plants, grown from physiologically aged seed, at different plant growth stages crucial to tuber production. However, these results are not to diminish the concern with glyphosate drift on ‘Red Norland’ potato grown for the fresh market, as it is still relevant and important for the producer. The lack of significance suggested that the effects from glyphosate drifting onto a potato crop already compromised aren’t as problematic due to the physiological changes within the plant and the decreased production potential from physiologically aged seed. Further research should be completed in the field on both

physiological new and old seed to see if there are any differences based on the age of the certified seed used.

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CHAPTER 5. EVALUATION OF 'RED NORLAND' SEED EXPOSED TO SIMULATED GLYPHOSATE DRIFT

5.1. Abstract

Successful commercial potato (*Solanum Tuberosum* L.) production begins with the availability of high-quality seed produced under strict standards. Glyphosate residue within seed tubers has been shown to decrease emergence and crop quality for russet potato cultivars. The evaluation of glyphosate residue within 'Red Norland' seed tubers was necessary due to the high number of hectares planted in the Red River Valley. Glyphosate was applied at sub-lethal doses to the mother plants in 2014 during three crucial tuber development stages, tuber initiation (TI), early tuber bulking (EB), and late tuber bulking (LB).

Generation 1 progeny harvested from the mother plants were harvested, stored over the winter and planted as seed in 2015 to evaluate growth characteristics. Glyphosate doses applied to the mother plants during EB or LB resulted in the lowest emergence of the generation 1 seed, at 60 % and 64 %, respectively. Glyphosate doses applied to the mother plant at TI was the plant growth stage least affected for the generation 1 seed performance. At all plant growth stages, as glyphosate dose increased on the mother plant, foliar plant height decreased for the generation 1 seed. Yield from the generation 1 seed was reduced as the glyphosate dose amount and the growth stage of the mother plant when the application was made increased. Yields of generation 1 seed ranked highest to lowest in tolerance to glyphosate were when glyphosate doses were applied at TI, LB, and EB to the mother plant. A glyphosate drift event occurring to the mother plants at the EB growth stage resulted in the most damage in the generation 1 seed. Precautions should be put in place to avoid glyphosate contamination of seed tubers.

5.2. Introduction

Successful commercial potato production begins with the availability of high-quality certified seed (United States Potato Board 2016). Inspectors at a federal and state level are involved in the regulation and audits of all certified seed acres in the United States. Strict standards are in place to monitor seed quality. North Dakota is one of the 15 states that produce certified seed. The cold winters experienced in North Dakota are beneficial and provide a natural method for pest incidence reduction.

Glyphosate has been found to affect progeny of plants contaminated by drift including both monocot and dicots. The presence of glyphosate has been confirmed to cause a decrease in germination and emergence in potato, soybean, dry bean, and wheat (Hatterman-Valenti and Robinson 2015; Hatterman-Valenti 2014; Hutchinson et al. 2014; Norsworthy 2004; Blackburn and Boutin 2003; Yenish and Young 2000).

The objective of this study was to evaluate the performance of 'Red Norland' seed from mother plants exposed to simulated glyphosate drift using sub-lethal doses applied during three different crucial growth stages of daughter tuber development. The glyphosate contaminated daughter tubers utilized as seed were evaluated for plant and tuber growth.

5.3. Materials and Methods

5.3.1. Experimental design and preparation

For clarification purposes, Generation 1 daughter tubers were harvested from the mother plants, which received foliar applied sub-lethal glyphosate doses in the previous growing season. Generation 2 daughter tubers were harvested from the plants grown from generation 1 daughter tuber seed in the current season.

Simulated glyphosate drift applied as sub-lethal doses were administered on the mother plants in the field at the Northern Plains Potato Growers Association Research Farm in 2014, as well as in the NDSU Waldron greenhouse in the winter of 2014/2015. Glyphosate treatments were 210, 105, 52.5, 26.5, and 0 g a.e. ha^{-1}. The doses are fractions (1/4, 1/8, 1/16, 1/32) of the standard field use rate 840 g a.e. ha^{-1}. Treatments were applied to mother plants during three crucial plant growth stages (PGS) of tuber initiation (TI), early tuber bulking (EB), and late tuber bulking (LB). Samples of generation 1 daughter tubers were harvested from both field and greenhouse studies and stored as individual plot samples over the winter in a 2.2 ^{0}C cooler with approximately 95 % relative humidity.

In the spring, the generation 1 daughter tubers were cut into 70 g ± 5 g seed pieces, and information was recorded about each piece. Seed piece identifying characteristics included differentiation between six seed piece types (whole piece, cut piece, eye end, stem end, split eye end or split stem end), whether or not physical damage was visible, and specific weight. Each seed piece was labeled with a letter. The identifying characteristics were collected to statistically reduce variation of results. At planting, 20 seed pieces were randomly selected from each individual plot sample and the corresponding letter labeled on each piece was recorded. Since some greenhouse plots did not have 20 seed pieces available, all pieces were planted. Seed pieces were planted in a randomized complete block design with three replications for Grand Forks samples and four replications for greenhouse samples. The locations were planted in separate blocks in the field.

The North Dakota State University research field in Fargo, ND was selected for the location of the plots for logistical purposes. The soil at this location is primarily Fargo silty clay

with poor drainage qualities that can cause temporary water pooling after precipitation. The plots were maintained by standard potato grower practices for North Dakota.

5.3.2. Evaluation of treatments

Simulated glyphosate drift was applied as sub-lethal doses to the 'Red Norland' mother plants in the previous season, therefore no additional glyphosate was applied during the current experiment. Evaluations and observations of growth characteristics were measured from the plants grown from generation 1 daughter tuber seed. Emergence and stem number data were collected 23, 43, 63, and 79 DAP, average plant heights were collected 43, 63, and 79 days after planting (DAP). All plots were subjected to a vine-kill desiccant at 80 DAP. Each emerged plant ≥ 20 cm in height prior to desiccation was hand harvested. Due to the high number of individual plants that required hand harvesting, the harvest took place over several weeks. Grand Forks plots were harvested September 14-18, greenhouse north plots were harvested October 7, and greenhouse south plots were harvested October 8-9. Data collected from the harvested plants included: tuber number per plant, yield, and unmarketable tubers per plant.

5.3.3. Data analysis

Foliar and tuber data collected from the plants grown from generation 1 daughter tuber seed were combined across all three locations (Grand Forks, greenhouse north, and greenhouse south) after testing homogeneity of variance confirmed variance ratio differed by less than 10, using the 10-fold f-test method, which proportions the largest variance of the treatment groups to the smallest and compares it to a critical value table (Tabachnik and Fidell 2001). The data collected were analyzed using PC SAS 9.3 (Statistical Analysis Software, version 9.3. SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513). Regression coefficients were estimated for plant emergence, and plant height as a function of evaluation time. Environments were

considered random and treatments fixed. Significance was interpreted at $P > 0.05$ for all characteristics measured. Pre-experimental observations of seed type and damage were analyzed as class variables to remove any variability that would be expressed in the measured characteristics based on the identification of the seed piece.

5.4. Results

5.4.1. Foliar Measurements

5.4.1.1. Emergence

North Dakota State Seed Department Potato Program currently does not have germination requirements for growers producing certified seed. Dr. Asunta Thompson, potato breeder for NDSU, stated that the standard potato grower expectation for emergence should not be below 92 %, and that anything less would cause concern (A. Thompson, personal communication, March 29, 2016). Other certified seed crops grown in North Dakota such as, small grains, flax, sunflower, soybean, field pea legally require a minimum of 85 % germination (Anonymous 2016). Using potato seed harvested from mother plants that had been contaminated by glyphosate drift enabled the evaluation of effects of glyphosate residue within the tubers on the emergence of seed tubers. Emergence was significant ($P < 0.05$) at the interactions, Time x PGS, Time x Glyphosate dose, PGS x Glyphosate dose, and Time x PGS x Glyphosate dose.

Plant emergence of generation 1 daughter tubers was affected as a result of simulated glyphosate drift applied as sub-lethal doses in the previous season to the mother plants at different plant growth stages (TI, EB, and LB), evaluated at four different times after planting (Figure 5.1.). Glyphosate applied to mother plants at the TI plant growth stage, had the most plants emerge, reaching 87 %, while LB and EB only had 64 %, and 60 %, 63 DAP, respectively. Initial plant emergence recorded at 23 DAP was greater than 50 % for TI, while EB and LB had

only 31, and 15 %, respectively. Maximum plant emergence across all PGS occurred at 63 DAP with no gain expressed at 79 DAP.

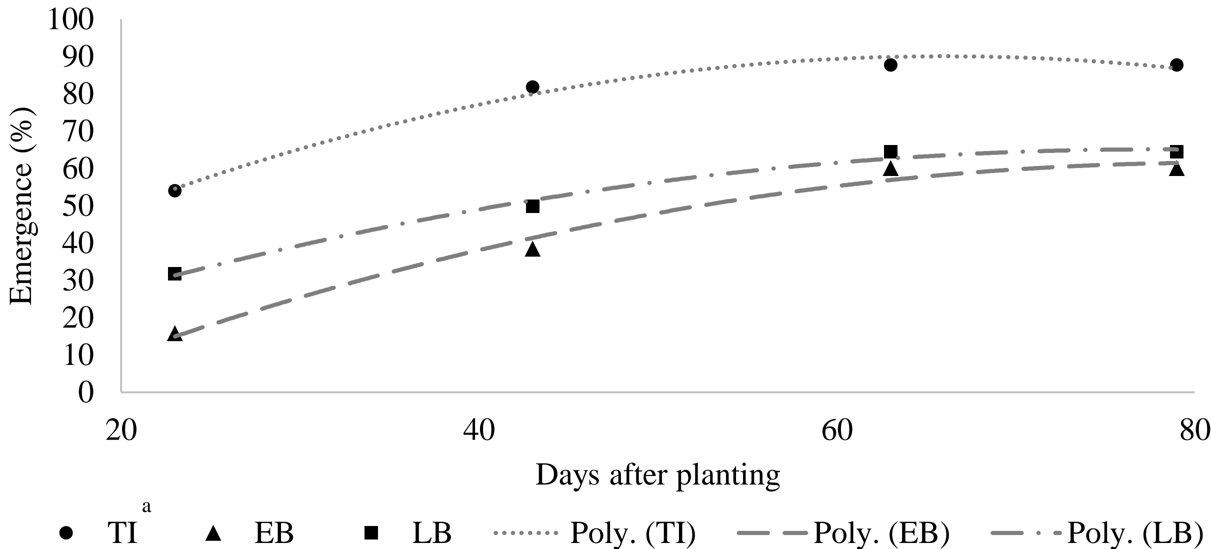

Figure 5.1. Emergence of plants grown from generation 1 daughter tubers evaluated at different times as a result of sub-lethal glyphosate doses applied at three different plant growth stages on mother-plants the previous season during production of seed tubers. Fargo, ND, 2015, combined over five sub-lethal glyphosate doses (0, 26.5, 52.5, 105, and 210 g a.e. ha^{-1}) and three locations (Grand Forks, ND, greenhouse north, and greenhouse South).
Logistic regression for TI: $Y = -0.0191x^2 + 2.5271x + 6.6123$ ($R^2 = 0.9876$). Logistic regression for EB: $Y = -0.0136x^2 + 2.2216x - 28.955$ ($R^2 = 0.9839$). Logistic regression for LB: $Y = -0.0112x^2 + 1.7507x - 3.028$ ($R^2 = 0.9913$).
[a] TI, EB, LB = tuber initiation, early tuber bulking, and late tuber bulking, respectively.

Plant emergence of generation 1 daughter tubers was affected by glyphosate sub-lethal doses (0, 26.5, 52.5, 105, and 210 g a.e. ha^{-1}) applied in the previous season to the mother plants at different plant growth stages (Figure 5.2.). Untreated seed had 81 % plant emergence by 23 DAP, and maximized at 88 % by 43 DAP. In contrast, the lowest sub-lethal glyphosate dose of 26.5 g a.e. ha^{-1}, which represents 1/32nd of the standard field use rate (840 g a.e. ha^{-1}), had ≥ 85 % emergence at 63 DAP. Plant emergence was < 80 % for plants grown from generation 1 daughter tubers that were utilized as seed after being harvested from mother plants that received ≥ 52.5g a.e. ha^{-1} glyphosate the previous year.

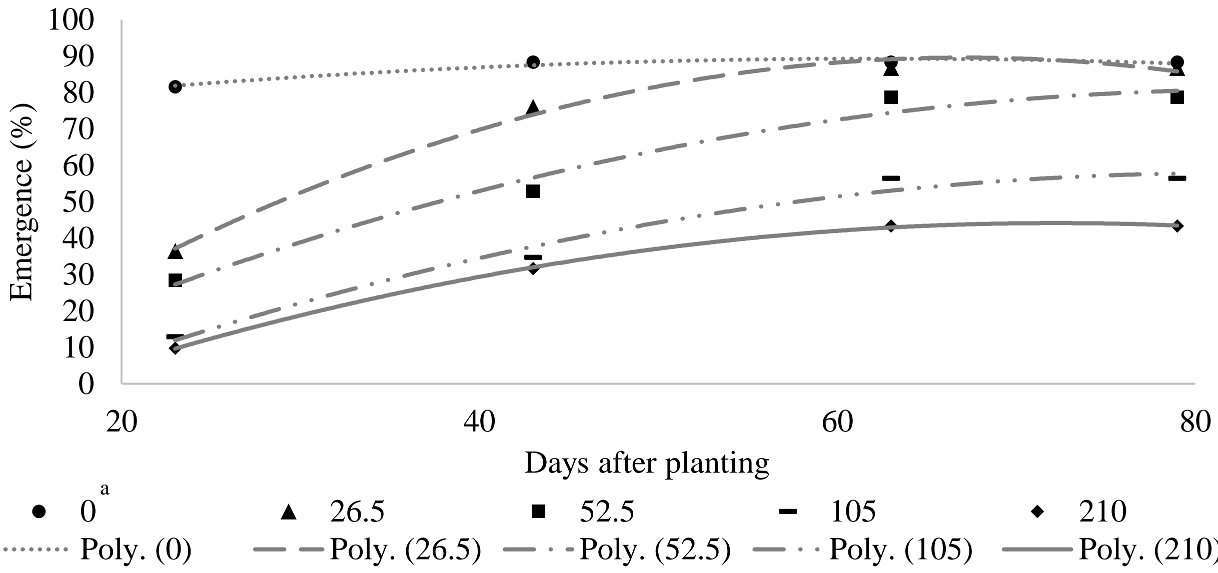

Figure 5.2. Emergence of plants grown from generation 1 daughter tubers evaluated at different times as a result of different sub-lethal glyphosate dose applied to mother-plants the previous season during production of seed tubers. Fargo, ND, 2015, combined over three plant growth stages (Tuber initiation, early tuber bulking and late tuber bulking) and three locations (Grand Forks, ND, greenhouse north, and greenhouse south).
Logistic regression for 0: $Y = -0.0048x^2 + 0.5982x + 70.657$ ($R^2 = 0.9464$). Logistic regression for 26.5: $Y = -0.0269x^2 + 3.615x - 31.72$ ($R^2 = 0.9932$). Logistic regression for 52.5: $Y = -0.0144x^2 + 2.4143x - 20.626$ ($R^2 = 0.9786$). Logistic regression for 105: $Y = -0.013x^2 + 2.1489x - 30.535$ ($R^2 = 0.9821$). Logistic regression for 210: $Y = -0.0142x^2 + 2.0558x - 30.061$($R^2 = 0.9996$).
[a] Glyphosate doses 0, 26.5, 55.5, 105, and 210 g a.e. ha^{-1}.

The significant interaction between the PGS when the mother plant received the sub-lethal glyphosate dose and the glyphosate dose indicated that plant emergence varied with increasing glyphosate doses for each PGS (Figure 5.3.). Regardless of PGS, as the sub-lethal glyphosate dose increased, the percentage of emergence declined. However, the rate of decline was greatest for plants grown from generation 1 daughter tuber harvested from mother plants that received glyphosate at the EB stage.

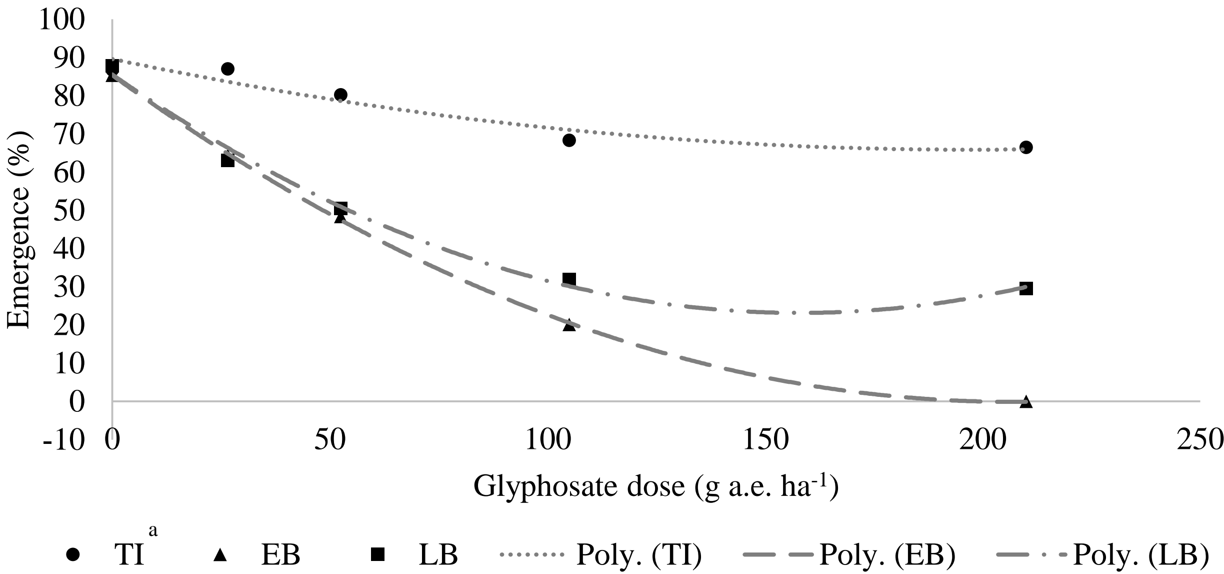

Figure 5.3. Emergence of plants grown from generation 1 daughter tubers for the interaction of plant growth stage and sub-lethal glyphosate dose applied to mother-plants the previous season during production of seed tubers. Fargo, ND, 2015, combined over evaluation times (23, 43, 63, and 79 days after planting) and three locations (Grand Forks, ND, greenhouse north, and greenhouse south).
Logistic regression for TI: Y= -0.0006x^2 – 0.2406x + 89.612 (R^2 = 0.925). Logistic regression for EB: Y= -0.002x^2 – 0.8273x + 85.379 (R^2 = 0.9997). Logistic regression for LB: Y= 0.0025x^2 – 0.7905x + 85.562 (R^2 = 0.9919).
[a] TI, EB, LB = tuber initiation, early tuber bulking, and late tuber bulking, respectively.

5.4.1.2. Foliar height

Foliar plant height may vary by available plant nutrients, cultivar, and growing regions. The above-ground foliage of the plant is considered the factory for tuber production of the plant; this represents a source-to-sink relationship and is one of the major determinants of growth (Lemoine et al. 2013). Foliar plant height was significant ($P < 0.05$) for main effects, as well as the interactions.

Foliar plant height was significantly different for the interaction of evaluation time and the plant growth stage the mother plant received the glyphosate dose. The plant growth stage TI had taller plants compared to EB and LB at each of the evaluation times (Figure 5.4.). All plant growth stages, TI, EB and LB, reached a maximum plant height 63 DAP measuring 55, 26, and

36 cm, respectively. Simulated glyphosate drift occurring to the mother plant at EB resulted in the lowest recorded plant height of 26 cm at 63 DAP.

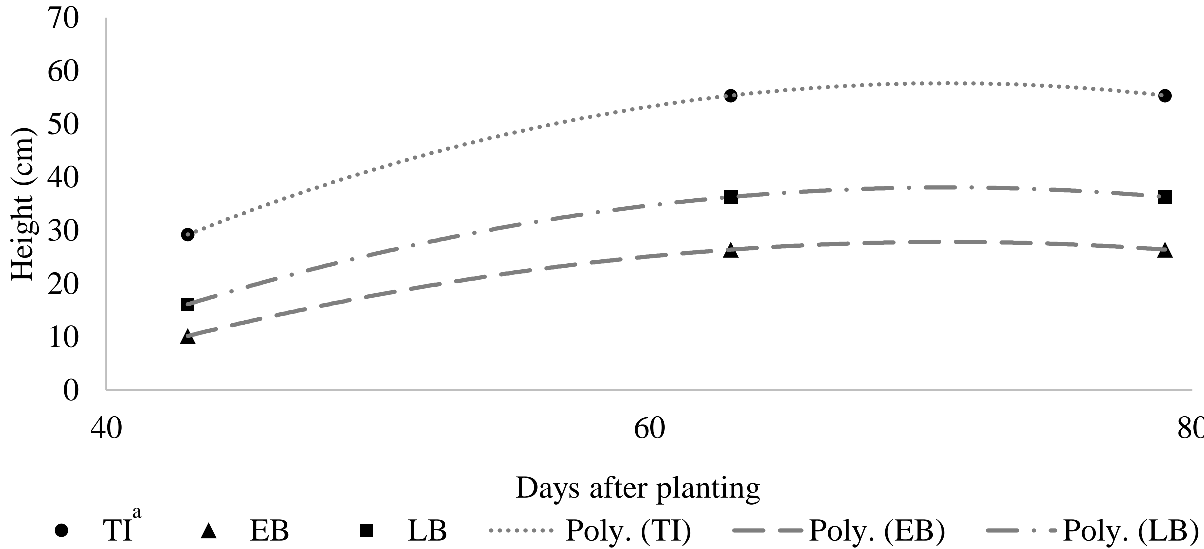

Figure 5.4. Average foliar height of plants grown from generation 1 daughter tubers evaluated at different times as a result of sub-lethal glyphosate dose applied at three different plant growth stages on mother-plants the previous season during production of seed tubers. Fargo, ND, 2015, combined over five sub-lethal glyphosate dosages (0, 26.5, 52.5, 105, and 210 g a.e. ha^{-1}) and three locations (Grand Forks, ND, greenhouse north, and greenhouse south).
Logistic regression for TI: $Y = -0.0363x^2 + 5.1507x - 125.18$ ($R^2 = 1$). Logistic regression for EB: $Y = -0.0225x^2 + 3.1992x - 85.719$ ($R^2 = 1$). Logistic regression for LB: $Y = -0.028x^2 + 3.9795x - 103.18$ ($R^2 = 1$).
[a] TI, EB, LB = tuber initiation, early tuber bulking, and late tuber bulking, respectively.

Analyzing foliar plant height for the interaction of evaluation time and different sub-lethal glyphosate doses indicated that as glyphosate increased, average foliar plant height decreased (Figure 5.5.). Maximum plant height was achieved approximately 70 DAP regardless of the glyphosate dose. Glyphosate doses of 105 and 210 g a.e. ha^{-1} applied to the mother plant had similar height measurements for plants grown from the generation 1 daughter tubers, representing the lowest recorded plant heights of 24, and 21 cm, respectively. The sub-lethal doses of 26.5 and 52.5 g a.e. ha^{-1} resulted in plants that were 20 and 38 % shorter when compared to the untreated.

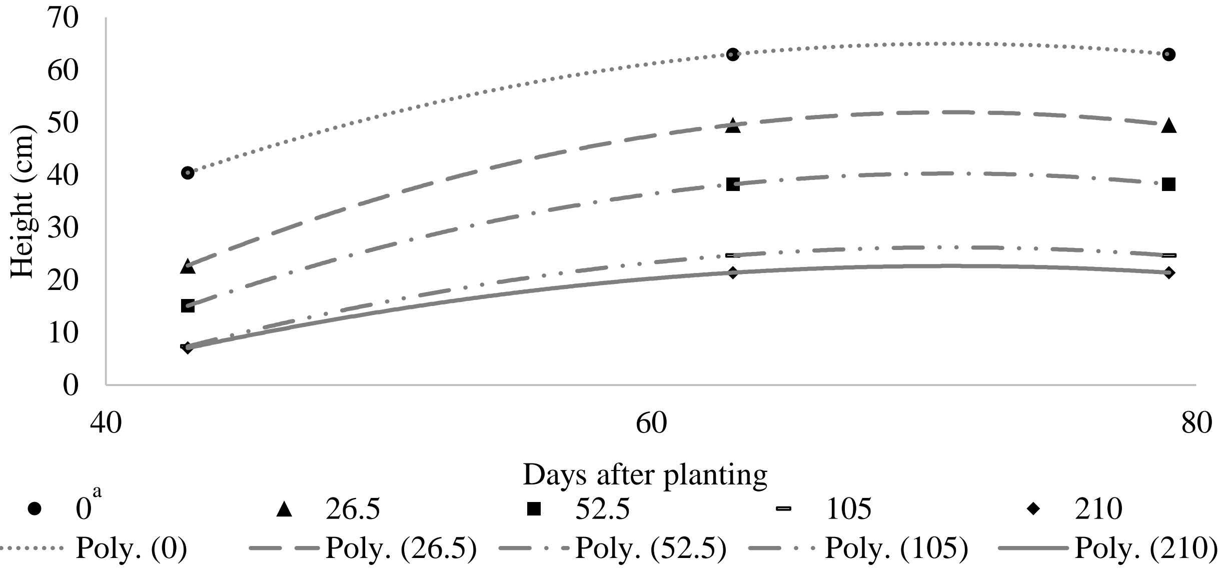

Figure 5.5. Average foliar height of plants grown from generation 1 daughter tubers evaluated at different times as a result of sub-lethal glyphosate dose applied on mother-plants the previous season during production of seed tubers. Fargo, ND, 2015, combined over three plant growth stages (Tuber initiation, early tuber bulking and late tuber bulking) three locations (Grand Forks, ND, greenhouse north, and greenhouse south).
Logistic regression for 0: $Y= -0.0313x^2 + 4.4495x – 92.99$ ($R^2 = 1$). Logistic regression for 26.5: $Y= -0.0372x^2 + 5.2855x – 135.71$ ($R^2 = 1$). Logistic regression for 52.5: $Y= -0.0322x^2 + 4.5675x – 121.87$ ($R^2 = 1$). Logistic regression for 105: $Y= -0.0241x^2 + 3.416x – 95.068$ ($R^2 = 1$). Logistic regression for 210: $Y= -0.0199x^2 + 2.8305x – 77.825$ ($R^2 = 1$).
[a] Glyphosate doses 0, 26.5, 55.5, 105, and 210 g a.e. ha^{-1}.

The interaction of sub-lethal glyphosate dose and the plant growth stage when the glyphosate dose was applied to the mother plants indicated that the ability of a glyphosate dose to stunt plants varied with the plant growth stage the mother plants received the glyphosate. Increasing glyphosate doses had the least effect on height of plants grown from the generation 1 daughter tubers, when the mother plant was subjected to the simulated glyphosate drift at the TI stage (Figure 5.6.). Early tuber bulking was the most affected PGS at all of the sub-lethal glyphosate doses. Plant height decreased 39 % at 26.5 g a.e. ha^{-1} compared to the untreated, while 52.5, 105, and 210 g a.e. ha^{-1} resulted in a 68, 92 and 100 % decrease, respectively.

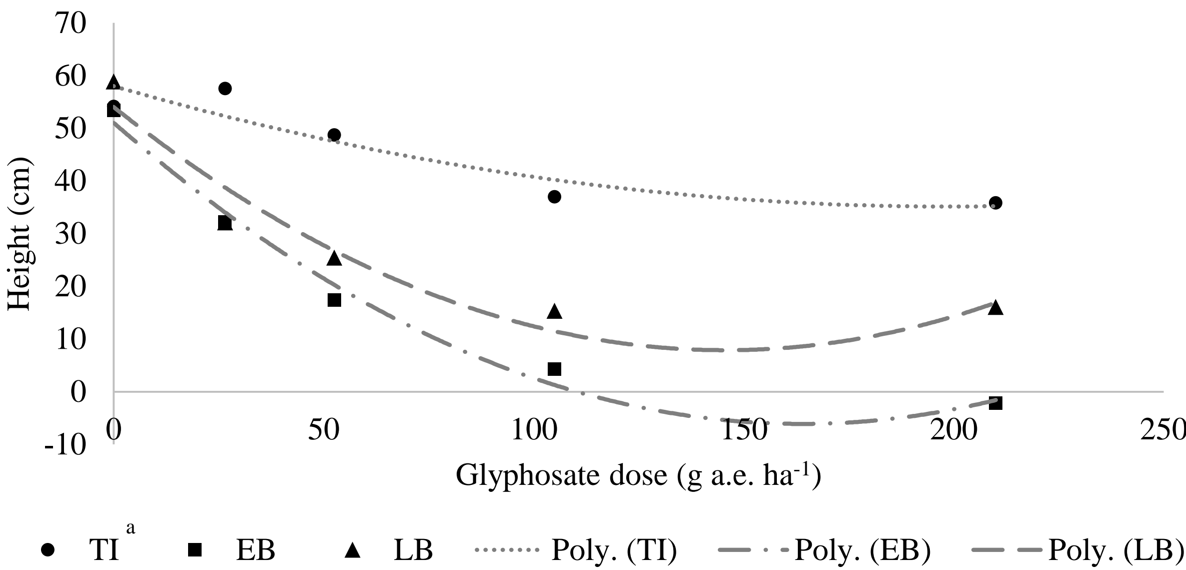

Figure 5.6. Average foliar height of plants grown from generation 1 daughter tubers for the interaction of plant growth stage and sub-lethal glyphosate dose applied to mother-plants the previous season during production of seed tubers. Fargo, ND, 2015, combined over evaluation times (43, 63, and 79 days after planting) three locations (Grand Forks, ND, greenhouse north, and greenhouse south).
Logistic regression for TI: Y= -0.0006x^2 – 0.2305x + 57.993 (R^2 = 0.8588). Logistic regression for EB: Y= -0.0022x^2 – 0.6336x + 54.021 (R^2 = 0.9326). Logistic regression for LB: Y= 0.0021x^2 – 0.6972x + 51.029 (R^2 = 0.9864).
[a] TI, EB, LB = tuber initiation, early tuber bulking, and late tuber bulking, respectively.

5.4.1.3. Stem number

Average stem number per plant grown from generation 1 daughter tubers was directly affected by plant emergence. If the treatment caused a number of plants to not emerge then a lower average stem number would be recorded. Stem number was influenced by glyphosate dose and the PGS the mother plant received the glyphosate dose. Glyphosate applied to mother plants at TI had no effect on average stem number for plants grown from generation 1 daughter tubers (Figure 5.7.). Since plant emergence was not drastically affected at this plant growth stage (Figure 5.1.) similar stem numbers were expected. Glyphosate doses 26.5 and 52.5 g a.e. ha^{-1} applied to the mother plants at EB and LB resulted in stem numbers comparable to the untreated.

However, higher glyphosate doses applied at either the EB or LB stage caused fewer stems per plant compared to the TI stage.

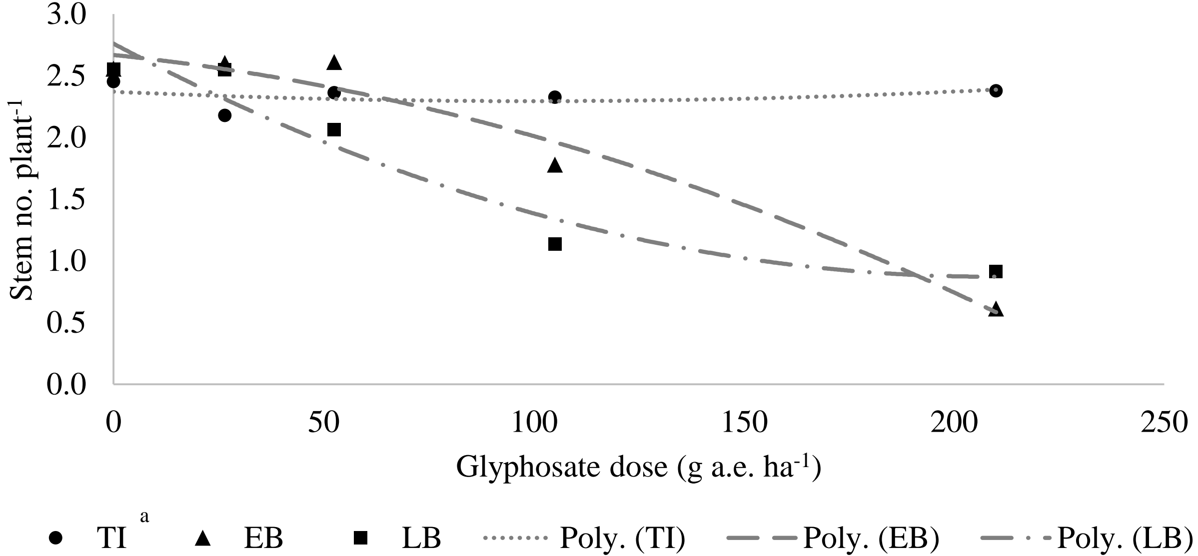

Figure 5.7 Average stem number per plant grown from generation 1 daughter tubers for the interaction of plant growth stage and sub-lethal glyphosate dose applied to mother-plants the previous season during production of seed tubers. Fargo, ND, 2015, combined over evaluation times (43, 63, and 79 days after planting) and three locations (Grand Forks, ND, greenhouse north, and greenhouse south).
Logistic regression for TI: Y= 8E-06x^2 – 0.0015x + 2.37 (R^2 = 0.1507). Logistic regression for EB: Y= -3E-05x^2 – 0.0035x + 2.6675 (R^2 = 0.9688). Logistic regression for LB: Y= 4E-05x^2 – 0.0181x + 2.759 (R^2 = 0.9342).
[a] TI, EB, LB = tuber initiation, early tuber bulking, and late tuber bulking, respectively.

5.4.2. Tuber Measurements

5.4.2.1. Tuber number

Tuber number per plant was directly related to number of developed stems per plant, which was directly related to plant emergence in this experiment. High plant emergence across all sub-lethal glyphosate doses applied to mother plants at TI, resulted in similar average tuber number per plant when compared to the untreated (Figure 5.8.). In contrast, no plant emergence at 210 g a.e. ha^{-1} glyphosate dose applied to the mother plant at EB stage resulted in no tuber production. The 105 g a.e. ha^{-1} glyphosate dose at the EB stage averaged 1 tuber per plant, while

52.5 and 26.5 g a.e. ha^{-1} glyphosate resulted in approximately half as many tubers as the untreated.

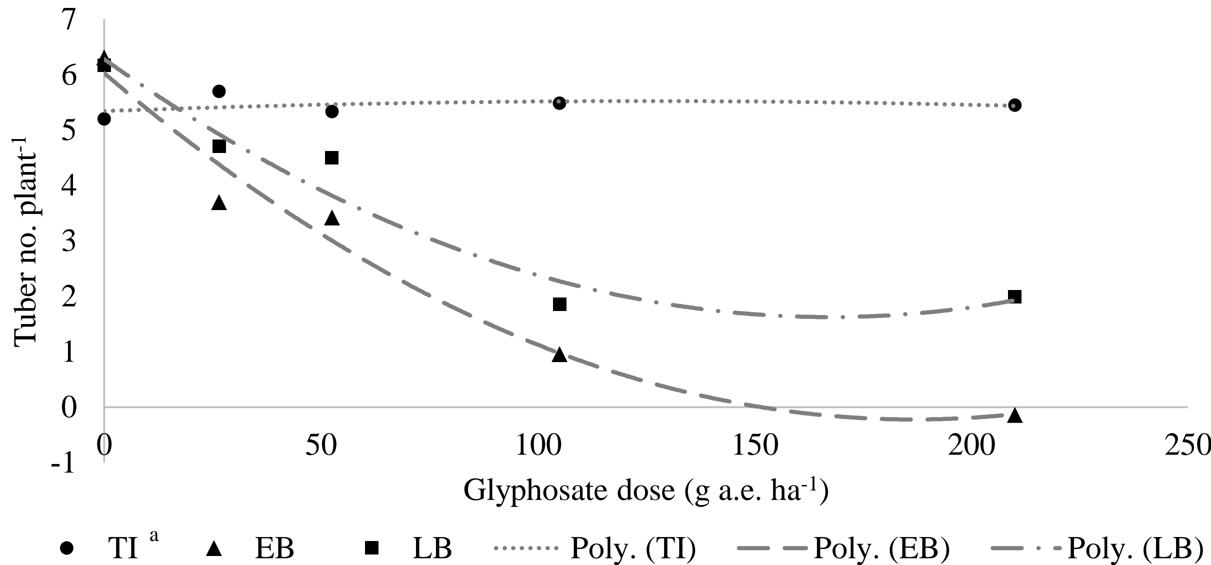

Figure 5.8. Average generation 2 daughter tuber number per plant grown from generation 1 daughter tuber for the interaction of plant growth stage and sub-lethal glyphosate dose applied to mother-plants the previous season during production of seed tubers. Fargo, ND, 2015, combined over three locations (Grand Forks, ND, greenhouse north, and greenhouse south).
Logistic regression for TI: Y= 1E-05x^2 + 0.00295x + 5.3403 (R^2 = 0.1237). Logistic regression for EB: Y= 0.0002x^2 – 0.0669x + 6.0242 (R^2 = 0.972). Logistic regression for LB: Y= 0.0002x^2 – 0.0556x + 6.2782 (R^2 = 0.9494).
[a] TI, EB, LB = tuber initiation, early tuber bulking, and late tuber bulking, respectively.

Sub-lethal glyphosate doses applied to the mother plants at LB resulted in a reduction of plant emergence and the similar reduction trend for tuber number per plant was expressed, with 26.5 and 52.5 g a.e. ha^{-1} reducing tubers per plant by 2 when compared to the untreated with 6 tubers per plant. Glyphosate doses 105 and 210 g a.e. ha^{-1} resulted in a third of tubers per plant compared to the untreated. The LB stage was not nearly as sensitive as EB, but also resulted in an undesirable reduction of tubers per plant.

5.4.2.2. Yield

Plant yield was influenced by the interaction of PGS and glyphosate dose. Foliar plant development is important for the production of tubers. Although TI displayed tolerance to

simulated glyphosate drift with similar plant emergence, plant height, stem number and tuber number as the untreated, this did not translate to comparable yields to the untreated (Figure 5.9.). At TI, the lowest glyphosate dose provided a minor spike in yield compared to the untreated, however each subsequent dose reduced yield further. The 210 and 105 g a.e. ha^{-1} glyphosate doses resulted in approximately 42 % lower yields compared to the untreated. At the EB stage, no sub-lethal glyphosate dose was comparable to the untreated. A yield decrease of 63 to 100 % occurred as glyphosate dose increased. Simulated glyphosate drift at the LB stage resulted in 50-75 % yield loss when compared to the untreated.

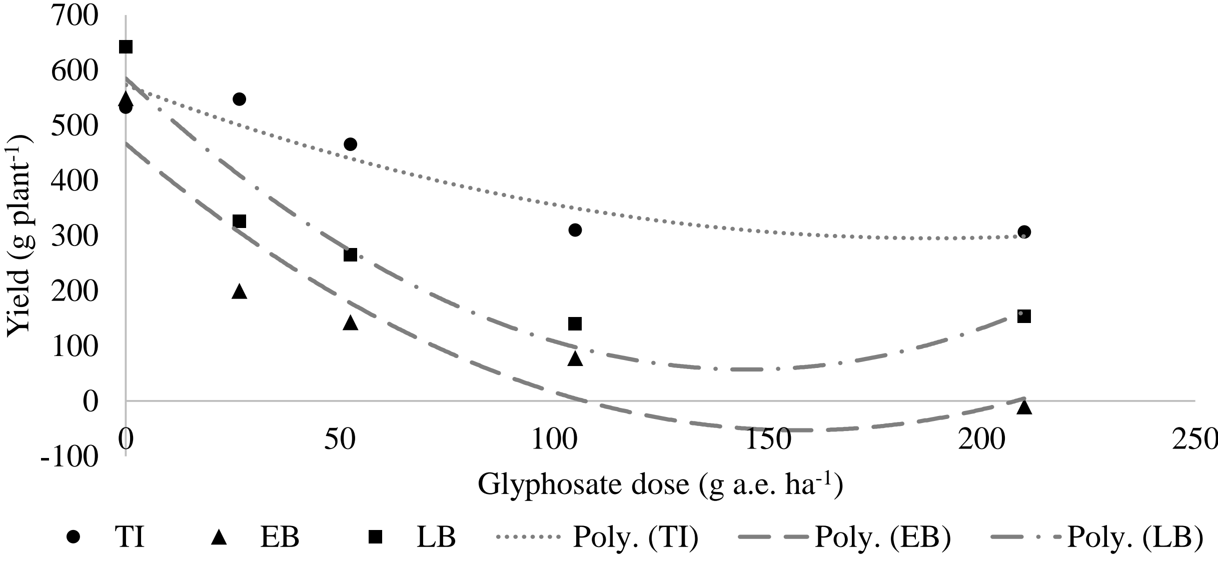

Figure 5.9. Average generation 2 daughter tuber yield per plant grown from generation 1 daughter tubers for the interaction of plant growth stage and sub-lethal glyphosate dose applied to mother-plants the previous season during production of seed tubers. Fargo, ND, 2015, combined over three locations (Grand Forks, ND, greenhouse north, and greenhouse south). Logistic regression for TI: $Y= 0.0078x^2 – 2.9507x + 573.05$ ($R^2 = 0.8888$). Logistic regression for EB: $Y= 0.0209x^2 – 6.5918x + 466.39$ ($R^2 = 0.8637$). Logistic regression for LB: $Y= 0.025x^2 – 7.2602x + 584.78$ ($R^2 = 0.926$).
[a] TI, EB, LB = tuber initiation, early tuber bulking, and late tuber bulking, respectively.

5.4.2.3. Unmarketable tubers

Unmarketable tubers were not analyzed for this experiment, although damaged tubers were found. It was suspected that they likely developed from glyphosate that drifted during the current growing season.

5.5. Discussion

The data from the three locations were combined for this seed study based on low variation between locations, despite the differing results found with mother plants amongst the field experiments (Chapter 3.) and the greenhouse experiments (Chapter 4.). Although the greenhouse experiment did not produce differences from the sub-lethal glyphosate doses, the daughter tubers used as seed had the same results as the daughter tubers harvested from the field experiment. This reconfirms that even when glyphosate drift damage was unnoticeable or unidentified, its effects will compromise the production of sound and healthy seed.

All glyphosate doses to mother plants resulted in seed that emerged slower or not at all when compared to untreated seed. Plant emergence declined as the sub-lethal glyphosate dose increased. Plant emergence for PGS ranked highest to lowest in tolerance to glyphosate was TI, LB, and EB. Similar results were reported by Hutchinson et al. (2014) who found mid-bulking to be the most sensitive plant growth stage to glyphosate drift. Generation 1 daughter tubers harvested from mother plants that received glyphosate during the TI stage was more tolerant to the glyphosate at the lower doses, however, 105 g a.e. ha^{-1} resulted in only 68 % plant emergence. Hatterman-Valenti (2014) observed plants treated with the highest glyphosate dose of 282 g a.e. ha^{-1} during mid-bulking had seed that expressed no sprout emergence throughout the entire growing season, similar to EB within this study.

Seedling vigor may inferred from this data based on the emergence evaluation at 43 DAP, since sprout emergence typically occurs between 14 and 30 DAP depending on weather. All three experiments were planted shallow to promote emergence, with ideal weather conditions (average temperature of 20 °C, and 30 mm total rainfall accumulation, between planting and 23 DAP) to promote germination, emergence and vigor. With this environment, all sound and healthy seed should have emerged by the evaluation at 43 DAP, however only TI had reached ≥ 80 %. Thus seed from mother plants that received glyphosate drift at early or late tuber bulking will not meet industry expectations for emergence and seedling vigor. Hutchinson et al. (2014) reported that seed from 'Ranger Russet' mother plants receiving a glyphosate drift at the mid-bulking stage had poor emergence and low seedling vigor.

Seed pieces that did not emerge were dug up for observations. The majority of these seed pieces were still intact and had not disintegrated after 80 days in the soil. The non-emerged seed pieces generally formed a large number of small, spindly sprouts at each eye, but these sprouts never reached the soil surface. More compacted cauliflower-curd sprouts were also seen, identical to symptoms described by Smid and Hiller (1981) and Worthington (1985), and also reported by Hatterman-Valenti (2014). Robinson and Hatterman-Valenti (2013) describe high levels of glyphosate causing "cauliflower" formation of shoots, moderate levels causing "candelabra" formation, and low levels causing a weakened plant. All of these symptoms were observed in this experiment. Hutchinson et al. (2014) found similar results that reinforced stem number remained stable across glyphosate doses at an early growth stage (TI), but had a linear decrease for later growth stages.

Glyphosate exposure to mother plants earlier in the season tended to have a lower effect on generation 2 yield from the plants grown from generation 1 daughter tubers used a seed

(Hutchinson et al. 2014). In most cases as glyphosate dose increased, the yield decreased (Hutchinson et al. 2014; Hatterman-Valenti 2014; Robinson and Hatterman-Valenti 2013), but a minimum dose of 71 g a.e. ha^{-1} glyphosate was required to result in a yield reduction (Smid and Hiller 1981). Glyphosate was also shown to reduce wheat yield up to 97 % when seed was used that had glyphosate applied to plants prior to harvest (Yenish and Young 2000).

Hutchinson et al. (2014) found an increase in culls of generation 2 daughter tubers as glyphosate dose increased, but this was only different during the mid-bulking growth stage. The plots were situated on the NDSU research farm where a number of glyphosate tolerant crops were planted in close proximity. In order to confidently identify tuber damage in the third generation seed, the seed would need to be planted in a glyphosate-free environment. However, finding an environment without glyphosate in the United States upper Midwest may be difficult to find, short of evaluating this in a controlled environment. A study completed in Mississippi, Iowa and Indiana observed glyphosate in > 60 % of the air and rain samples, having the highest concentrations when samples were collected during intense glyphosate application periods (Chang et al. 2011). Since one can assume that glyphosate is prevalent in the atmosphere in locations where it is widely used in agriculture, it is understandable that it could easily inflict unknown damage to a sensitive crop like potato.

5.6. Conclusion

The results from this experiment emphasize the importance of avoiding glyphosate drift on a 'Red Noland' potato field grown for seed production during the plant growth stages of early and late tuber bulking. A glyphosate drift event occurring during this time would be from glyphosate applied as a desiccant in many registered crops such as barley, flax, lentils, peas, safflower, sunflower, and wheat. Glyphosate promotes crop drying and even maturation for these

crops at harvest. However due to the determinate growth habit of the Red Norland cultivar, a glyphosate drift event occurring after tuber initiation becomes undetectable by foliar chlorosis and may go unnoticed.

Glyphosate drift on a certified seed production field may not only be undetectable, but also detrimental to the quality of the seed. A certified seed grower could jeopardize their reputation by selling seed that was compromised by glyphosate drift, as the commercial grower would have to overcome the problems associated with glyphosate residues in the seed after the seed pieces were planted. Depending on the glyphosate dose and timing of drift, poor emergence would result, and cause a variable and sporadic plant stand, which could cause weed problems, increased soil temperature on the hill, variability in tuber size, misshapen tubers, loss of soil moisture and inefficient use of nutrients. Weak seed with low vigor generally produces a weaker plant, allowing for pathogens to invade and cause infection, putting the entire field at risk for a disease epidemic.

The detection of glyphosate residue within the seed can only be positively confirmed by lab testing, which is time consuming and costly. Although services like that are available, it is not a mandatory requirement from the state seed department. The most important and effective management strategy is to prevent glyphosate from drifting onto ‘Red Norland’ fields that are destined to be certified seed. Techniques such as communicating with neighbors who use glyphosate in their production system, planting a border buffer crop, and if possible have a dedicated sprayer to apply potato specific products in potato fields to avoid tank contamination, should be used to reduce the risk of glyphosate contamination in certified seed fields.

5.7. Literature Cited

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CHAPTER 6. ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) METHOD FOR DETECTION OF GLYPHOSATE RESIDUE IN 'RED NORLAND' POTATO TUBERS

6.1. Abstract

The presence of glyphosate residue in 'Red Norland' potato tubers can only be positively confirmed through laboratory analysis. Many different analytical methods have been used successfully over the last 20 years, however with the development of an enzyme-linked immunosorbent assay (ELISA) to detect glyphosate, it may become the chosen technique in the near future. Information regarding glyphosate contamination is important for consumers as well as for commercial growers, as glyphosate drift has been shown to reduce emergence in affected seed. Simulated glyphosate drift was applied to mother plants during field experiments in 2014 and 2015. Sub-lethal glyphosate doses were applied during three crucial tuber development stages, tuber initiation (TI), early tuber bulking (EB) and late tuber bulking (LB).

Results of the offspring differed between years, possibly due to the storage of the samples. In 2014, regardless of dose, glyphosate applied to mother plants during TI, resulted in accumulation of < 0.2 ppm of glyphosate residue in the daughter tubers. While doses applied during EB and LB resulted in > 1.5 ppm at the highest dose applied. In 2015, glyphosate doses applied during TI resulted in accumulation similar to EB, reaching 0.5 ppm at the dose of 105 g a.e. ha^{-1}. The same dose applied during LB had the least amount of residue accumulation at < 0.2 ppm. Glyphosate residue was also detected in all untreated samples, which suggests a prevalence of glyphosate drift. More research should be completed to discover the repercussions on sensitive crops like potato. These are considered preliminary results and indicate that more work needs to be completed to perfect the immunoassay procedure process.

6.2. Introduction

As described in Chapter 3, “Evaluation of ‘Red Norland’ seed exposed to simulated glyphosate drift”, seed contaminated by glyphosate caused a number of problems in production. The detection of glyphosate in the seed prior to seed sales or field planting is key to avoiding production issues. Glyphosate residue in potatoes sold for the fresh market should also be addressed, as potatoes are not a processed food, and glyphosate would be present in the tuber tissue that consumers ingest. February of 2016, Newsweek reported that the U.S. Food and Drug Administration would begin testing food for glyphosate residue later in the fiscal year (Schlanger 2016).

Many analytical methods have been used to successfully detect glyphosate residue in crops over the last 20 years (Stalikas and Konidari 2001). Methods such as gas (GC) and liquid (HPLC) chromatographic techniques continue to be the most popular, and most laboratories are outfitted with appropriate equipment. However, the development of immunoassays may become the chosen technique in the near future, since it’s efficient, affordable, and less time-consuming than GC or HPLC methods.

The objective of this study was to evaluate tubers harvested from mother-plants that were exposed to simulated glyphosate drift as sub-lethal doses during tuber development utilizing the enzyme-linked immunosorbent assay (ELISA) method. The contaminated seed were subjected to a lab analysis to identify glyphosate presence as well as attempt to determine a threshold for the cultivar ‘Red Norland’ where glyphosate does not affect the tubers when used as certified seed.

6.3. Materials and Methods

6.3.1. Experimental design and preparation

Tuber samples collected from two years of field experiments were used to test for glyphosate residue. Field experiments were conducted in 2014 and 2015 at the Northern Plains Potato Growers Association (NPPGA) Research Farm, located five miles south of Grand Forks, North Dakota. Sub-lethal glyphosate doses were applied in combination with different plant growth stages and replicated three times in the field. Glyphosate treatments were 210, 105, 52.5, 26.5, and 0 g a.e. ha^{-1}. The doses are fractions (1/4, 1/8, 1/16, 1/32) based off the standard field use of 840 g a.e. ha^{-1}. Treatments were applied to the mother plants during three crucial plant growth stages (PGS) tuber initiation (TI), early tuber bulking (EB), and late tuber bulking (LB) during the production of seed potato tubers. Samples of daughter-tubers from two plants were harvested at the end of the growing season, 93 and 90 days after planting (DAP), for 2014, and 2015, respectively, and placed into a household freezer to halt tuber respiration.

Tuber samples were removed from the freezer unit and thawed prior to sample homogenization. An unidentified and inconsistent portion of all tubers from each EU was placed into a food processor and blended thoroughly. Approximately 20 g of the homogenized sample was placed into a disposable plastic container with the capacity of 28 g, then the samples were placed back into the freezer until all EU's were prepared to this step. Four 96 well microtiter plates were used, with six samples, one control and 25 samples triplicated within the plate. Plates 1 and 2 were completed 30 November 2015, and 11 January 2016, respectively, while plates 3 and 4 were completed simultaneously on 12 January, 2016.

Highly-detailed procedures were attained from the assay developer Abraxis LLC (124 Railroad Drive, Warminster, PA). An extraction procedure was completed on all samples prior to

introduction to the plate. The homogenized samples were removed from the freezer the evening prior to the procedure to allow thawing. A 10 g homogenized sample was weighed and placed into a vial, and 10 mL 1 N hydrochloric acid was added before holding for 2 min on a vortex mixer. The sample was allowed to separate for a minimum of 2 min, before removing 1 mL supernatant and placing into a micro-centrifuge tube. The samples were centrifuged for 5 min at 6000 rpm before 40 µL of the sample supernatant was placed into a 4 mL glass vial with 3.96 mL of Glyphosate Diluent and vortexed. Sample derivatization and addition of plate additive steps were followed according to the Abraxis procedures found in Appendix B Supplementary Material for ELISA Procedure.

6.3.2. Evaluation of treatments

The measurement of absorbance within the microtiter plate was completed with a spectrophotometer ELISA reader at 450 nm wavelength. Multimode Detection Software was used to measure and calculate the raw data. A solver worksheet provided by Abraxis was used to input the raw data and calculate the R^2- value based off the standards as well as the % CV and detected glyphosate (ng/mL) for all samples. The glyphosate concentration in the samples was determined by multiplying the ELISA results by a factor of 180 (based on the sample preparation and the natural water content (80 %) of potatoes). Samples showing a concentration lower than 0.075 ppb (0.000075 ppm) should be reported as containing < 13.5 ppb (0.0135 ppm) of glyphosate. Samples reporting a higher concentration than 4.0 ppb (0.004 ppm) should be reported as containing > 720 ppb (0.72 ppm). Any result found outside of the parameters was reported as a missing data point. In these cases, the samples should be further diluted and re-analyzed to obtain an accurate quantitative result, however the samples were destroyed with the first attempt and re-analyzation was not an option.

6.3.3. Data analysis

The data from each year were not combinable after testing homogeneity of variance confirmed variance ratio differed by more than 10, using the 10-fold f-test method, which proportions the largest variance of the treatment groups to the smallest and compares it to a critical value table (Tabachnik and Fidell 2001). The data collected were analyzed and reported separately. Data was analyzed using PC SAS 9.3 (Statistical Analysis Software, version 9.3. SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513). Repetitions were considered random and treatments fixed. A significance level of $P < 0.05$ was used for all characteristics measured. Linear regression analysis was completed for treatment separation.

6.4. Results

6.4.1. Glyphosate residue 2014

The standards supplied in the ELISA kit have limits of detectability between 0.0135 and 0.720 ppm. The results for 2014 samples exceeded the plate limitations, which reduced the accuracy and reliability of the results. The 2014 samples were completed in ELISA plate 1 and 2, which resulted in variability of analysis (Table 6.1.). The high CV % in standards 0.0005 and 0.001 ppm in plate 2 also reduced accuracy of the samples.

In 2014, 45 samples, subjected to different sub-lethal glyphosate doses at different plant growth stages were harvested for glyphosate residue detection. Out of those 45 samples, 9 were untreated, however, glyphosate was detected in all 45 samples (Figure 6.1.).

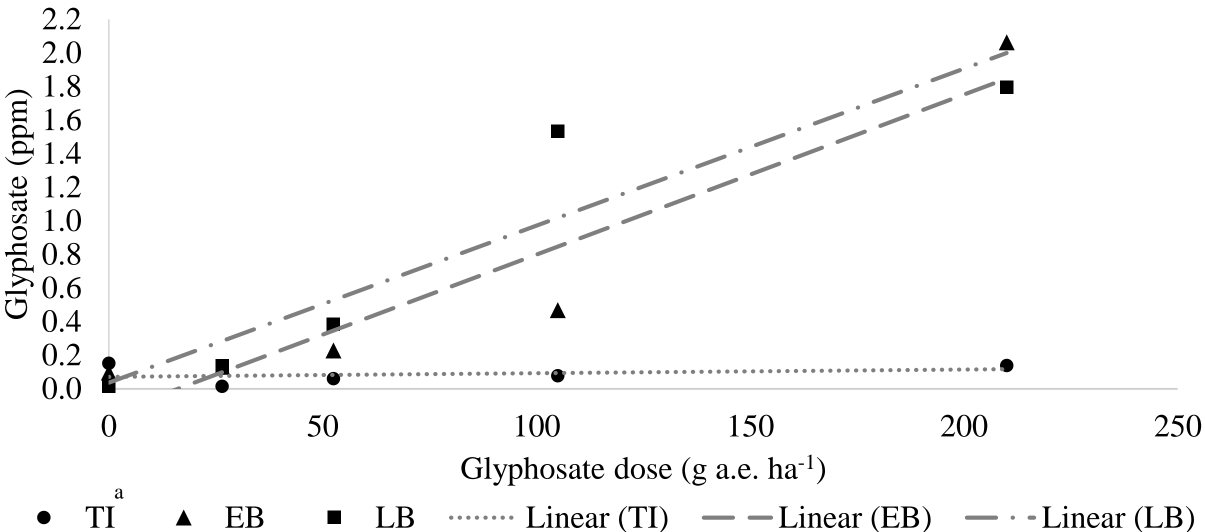

Figure 6.1. Glyphosate residue as a result of the interaction of sub-lethal dose and plant growth stage, Grand Forks, ND, 2014.
Logistic regression for TI: Y= 0.0002x + 0.0723 (R2 = 0.0996). Logistic regression for EB: Y= 0.0095x + 0.1525 (R2 = 0.903). Logistic regression for LB: Y= 0.0093x + 0.0367 (R2 = 0.8727).
[a] TI, EB, LB = tuber initiation, early tuber bulking, and late tuber bulking, respectively.

Any glyphosate dose applied to the mother plants during TI, resulted in lower levels of glyphosate residue accumulation within the daughter tubers, < 0.2 ppm. The highest glyphosate dose of 210 g a.e. ha^{-1} applied to the mother plants at EB resulted in the highest accumulation, where mother plants receiving the glyphosate dose at LB had a similar level of glyphosate residue detected at 105 g a.e. ha^{-1}. These results indicate that glyphosate drift occurring early in the season during TI, does not accumulate as much glyphosate within the tubers compared to glyphosate drift during EB or LB. This may explain why daughter-tubers from mother plants exposed to glyphosate doses at TI and used as seed were not affected compared to those from EB or LB stages (Chapter 5.).

6.4.2. Glyphosate residue 2015

The 2015 sample results were much different than the sample results from 2014. The glyphosate dose, 210 g a.e. ha^{-1}, was not displayed due to the high number of missing data for

that dose class. This was presumably due to the glyphosate level in the samples receiving 210 g a.e. ha^{-1} resulted in glyphosate residues above the limitation of detection (0.72 ppm) for the ELISA plate, and were reported as exceeding 0.72 ppm. The 2015 samples were completed in ELISA plates 3 and 4, and had acceptable CV values (Table 6.1.), but poor sensitivity between standards based on the B/B^0 ratio (Table 6.2.). This had an effect on the accuracy and reliability of the results.

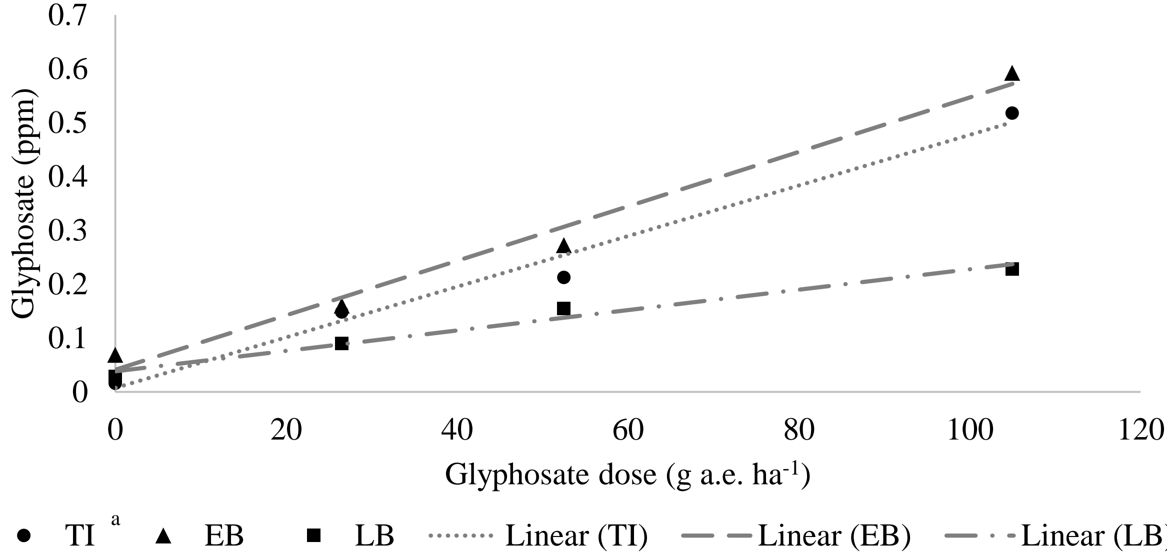

Figure 6.2. Glyphosate residue as a result of the interaction of sub-lethal dose and plant growth stage, Grand Forks, ND, 2015.
Logistic regression for TI: $Y= 0.0047x + 0.0074$ ($R^2 = 0.9826$). Logistic regression for EB: $Y= 0.0051x + 0.0411$ ($R^2 = 0.9834$). Logistic regression for LB: $Y= 0.0019x + 0.0385$ ($R^2 = 0.9789$).
[a] TI, EB, LB = tuber initiation, early tuber bulking, and late tuber bulking, respectively.

Similar to samples in 2014, even the untreated samples had low levels of glyphosate residue. Glyphosate residue was detected at higher levels for mother plants receiving glyphosate at TI in 2015 (Figure 6.2.) compared to 2014 (Figure 6.1.). Glyphosate accumulation in the daughter tubers for mother plants receiving glyphosate doses at LB was lower than TI and EB, but resulted in a reduction in performance when the same tubers were used as certified seed

(Chapter 5.). Mother plants receiving glyphosate at EB had the highest values of glyphosate residue accumulation in the daughter tuber samples.

6.4.3. ELISA Plate Validation

As with all immunoassays, a consistent technique is required to ensure optimal performance of the procedure. In order to achieve precision, each well must be treated in an identical manner. Coefficients of variation percentages (CV) are calculated for each standard and sample to determine the distribution of the sample replication within the plate. A CV % less than 10 is desired for this procedure. Executing the ELISA procedure for plates 2 and 4 resulted in large variation of the standard data, compared to plates 1 and 3, which could have compromised the results of glyphosate detected (Table 6.1.).

Table 6.1. Coefficients of variation for glyphosate standards and samples across four ELISA plates.

ELISA	Glyphosate standards (ppm)						Sample averages		
plate	0	0.00075	0.0002	0.0005	0.001	0.004	Min.	Max.	Ave.
	CV[a] (%)								
1	1.154	6.515	4.294	4.714	5.372	3.329	0.968	13.968	4.848
2	6.190	6.177	2.569	12.458	15.075	10.064	1.125	27.690	6.364
3	4.673	2.877	0.971	6.852	6.523	3.156	0.114	21.472	4.343
4	5.959	5.344	5.886	3.396	19.411	4.721	0.725	8.026	4.781

[a] coefficients of variation

The accuracy/sensitivity of the ELISA assay was also confirmed with the B/B_0 ratio based off of the zero standard provided in the kit. A value higher than 0.950 for the standard 0.00075, would mean the assay was not deciphering between 0 ppb and 0.00075 ppm very well. Plate 2 had the only B/B_0 value below 0.950, which means plates 1, 3 and 4 had non-ideal sensitivity between the standards (Table 6.2.). Plate 4 had the worst sensitivity, with virtually no differences between 0.00, 0.00075, and 0.0002 ppm standards. Poor assay sensitivity will have a negative effect on the accuracy of the plate and the results of the glyphosate detected.

Table 6.2. Sensitivity of ELISA assay for glyphosate standards across four ELISA plates.

ELISA plate	Glyphosate standards (ppm)					
	0	0.00075	0.0002	0.0005	0.001	0.004
	---------------------------------- B/B_0[a] ----------------------------------					
1	-	0.965	0.849	0.637	0.462	0.233
2	-	0.916	0.795	0.580	0.390	0.202
3	-	0.972	0.873	0.597	0.409	0.180
4	-	1.001	0.954	0.640	0.381	0.190

[a] B/B_0 = the average absorbance of the 0 ppb standard (B_o) divided by the average absorbance value of each standard (B).

A 4-parameter (Y= (A-D)/ (1+(X/C) ^B) +D)) logistic curve model was generated to fit the standard data points and calculate an R^2 value. Plates 1, 2, 3, and 4 resulted in R^2 values of 0.99964, 0.99955, 0.99480, and 0.99116, respectively. These values represent the variation of the standards among the curve, which are still acceptable but not ideal for plates 3 and 4.

6.5. Discussion

A study by Rubio et al. (2014), employed by Abraxis, LLC, completed a glyphosate residue experiment utilizing the Abraxis Glyphosate Plate, and positively confirmed the presence of glyphosate in honey, and soy sauce. They found 59 % of honey samples contained glyphosate at a mean of 0.064 ppm and 36 % of the soy sauce samples with a mean of 0.242 ppm. The determined limit of quantification (LOQ) for honey and soy sauce was 0.015 to 0.08 and 0.75 to 4 ppm, respectively. The soy sauce LOQ was the same limit available for detection of glyphosate residue within the potato ELISA completed in this experiment. Personal communication (18 January, 2016) with an Abraxis Sales Associate/procurement employee, explained that ELISA kit procedures completed in the Abraxis laboratory are all completed with a syringe-type electronic repeating pipette, ensuring accuracy within the plate and providing reliable results.

All samples analyzed in this experiment, including the untreated, had a detectable level of glyphosate residue present in the tissue sample, which suggested a high probability of glyphosate

drift in the agriculture system in the Red River Valley during peak application times. In 2014, the experimental plots were surround by a glyphosate tolerant variety of corn, and in 2015, surround by wheat that was desiccated late in the summer, which may have provided additional glyphosate exposure. Cessna et al. (2001) also found that although the untreated plots were not directly subjected to glyphosate, they had low levels of glyphosate detected.

Cessna et al. (1994) reported on wheat, and Cessna et al. (2001) on field pea, barley and flax, that at physiological maturity, glyphosate application dose and environmental conditions all played a part in the level of glyphosate residue detected. Similar conclusions were found in this study but with the stage of tuber development instead of physiological maturity.

6.6. Conclusion

The data sets had too much variation to combine for analysis, which could be the result of how the samples were handled. The 2014 samples, remained in the freezer unit for one year longer than the 2015 samples, and were also subjected to some freezer unit failure and potentially thawed before being refrozen. The freezer unit problem was resolved for the inclusion of the 2015 samples.

The inconsistent portion of tubers used could also have had a negative effect on the results obtained from the studies. Two samples varying in size with presumably the same amount of glyphosate residue in the tissue would result in different residue levels detected if half of each tuber was used, compared to the entire tuber. Utilizing a set portion of each tuber could have mitigated this inconsistency.

This experiment with an ELISA assay for detection of glyphosate residue was the first of its kind completed at NDSU and is considered preliminary results, which requires revision of procedures to achieve reliable results. With assurance of quality and precision, this method could

be implemented into state procedures to positively identify glyphosate residue within the LOQ and assist both certified seed growers and commercial growers to avoid planting glyphosate contaminated seed. It would also be a useful tool for the detection of glyphosate within the potato fresh market food system to ensure consumer safety. At this point a threshold value cannot be determined using the results from this experiment, but should be continued in order to identify a threshold value for certified seed.

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APPENDIX A. ANOVA TABLES

Chapter 3

Table A1. Type 3 Test of Fixed Effects. Foliar fresh weight, Grand Forks, ND, combined over two years (2014 and 2015).

Effect	Num DF	Den DF	F Value
PGS	2	70	2.13
Glyphosate dose	4	70	2.94 *
PGS x Glyphosate dose	8	70	1.09

Table A2. Type 3 Tests of Fixed Effects. Above ground plant height, Grand Forks, ND, combined over years (2014 and 2015).

Effect	Num DF	Den DF	F Value
PGS	2	70	9.15 *
Glyphosate dose	4	70	1.84
PGS x Glyphosate dose	8	70	1.11

Table A3. Type 3 Tests of Fixed Effects. Stem number, Grand Forks, ND, combined over years (2014 and 2015).

Effect	Num DF	Den DF	F Value
PGS	2	70	3.58 *
Glyphosate dose	4	70	1.39
PGS x Glyphosate dose	8	70	1.16
Time	4	4	0.10
PGS x Time	8	280	0.98
Glyphosate dose x Time	16	280	1.00
PGS x Glyphosate dose x Time	32	280	1.06

Table A4. Type 3 Tests of Fixed Effects. Average number of tuber per plant, Grand Forks, ND, combined over two years (2014 and 2015).

Effect	Num DF	Den DF	F Value
PGS	2	70	80.12 *
Glyphosate dose	4	70	3.40 *
PGS x Glyphosate dose	8	70	2.33 *
Time	4	4	1.66
PGS x Time	8	280	11.42 *
Glyphosate dose x Time	16	280	3.78 *
PGS x Glyphosate dose x Time	32	280	3.2 *

Table A5. Type 3 Tests of Fixed Effects. Average yield per plant, Grand Forks, ND, combined over two years (2014 and 2015).

Effect	Num DF	Den DF	F Value
PGS	2	70	272.86 *
Glyphosate dose	4	70	2.83 *
PGS x Glyphosate dose	8	70	0.87
Time	4	4	28.29 *
PGS x Time	8	280	24.46 *
Glyphosate dose x Time	16	280	2.07 *
PGS x Glyphosate dose x Time	32	280	2.00 *

Table A6. Type 3 Tests of Fixed Effects. Average number of unmarketable tubers per plant, Grand Forks, ND, combined over two years (2014 and 2015).

Effect	Num DF	Den DF	F Value
PGS	2	70	168.38 *
Glyphosate dose	4	70	21.80 *
PGS x Glyphosate dose	8	70	13.92 *
Time	4	4	9.93 *
PGS x Time	8	280	56.81 *
Glyphosate dose x Time	16	280	6.90 *
PGS x Glyphosate dose x Time	32	280	3.88 *

Chapter 4

Table A7. Type 3 Tests of Fixed Effects. Average foliar fresh weight per plant, combined across greenhouse locations (2014).

Effect	df	MS	F Value
PGS	2	136758.8	0.97
Glyphosate dose	4	3675.2	2.29
PGS x Glyphosate dose	8	7075.8	0.72

Table A8. Type 3 Tests of Fixed Effects. Average foliar length per plant, combined across greenhouse locations (2014).

Effect	df	MS	F Value
PGS	2	2809.1	2.28
Glyphosate dose	4	1127.2	5.91
PGS x Glyphosate dose	8	527.7	2.64

Table A9. Type 3 Tests of Fixed Effects. Average stem number per plant, combined across greenhouse locations (2014).

Effect	df	MS	F Value
PGS	2	6.9	3.73
Glyphosate dose	4	1.7	0.63
PGS x Glyphosate dose	8	3.9	0.32

Table A10. Type 3 Tests of Fixed Effects. Average tuber number per plant, combined across greenhouse locations (2014).

Effect	df	MS	F Value
PGS	2	154.8	2.58
Glyphosate dose	4	69.6	1.65
PGS x Glyphosate dose	8	22.2	1.77

Table A11. Type 3 Tests of Fixed Effects. Average yield per plant, combined across greenhouse locations (2014).

Effect	df	MS	F Value
PGS	2	35000.2	0.12
Glyphosate dose	4	19521.4	0.32
PGS x Glyphosate dose	8	43590.9	1.31

Table A12. Type 3 Tests of Fixed Effects. Average number of unmarketable tubers per plant, combined across greenhouse locations (2014).

Effect	df	MS	F Value
PGS	2	61.8	2.03
Glyphosate dose	4	87.9	3.18
PGS x Glyphosate dose	8	24.9	1.53

Chapter 5

Table A13. Type 3 Tests of Fixed Effects. Emergence evaluated at 23, 43, 63, and 79 DAP, Fargo, ND, 2015, combined across locations (Grand Forks, ND, greenhouse north, and greenhouse south).

Effect	df	MS	F Value
Type	6	3.83	43.37 *
Damage	1	3.87	43.86 *
Time	3	41.01	370.86 *
PGS	2	50.62	52.18 *
Time x PGS	6	0.86	6.35 *
Glyphosate dose	4	184.90	45.41 *
Time x Glyphosate dose	12	2.37	17.46 *
PGS x Glyphosate dose	8	5.51	5.28 *
Time x PGS x Glyphosate dose	24	1.39	10.22 *

Table A14. Type 3 Tests of Fixed Effects. Foliar height evaluated at 43, 63, and 79 DAP, Fargo, ND, 2015, combined across locations (Grand Forks, ND, greenhouse north, and greenhouse south).

Effect	df	MS	F Value
Type	6	11467.00	45.53 *
Damage	1	20788.00	82.54 *
Time	3	196156.00	140.64 *
PGS	2	206600.00	45.91 *
Time x PGS	4	3758.36	18.46 *
Glyphosate dose	4	183379.00	38.77 *
Time x Glyphosate dose	8	2174.27	10.68 *
PGS x Glyphosate dose	8	18066.00	3.72 *
Time x PGS x Glyphosate dose	16	1299.27	6.39 *

Table A15. Type 3 Tests of Fixed Effects. Stem number per plant, Fargo, ND, 2015, combined across locations (Grand Forks, ND, greenhouse north, and greenhouse south).

Effect	df	MS	F Value
Type	6	44.38	29.56 *
Damage	1	8.55	5.70 *
PGS	2	25.67	8.90 *
Glyphosate dose	4	62.68	21.18 *
PGS x Glyphosate dose	8	21.83	7.28 *

Table A16. Type 3 Tests of Fixed Effects. Tuber number per plant, Fargo, ND, 2015, combined across locations (Grand Forks, ND, greenhouse north, and greenhouse south).

Effect	df	MS	F Value
Type	6	51.05	4.00 *
Damage	1	91.02	7.14 *
PGS	2	686.95	27.20 *
Glyphosate dose	4	473.98	18.27 *
PGS x Glyphosate dose	8	158.41	6.02 *

Table A17. Type 3 Tests of Fixed Effects. Average yield per plant, Fargo, ND, 2015, combined across locations (Grand Forks, ND, greenhouse north, and greenhouse south).

Effect	df	MS	F Value
Type	6	1139637.00	12.56 *
Damage	1	1260270.00	13.89 *
PGS	2	5876573.00	25.82 *
Glyphosate dose	4	6910951.00	29.41 *
PGS x Glyphosate dose	8	639667.00	2.68 *

Table A18. Type 3 Tests of Fixed Effects. Unmarketable tuber number per plant, Fargo, ND, 2015, combined across locations (Grand Forks, ND, greenhouse north, and greenhouse south).

Effect	df	MS	F Value
Type	6	14.16	4.41 *
Damage	1	38.23	11.90 *
PGS	2	56.22	10.38 *
Glyphosate dose	4	54.50	9.83 *
PGS x Glyphosate dose	8	16.37	2.92 *

Chapter 6

Table A19. Type 3 Tests of Fixed Effects. Glyphosate residue detected in ‘Red Norland’ potato tuber samples, Grand Forks, ND, 2014.

Effect	df	MS	F Value
PGS	2	4.62	16.91 *
Glyphosate dose	4	5.42	19.86 *
PGS x Glyphosate dose	8	1.98	7.28 *

Table A20. Type 3 Tests of Fixed Effects. Glyphosate residue detected in ‘Red Norland’ potato tuber samples, Grand Forks, ND, 2015.

Effect	df	MS	F Value
PGS	2	0.19	63.45 *
Glyphosate dose	4	0.69	235.76 *
PGS x Glyphosate dose	8	0.05	18.35 *

APPENDIX B. SUPPLEMENTARY MATERIAL FOR ELISA PROCEDURE

1. **Intended Use**
   For the detection of Glyphosate in potatoes.
2. **Sensitivity**
   13.5 ppb in matrix
3. **Materials and Reagents Required**
   Analytical balance
   40 mL glass vials with Teflon-lined caps
   Microcentrifuge tubes
   4 mL glass vials with Teflon-lined caps
   Disposable pipettes
   Scoopula
   Blender or food processor
   Micropipettes with disposable plastic tips
   Vortex mixer
   Microcentrifuge
   Timer
   Plate shaker or Micro-well plate holder with insert retainer for vortex mixer
   1 N Hydrochloric Acid (HCl)
   Glyphosate sample diluent
   Abraxis Glyphosate Plate ELISA kit
4. **Notes and Precautions**
   This procedure is intended for use with potatoes. Other matrices should be thoroughly validated before use with this procedure.
   Samples much be homogenized before extraction. To prepare samples, place entire potato, including skin, into blender or food processor (large potatoes should be chopped into pieces before placing in blnder or food processor). Blend thoroughly.
   Hydrochloric Acid must be handled with care. Wear appropriate protective clothing (gloves, glasses, etc). Avoid contact with skin and mucous membranes. If contact occurs, wash with copious amounts of water and seek appropriate medical attention.
   Due to the viscous nature of the sample extracts, the microtiter plate should be placed on a plate shaker or vortex mixer fitted with a micro-plate holder adapter for the incubations with the antibody and conjugate solutions. This will allow for the appropriate mixing of all reagents in the microtiter wells.
5. **Extraction Procedure**
   a. Weight 10 g of homogenized sample into an appropriately labeled 40 mL glass vial.
   b. Add 10 mL of 1 N HCl. Vortex for 2 minutes.
   c. Allow the sample to separate for 2 minutes.
   d. Pipette approximately 1 mL of the supernatant into an appropriately labeled microcentrifuge tube.
   e. Centrifuge at 6000 rpm for 5 minutes.
   f. Pipette the supernatant into an appropriately labeled 4 mL glass vial with a Teflon-lined cap.

Figure B1. Glyphosate in Potatoes Sample Preparation

g. Add 3.96 mL of Glyphosate Diluent to a clean, appropriately labeled 4 mL glass vial. Add 40 µL of the supernatant (from step f) to the Glyphosate Diluent in the vial (1:100 sample dilution). Vortex. This will then be analyzed as sample, see *Derivatization of Standards, Control, and Samples* in the Reagent Preparation section of the Glyphosate Plate ELISA kit user's guide.

6. **Evaluation of Results**
The glyphosate concentration in the samples is determined by multiplying the ELISA results by a factor of 180 (based on sample preparation procedure and natural water content (80%) of potatoes). Sample extracts showing a concentration lower than standard 1 (0.075 ppb) should be reported as containing < 13.5 ppb of glyphosate. Samples showing a higher concentration than standard 5 (4.0 ppb) can be reported as containing > 720 ppb of glyphosate or diluted further and reanalyzed to obtain an accurate quantitative result.
7. **Performance Data**
Potato samples were spiked with various amounts of glyphosate, prepared as described above, and then derivatized and assayed using the Glyphosate Plate Assay. Average recovery was 123%.
8. **Assistance**

For ordering or technical assistance contact:
Abraxis LLC
54 Steamwhistle Drive
Warminster, PA 18974
Tel: (215) 357-3911
Fax: (215) 357-5232
Email: infor@abraxiskits.com
Web: www.abraxiskits.com

Figure B1. Glyphosate in Potatoes Sample Preparation (continued)

→ Glyphosate Plate

• Intended Use

For the detection and quantitation of glyphosate in water (groundwater, surface water, well water). For soil, crop, and food use contact the company for application bulletins and/or specific matrix validation guidelines.

• Principle

The Abraxis Glyphosate Plate Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of glyphosate. The sample (please refer to reagent preparation section) to be tested is derivatized and then added, along with an antibody specific for Glyphosate to microtiter wells coated with Goat Anti-Rabbit Antibody and incubated for 30 minutes. The glyphosate enzyme conjugate is then added. At this point a competitive reaction occurs between the glyphosate, which may be in the sample, and the enzyme labeled glyphosate analog for the antibody binding sites on the microtiter well. The reaction is allowed to continue for sixty (60) minutes. After a washing step and addition of a substrate (color solution), a color signal (blue color) is generated.

The presence of glyphosate is detected by adding the "Color Solution", which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled glyphosate bound to the glyphosate antibody catalyzes the conversion of the substrate/chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of a diluted acid (Stopping Solution). Since the labeled glyphosate (conjugate) was in competition with the unlabeled glyphosate (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of glyphosate in the sample.**

• Reagents

The Abraxis Glyphosate Plate Kit contains the following items:

1. *Microtiter Plate coated with Goat Anti Rabbit Antibody*
   96 test kit: 8 X 12 strips
2. *Glyphosate Antibody Solution*
   Glyphosate antibody (rabbit anti-glyphosate) solution in a buffered saline solution with a non-mercury preservative and stabilizers.
   96 test kit: one 6 mL vial
3. *Glyphosate Enzyme Conjugate*
   Horseradish peroxidase (HRP) labeled glyphosate analog diluted in a buffered solution with a non-mercury preservative and stabilizers.
   96 test kit: one 6 mL vial
4. *Glyphosate Standards*
   Six concentrations (0, 0.075, 0.2, 0.5, 1.0, 4.0 ppb) of glyphosate standards in distilled water with a non-mercury preservative and stabilizers.
   96 test kit: one 2 mL vial each
5. *Control*
   A concentration (approximately 0.75 ppb) of glyphosate in distilled water with a non-mercury preservative and stabilizers.
   96 test kit: one 2 mL vial
6. *Diluent/Zero Standard (Sample Diluent)*
   Distilled water with a non-mercury preservative and stabilizers without any detectable glyphosate.
   96 test kit: one 30 mL vial
7. *Color Solution*
   A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.
   96 test kit: one 16 mL vial
8. *Stopping Solution*
   A solution of diluted acid.
   96 test kit: one 12 mL vial
9. *Washing Buffer 5X Concentrate*
   Buffer salts with detergent and a non-mercury preservative.
   96 test kit: one 100 mL vial
10. *Assay Buffer*
    Dissolved buffer salts.
    96 test kit: one 125 mL vial
11. *Derivatization Reagent*
    96 test kit: three 100 µL vials
12. *Derivatization Reagent Diluent*
    Dimethyl Sulfoxide (DMSO):
    96 test kit: three 4 mL vials

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box, except for derivatization reagent (use the same day as diluted). *The Washing Solution requires no special storage condition and may be stored separately from the reagents to conserve refrigerator space.*

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Pipets*	Precision pipets capable of delivering 50, 100, 150, 250 µL A 1.0 mL repeating pipet Disposable 5 mL pipette
Parafilm	
Disposable Test Tubes	
Distilled or deionized Water	
Vortex Mixer*	Thermolyne Mini Mix, Scientific Industries Vortex Genie, or equivalent
Plate Reader* capable of readings at 450 nm	

* Please contact Abraxis for supplier information.

• Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated.
Samples containing gross particulate matter should be filtered (e.g. 0.2 um Anotop™ 25 Plus, Whatman, Inc.) to remove particles.

Samples which have been preserved with monochloroacetic acid or other acids, should be neutralized with strong base, e.g. 6N NaOH, prior to assay.

If the glyphosate concentration of a sample exceeds 4 ppb, the sample is subject to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard or Sample Diluent. For example, in a separate test tube make a ten-fold dilution by adding 100 µL of the sample to 900 µL of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtain by the dilution factor e.g. 10.

The presence of the following substances up to 10,000 ppm were found to have no significant effect on the Glyphosate Assay results: nitrate, phosphate, sulfate, sodium fluoride, calcium, magnesium, copper, zinc, iron and sodium thiosulfate. Manganese up to 100 ppm. Humic acid up to 10 ppm. Sodium chloride up to 1.0 M.

Solvents usually used to extract pesticides from soil or plant matrices such as methanol and acetone were found to be acceptable for use in the Glyphosate Plate immunoassay up to 100%.

• Reagent Preparation

All reagents must be allowed to come to room temperature.

Wash Buffer

In a 1000 mL container, dilute the wash buffer concentrate 1:5 by the addition of deionized or distilled water (i.e. 100 mL of wash buffer 5X concentrate plus 400 mL of water.

Derivatization of Standards, Control, and Samples

1. Dilute Derivatization Reagent with 3.5 mL of Derivatization Reagent Diluent (Diluted Reagent needs to be used within the same day). Mixed thoroughly.
2. Label single test tubes for standards, control, and samples.
3. Pipette 250 µL of standard, control, sample(s) into separate disposable tubes.
4. Add 1.0 mL of Assay buffer, vortex to mix.
5. Add 100 µL of the diluted derivatization reagent, vortex each tube immediately after addition of reagent until no swirling lines are present.
6. Incubate at room temperature for 10 minutes.
7. Perform the ELISA as in Assay Procedure, start with step 1 of Assay procedure.

• Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each well in an identical manner.

Add reagents directly to the bottom of the well while **avoiding contact between the reagents and the pipet tip.** This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the wells and pipet tips.

Avoid foam formation during vortexing.

The microtiter plate consists of 12 strips of 8 wells, when you use fewer than 12 strips, remove the unneeded strips and store them refrigerated in the re-sealable bag (with desiccant) provided.

If more than three strips are being used per run, it is recommended that a multi-channel pipette be used for the addition of antibody, conjugate, color, and stopping solution.

Do not use any reagents beyond their stated shelf life.

Do not use the diluted derivatization reagent after 8 hours from dilution.

Avoid contact of Stopping Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

The Abraxis Glyphosate Plate Assay will detect glyphosate. Refer to specificity table for data on several of related compounds. The Abraxis Glyphosate Plate Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

• Quality Control

A control solution at approximately 0.75 ppb of Glyphosate is provided with the Abraxis Glyphosate Plate Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

Figure B2. Glyphosate Plate ELISA kit's User Guide

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

St 0-St 5: Standards
C: Control sample
S1-Sx: Samples

1. Add 50 µL of the appropriate derivatized standard, control, or sample (see Reagent Preparation). We recommend using duplicates or triplicates (See example above.)
2. Add 50 µL of the anti-Glyphosate antibody solution successively to each well. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop. Be careful not to spill the contents. Incubate at room temperature for 30 minutes.
3. After the incubation, remove the covering and add 50 µL of enzyme conjugate solution to the individual wells successively. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop. Be careful not to spill the contents. Incubate at room temperature for 60 minutes.
4. After the incubation, remove the covering and vigorously shake the contents of the wells into a waste container. Wash the strips 3 times using the 1X wash solution (see Reagent Preparation) with a volume of at least 250 µL per each wash step. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels.
5. Add 150 µL of color solution successively to each well. Incubate for 20-30 minutes.
6. Add 100 µL of Stopping Solution to each well in the same sequence as for the other reagents.
7. Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

Manual Calculations

1. Calculate the mean absorbance value for each of the standards.
2. Calculate the %B/B$_0$ for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the Diluent/Zero Standard.
3. Construct a standard curve by plotting the %B/B$_0$ for each standard on vertical linear (Y) axis versus the corresponding glyphosate concentration on horizontal log (X) axis on the graph paper provided. The analysis can also be performed using a a Log/Logit or 4-Parameter data calculation programs.
4. %B/B$_0$ for controls and samples will then yield levels in ppb of glyphosate by interpolation using the standard curve.

(Contact Abraxis for detailed application information on specific photometers.)

NOTE: Any results obtained with a calculated glyphosate concentration of less than 0.05 ppb should be assumed to be below the detection limit of the assay.

• Expected Results

In a study with water samples from various locations, the Abraxis Glyphosate Plate Assay was shown to correlate well with another analytical technique.

• Performance Data

Precision

The following results were obtained:

Control	1	2	3	4
Replicates	5	5	5	5
Days	3	3	3	3
n	15	15	15	15
Mean (ppb)	0.41	0.77	1.54	2.81
% CV (within assay)	12.2	8.2	4.1	5.5
% CV (between assay)	16.9	11.8	8.0	11.7

Sensitivity

The Abraxis Glyphosate Plate Assay has an estimated minimum detectable concentration based on a 90% B/B$_0$ of 0.05 parts per billion (ppb).

Recovery

Five (5) groundwater samples were spiked with various levels of glyphosate and then assayed using the Abraxis Glyphosate Plate Assay. The following results were obtained:

Amount of Glyphosate Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	%
0.25	0.25	0.05	102
0.5	0.53	0.05	105
1.0	1.03	0.14	103
2.0	2.12	0.16	106
Average			104

Specificity

The cross reactivity of the Abraxis Glyphosate Plate Assay for various related analogues can be expressed as the least detectable dose (LDD) which is estimated at 90% B/B$_0$, or as the dose required for 50% absorbance inhibition (50% B/B$_0$).

B/B$_0$ Compound	LDD (ppb)	50% (ppb)
Glyphosate	0.05	0.5
Glyphosine	50	3,000
Glufosinate	2000	70,000
AMPA	35,000	> 1,000,000
Glycine	> 10,000	> 1,000,000

The following compounds demonstrated no reactivity in the Abraxis Glyphosate Assay at concentrations up to 1000 ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, acetochlor, alachlor, atrazine, ametryn, benomyl, butylate, captan, carbaryl, carbendazim, carbofuran, cyanazine, 2,4-D, 1,3-dichloropropene, dinoseb, MCPA, metolachlor, metribuzin, pentachlorophenol, picloram, propazine, simazine, terbufos, thiabendazole, and thiophanate-methyl.

• Ordering information

Microtiter Plate Kit

Abraxis Glyphosate Plate Assay Kit, 96T	PN 500086
Sample Diluent	PN 500082
Derivatization Reagent Set	PN 500087
Plate Standard Set	PN 500088

Magnetic Particle Tube Kit

Abraxis Glyphosate HS Assay Kit, 120T	PN 500081
Sample Diluent	PN 500082
HS Derivatization Reagent Set	PN 500084
HS Standard Set	PN 500085

• Assistance

For ordering or technical assistance contact:

Abraxis LLC
Sales Department
Northampton Center
54 Steamwhistle Drive
Warminster, Pennsylvania, 18974

Phone: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

• General Limited Warranty

Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

Evaluation of residue within
15x 4 = 60 pots
5 rates.
3 timings.
1 harvest.
15 trts
x 4 reps
60 pot.
2x replication in space (at the same time)

Figure B2. Glyphosate Plate ELISA kit's User Guide (continued)

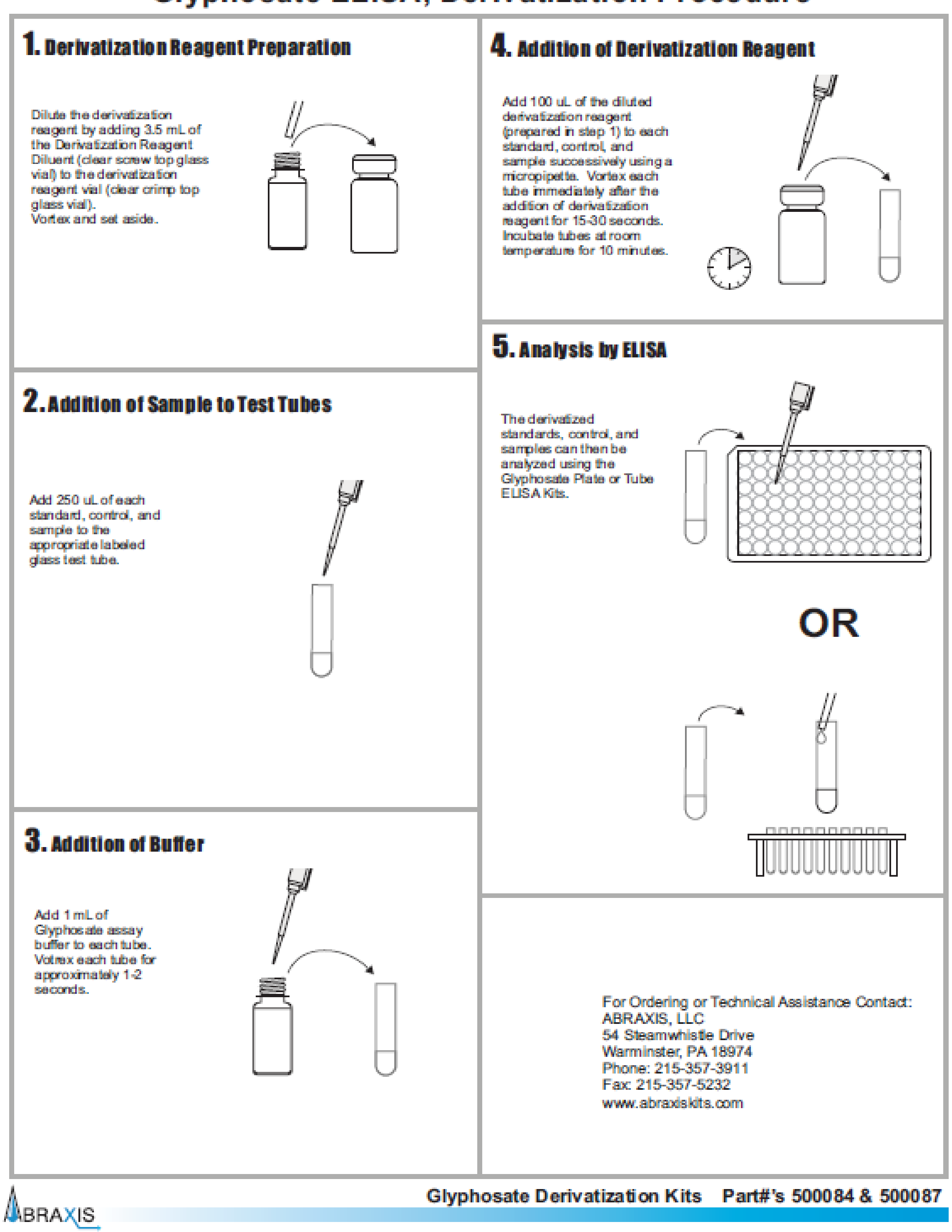


Figure B3. Glyphosate ELISA, Derivatization Procedure

Glyphosate Plate, Detailed ELISA Procedure

1. Addition of Standards, Samples

Add 50 uL of the derivatized standard solutions, control, or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.

2. Addition of Antibody Solution

Add 50 uL of the anti-Glyphosate Antibody Solution into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Incubate for 30 minutes.

3. Addition of Enzyme Conjugate

Add 50 uL of the enzyme conjugate to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill contents. Incubate for 60 minutes at room temperature.

4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times with a multi-channel pipette or wash bottle using the 1X washing buffer solution. Please use at least a volume of 250 uL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.

OR

5. Addition of Substrate/Color Solution

Add 150 uL of substrate/color solution to the individual wells successively using a multi-channel pipette or a stepping pipette. cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 20-30 minutes at room temperature.

6. Addition of Stopping Solution

Add 100 uL of stop solution to the wells in the same sequence as for the substrate solution using a multi-channel pipette or a stepping pipette.

7. Measurement of Color

Read the absorbance at 450nm using a microplate ELISA reader. Calculate the results.

For Ordering or Technical Assistance Contact:
ABRAXIS, LLC
54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
www.abraxiskits.com

Glyphosate Plate Kit Part # 500086

ABRAXIS

Figure B4. Glyphosate Plate, Detailed ELISA Procedure

1. **Intended Use**
   A step-by-step procedure for the extraction and detection of Glyphosate in dried lentils, white beans, soybeans, corn and barley.

2. **Range of Detection**
   The range of detection is 75.75ppb to 4,040ppb in matrix. If samples exceed calibration, are known to contain higher analyte levels, or a higher detection range is necessary, samples should be diluted further prior to analysis.

3. **Materials Required (not provided)**
   Coffee-bean grinder, or food processor, to homogenize sample(s)
   Analytical balance
   Scoopula or disposable tongue-depressors
   10mL glass serological pipettes
   Rubber bulb or electronic pipettor
   20mL glass vials with Teflon-lined caps
   1N Hydrochloric Acid (HCL)
   Vortex mixer
   Timer
   Micro-centrifuge tubes
   Micro-centrifuge
   4mL glass vials with Teflon-lined caps
   Pipettes capable of dispensing 20uL-200uL and 100uL-1mL
   Disposable pipette tips
   12 x 75mm glass test tubes
   Test tube rack
   Plate shaker or Micro-well plate holder with insert retainer for vortex mixer
   Plate-covers or parafilm
4. **Notes and Precautions**
   4.1 To prepare a truly representative sample, homogenize at least 50g of sample.
   4.2 Condition each pipette tip by drawing the liquid to be dispensed in and out of the tip 3 times before the final dispense. This will ensure that an accurate volume is transferred.
   4.3 Reagent basins should be labeled for each reagent (e.g. Antibody Solution, Conjugate Solution, Color Solution, etc.). Basins can be washed with deionized water and re-used for later assays, but only for use with the previously labeled reagent. Using the same basin for multiple reagents may lead to contamination—which could adversely affect assay performance.
5. **Extraction Procedure**
   5.1 Weigh 1g of homogenized sample into an appropriately labeled 20mL glass vial with a Teflon-lined cap.
   5.2 Add 10mL of 1N HCL to the 20mL glass vial.
   5.3 Vortex the 20mL glass vial for 2 minutes (be sure a full vortex is formed inside the vial).
   5.4 Allow the 20mL vial to sit on the bench-top for at least 5 minutes to allow solid matter to settle.

Figure B5. High-Detail Procedure for the Extraction and Detection of Glyphosate in Grains and Legumes

5.5 Condition a new pipette tip (see Step 4.2) then transfer 1mL of the supernatant (top layer) into an appropriately labeled micro-centrifuge tube.
5.6 Centrifuge the micro-centrifuge tube for 5 minutes at 6,000 rpm.
5.7 Condition a new pipette tip (see Step 4.2) then add 4mL of Glyphosate Sample Diluent to an appropriately labeled 4mL glass vial with a Teflon-lined cap.
5.8 Condition a new pipette tip (see Step 4.2) then transfer 40uL of supernatant from the Micro-centrifuge tube into the 4mL glass vial from Step 5.7.
5.9 Thoroughly mix by vortexing the 4mL glass vial (at highest speed setting) for 2 seconds then removing it for 1 second. Repeat this process 4 more times—for a total of 5 times.
5.10 The sample is now prepared and can be stored for future analysis or derivatized and analyzed.

6. Derivatization Procedure

6.1 Appropriately label 1 glass test tube for each standard (0, 0.075, 0.2, 0.5, 1 and 4ppb), the control (0.75ppb), and each sample (Sample #1, Sample #2, etc.) and place them into a test tube rack (e.g. If the user is testing 2 samples, they would need 6 test tubes for the standards, 1 test tube for the control, and 2 test tubes for the samples—for a total of 9 test tubes).
6.2 Dispense 250uL of each standard into the appropriate test tube (use a new pipette tip for dispensing each standard and condition each tip as described in Step 4.2).
6.3 Dispense 250uL of the control into the appropriate test tube (use a new pipette tip for dispensing the control and condition the tip as described in Step 4.2).
6.4 Dispense 250uL of each sample into the appropriate test tube (use a new pipette tip for dispensing each sample and condition each tip as described in Step 4.2).
6.5 Condition a new pipette tip and dispense 1mL of the Glyphosate Assay Buffer into each test tube (each test tube should now have 250uL + 1mL of liquid).
6.6 Thoroughly mix by vortexing the test tubes (at highest speed setting) for 2 seconds then removing it for 1 second. Repeat this process 4 more times—for a total of 5 times.
6.7 Condition a new pipette tip and transfer 3.5mL of the Glyphosate Derivatization Diluent (clear vial) into the Glyphosate Derivatization Reagent (amber vial) and vortex (see Step 6.6 for proper vortexing technique).
6.8 Condition a new pipette tip and dispense 100uL of the diluted Derivatization Reagent (solution in the amber vial prepared in Step 6.7) into each test tube (each test tube should now have 250uL + 1mL + 100uL of liquid). Vortex each test tube (see Step 6.6). It is vital to vortex the test tubes as quickly as possible because the reaction begins as soon as the 100uL is dispensed into the test tube.
6.9 Allow the test tubes to sit and incubate for at least 10 minutes.
6.10 The standards, the control and the samples are now derivatized and ready to be dispensed into the micro-titer plate wells.

Figure B5. High-Detail Procedure for the Extraction and Detection of Glyphosate in Grains and Legumes (continued)

7. Assay Procedure

7.1 Without touching the inside walls of the wells with the pipette tip (touching the inside walls of the wells with the pipette tip can cause air bubbles to form inside the wells—which can result in poor reproducibility between each duplicate). Dispense 50uL of Standard 0 (0ppb) into wells A1 and B1 (the standards, the control, and the samples should always be dispensed in duplicate).

7.2 Condition a new pipette tip and dispense 50uL of Standard 1 (0.075ppb) into wells C1 and D1.

7.3 Using new, conditioned pipette tips for each standard, control and sample, continue dispensing 50uL of the remaining standards, control and samples (in duplicate) into the appropriate wells. See the Abraxis Glyphosate user's guide for a computer-generated image of the micro-titer plate template.

7.4 Pour the contents of the Glyphosate Antibody Solution (red liquid) into a clean, appropriately labeled reagent basin. The basin can be re-used, but be sure to only use the basin for Glyphosate Antibody Solution (see Step 4.3).

7.5 Using a multichannel pipette, condition the pipette tips then dispense 50uL of the Glyphosate Antibody Solution into each well without touching the pipette tips to the inside of the wells. Cover the micro-titer plate with a plate-cover, or parafilm, and then rotate the micro-titer plate in a figure-8 motion on the bench-top for 1 minute.

7.6 Incubate the micro-titer plate for 30 minutes at room temperature using the plate shaker on lowest setting (alternatively place the plate inside the foam adapter that fits on the vortex and turn vortex speed to lowest setting).

7.7 Pour the contents of the Glyphosate Conjugate Solution (small, plastic amber bottle) into a clean, appropriately labeled reagent basin. The basin can be re-used, but be sure to only use the basin for Glyphosate Conjugate Solution (see Step 4.3).

7.8 Using a multichannel pipette, condition the pipette tips then dispense 50uL of the Glyphosate Conjugate Solution into each well without touching the pipette tips to the inside of the wells. Cover the micro-titer plate with a plate-cover, or parafilm, and then rotate the micro-titer plate in a figure-8 motion on the bench-top for 1 minute.

7.9 Incubate the micro-titer plate for 60 minutes at room temperature using the plate shaker or the vortex with the attached-adapter.

7.10 During the 60-minute incubation prepare the 1X Wash Buffer by adding the entire contents of the 5X Wash Buffer (100mL) into a larger bottle containing 400mL of deionized/distilled water and mix the solution by shaking/inverting the bottle several times.

7.11 After the 60-minute incubation, empty the contents of the micro-titer plate wells into a sink and forcefully pat the inverted micro-titer plate on dry paper towels.

7.12 Pour about 50mL of the 1X Wash Buffer into a clean, appropriately labeled reagent basin. The basin can be re-used, but be sure to only use the basin for 1X Wash Buffer (see Step 4.3).

Figure B5. High-Detail Procedure for the Extraction and Detection of Glyphosate in Grains and Legumes (continued)

7.13 Using a multichannel pipette, condition the pipette tips then dispense 250uL of the 1X Wash Buffer into the wells of the micro-titer plate without touching the pipette tips to the inside of the wells. Briefly swirl the micro-titer plate then empty contents into a sink and forcefully pat the inverted micro-titer plate on dry paper towels. Repeat this process 2 more times (for a total of 3 washes).

7.14 Pour the contents of the Color Solution into a clean, appropriately labeled reagent basin. The basin can be re-used, but be sure to only use the basin for Color Solution (see Step 4.3).

7.15 Using a multichannel pipette, condition the pipette tips then dispense 150uL of the Color Solution into the wells of the micro-titer plate without touching the pipette tips to the inside of the wells. Cover the micro-titer plate with a plate-cover, or parafilm, and then rotate the micro-titer plate in a figure-8 motion on the bench-top for 1 minute.

7.16 Incubate the micro-titer plate for 20-30 minutes at room temperature (**do not** place the micro-titer plate in the plate shaker or the vortex with the attached-adapter).

7.17 Pour contents of the Stop Solution into a clean, appropriately labeled reagent basin. The basin can be re-used, but be sure to only use the basin for Stop Solution (see Step 4.3).

7.18 Using a multichannel pipette, condition the pipette tips then dispense 100uL of the Stop Solution into the wells of the micro-titer plate without touching the pipette tips to the inside of the wells.

7.19 Measure the absorbance values (O.D. values) of the micro-titer plate wells at 450nm wavelength within 15 minutes of adding the Stop Solution.

8. Evaluation of Results

8.1 The ELISA results must be multiplied by a factor of 1010 to account for the extraction and dilution. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted and re-analyzed.

8.2 See the Abraxis Glyphosate user's guide for additional information.

9. Assistance

For ordering or technical assistance contact:
Abraxis LLC.
54 Steamwhistle Dr.
Warminster, PA 18974
Tel.: (215) 357-3911 Fax: (215) 357-5232

Figure B5. High-Detail Procedure for the Extraction and Detection of Glyphosate in Grains and Legumes (continued)

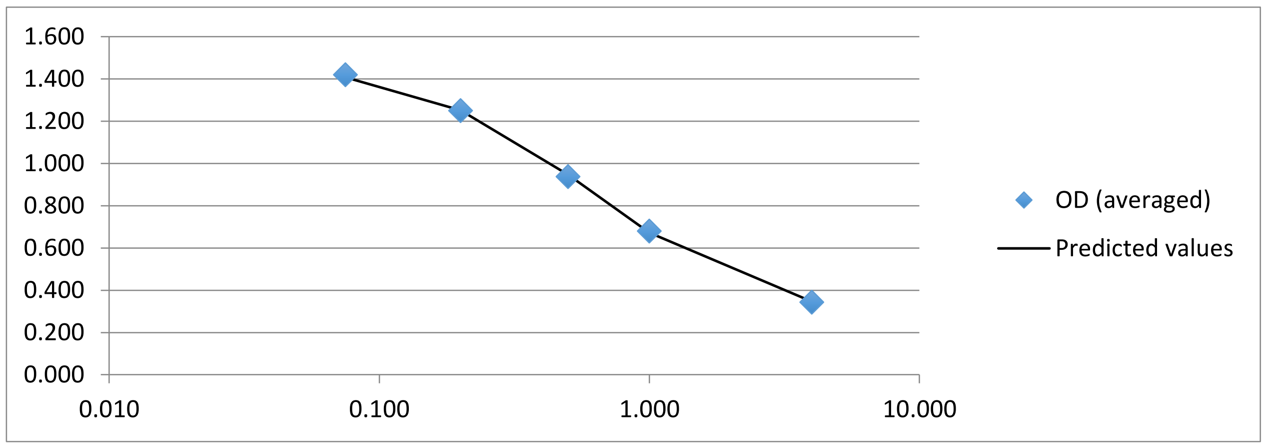
ELISA PLATE 1

4-parametric logistic fitting with Excel Solver for GLYPHOSATE ELISA

**** USER MUST ONLY INPUT VALUES IN GREY-COLORED CELLS ****

Kit Lot # __________

Std 0	Std 1	Std 2	Std 3	Std 4	Std 5
Amax (0 ppb)	0.075 ppb	0.2 ppb	0.5 ppb	1 ppb	4 ppb
1.4576	1.4228	1.2958	0.9505	0.6396	0.3362
1.4909	1.3272	1.191	0.8895	0.7102	0.3381
1.4684	1.5123	1.2636	0.9755	0.6913	0.3569



GLYPHOSATE (ng/ml)	OD (averaged)	Predicted values	Residual Squares	B/Bo	Std Dev	%CV
0.000	1.472	1.480	5.91E-05		0.017	1.154
0.075	1.421	1.408	1.52E-04	0.965	0.093	6.515
0.200	1.250	1.252	5.36E-06	0.849	0.054	4.294
0.500	0.939	0.947	6.98E-05	0.637	0.044	4.714
1.000	0.680	0.672	7.08E-05	0.462	0.037	5.372
4.000	0.344	0.346	5.68E-06	0.233	0.011	3.329
Sum Of Squares			3.63E-04			

Figure B6. ELISA Plate 1 Raw Data Results

Parameter (Y=(A-D)/(1+(X/C)^B)+D)		
A	1.48	(Max.)
B	1.33	(Slope)
C	0.61	(IC_{50})
D	0.25	(Min.)

R-Squared
0.99964

Sample	Absorbances	Std Dev	%CV	GLYPHOSATE (ng/ml)	GLYPHOSATE AVG (ng/ml)
Sample 1	0.922			0.532	0.540
Sample 1	0.9372			0.512	
Sample 1	0.8887	0.025	2.708	0.577	
Sample 2	0.9184			0.537	0.435
Sample 2	0.999			0.438	
Sample 2	1.1045	0.093	9.265	0.329	
Sample 3	1.2392			0.211	0.242
Sample 3	1.1389			0.297	
Sample 3	1.2292	0.055	4.595	0.219	
Sample 4	1.5015			#NUM!	#NUM!
Sample 4	1.4756			0.009	
Sample 4	1.4518	0.025	1.684	0.036	
Sample 5	1.7828			#NUM!	#NUM!
Sample 5	1.6589			#NUM!	
Sample 5	1.6391	0.078	4.599	#NUM!	
Sample 6	0.3336			4.495	3.873
Sample 6	0.3626			3.500	
Sample 6	0.3581	0.016	4.441	3.623	
Sample 7	0.6186			1.163	1.241
Sample 7	0.5983			1.235	
Sample 7	0.5753	0.022	3.626	1.326	
Sample 8	0.7883			0.740	0.877
Sample 8	0.69			0.952	
Sample 8	0.6954	0.055	7.627	0.939	
Sample 9	0.1625			#NUM!	#NUM!
Sample 9	0.1675			#NUM!	
Sample 9	0.1693	0.004	2.117	#NUM!	

Figure B6. ELISA Plate 1 Raw Data Results (continued)

Sample 10	0.2751			12.277	8.093
Sample 10	0.3073			6.148	
Sample 10	0.3108	0.020	6.610	5.854	
Sample 11	0.4142			2.528	2.475
Sample 11	0.4223			2.422	
Sample 11	0.4182	0.004	0.968	2.474	
Sample 12	0.9547			0.490	0.564
Sample 12	0.8691			0.606	
Sample 12	0.8762	0.048	5.278	0.595	
Sample 13	1.3978			0.084	#NUM!
Sample 13	1.4531			0.035	
Sample 13	1.571	0.088	6.002	#NUM!	
Sample 14	0.9027			0.558	0.504
Sample 14	0.965			0.478	
Sample 14	0.9672	0.037	3.875	0.475	
Sample 15	1.5424			#NUM!	#NUM!
Sample 15	1.5052			#NUM!	
Sample 15	1.4292	0.058	3.866	0.057	
Sample 16	1.5065			#NUM!	#NUM!
Sample 16	1.5358			#NUM!	
Sample 16	1.5007	0.019	1.242	#NUM!	
Sample 17	1.4724			0.013	#NUM!
Sample 17	1.4856			#NUM!	
Sample 17	1.344	0.078	5.455	0.127	
Sample 18	0.6956			0.938	0.924
Sample 18	0.6973			0.934	
Sample 18	0.7113	0.009	1.228	0.900	
Sample 19	0.8748			0.597	0.637
Sample 19	0.8417			0.648	
Sample 19	0.8307	0.023	2.703	0.666	
Sample 20	0.6935			0.944	0.923
Sample 20	0.7322			0.853	
Sample 20	0.6823	0.026	3.726	0.972	
Sample 21	1.0444			0.389	0.413
Sample 21	1.0695			0.363	
Sample 21	0.9579	0.059	5.718	0.486	
Sample 22	0.5353			1.514	1.407
Sample 22	0.5866			1.280	

Figure B6. ELISA Plate 1 Raw Data Results (continued)

Sample 22	0.5532	0.026	4.663	1.425	
Sample 23	0.2119			#NUM!	#NUM!
Sample 23	0.2616			24.805	
Sample 23	0.2032	0.032	13.968	#NUM!	
Sample 24	0.4102			2.583	2.777
Sample 24	0.3963			2.796	
Sample 24	0.3875	0.011	2.876	2.951	
Sample 25	0.2665			17.775	15.697
Sample 25	0.2618			24.391	
Sample 25	0.3249	0.035	12.360	4.925	

Figure B6. ELISA Plate 1 Raw Data Results (continued)

ELISA PLATE 2

4-parametric logistic fitting with Excel Solver for GLYPHOSATE ELISA

**** USER MUST ONLY INPUT VALUES IN GREY-COLORED CELLS ****

Kit Lot # ___________

Std 0	Std 1	Std 2	Std 3	Std 4	Std 5
Amax (0 ppb)	0.075 ppb	0.2 ppb	0.5 ppb	1 ppb	4 ppb
1.6116	1.3513	1.1776	0.8565	0.4893	0.3334
1.4255	1.3233	1.1943	0.7801	0.6206	0.3101
1.5006	1.4836	1.2375	0.9957	0.6587	0.2725

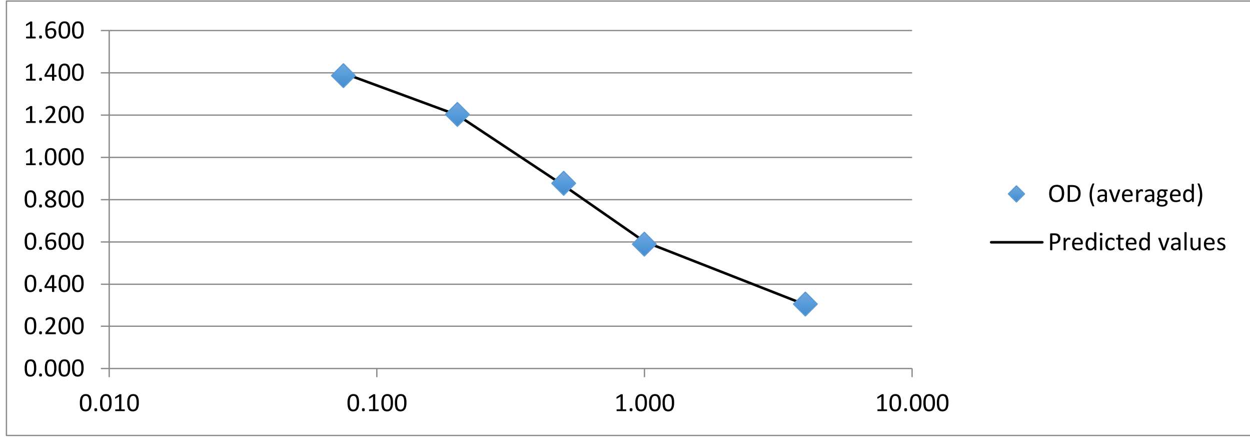


Figure B7. ELISA Plate 2 Raw Data Results

GLYPHOSATE (ng/ml)	OD (averaged)	Predicted values	Residual Squares	B/Bo	Std Dev	%CV
0.000	1.513	1.506	4.45E-05		0.094	6.190
0.075	1.386	1.398	1.46E-04	0.916	0.086	6.177
0.200	1.203	1.200	1.04E-05	0.795	0.031	2.569
0.500	0.877	0.867	1.05E-04	0.580	0.109	12.458
1.000	0.590	0.601	1.36E-04	0.390	0.089	15.075
4.000	0.305	0.302	1.29E-05	0.202	0.031	10.064
Sum Of Squares			4.55E-04			

Parameter (Y=(A-D)/(1+(X/C)^B)+D)

A	1.51	(Max.)
B	1.25	(Slope)
C	0.51	(IC_{50})
D	0.21	(Min.)

R-Squared
0.99955

Sample	Absorbances	Std Dev	%CV	GLYPHOSATE (ng/ml)	GLYPHOSATE AVG (ng/ml)
Sample 1	0.3916			2.181	1.805
Sample 1	0.4486			1.682	
Sample 1	0.4689	0.040	9.184	1.551	
Sample 2	1.066			0.300	0.345
Sample 2	0.9458			0.411	
Sample 2	1.038	0.063	6.187	0.324	
Sample 3	1.4654			0.033	0.046
Sample 3	1.4713			0.029	
Sample 3	1.3962	0.042	2.891	0.076	
Sample 4	1.3716			0.091	0.103
Sample 4	1.4207			0.061	
Sample 4	1.2634	0.080	5.953	0.158	
Sample 5	0.2374			10.876	14.888
Sample 5	0.223			19.678	
Sample 5	0.2298	0.007	3.131	14.110	
Sample 6	0.4193			1.908	1.777
Sample 6	0.4271			1.843	
Sample 6	0.4641	0.024	5.479	1.580	

Figure B7. ELISA Plate 2 Raw Data Results (continued)

Sample 7	1.3096			0.129	
Sample 7	1.4218			0.061	0.104
Sample 7	1.3223	0.061	4.547	0.121	
Sample 8	0.253			7.541	
Sample 8	0.2701			5.718	8.024
Sample 8	0.2376	0.016	6.411	10.813	
Sample 9	0.9294			0.429	
Sample 9	0.9263			0.432	0.422
Sample 9	0.951	0.013	1.438	0.406	
Sample 10	1.2402			0.173	
Sample 10	1.3515			0.103	0.120
Sample 10	1.3819	0.075	5.632	0.085	
Sample 11	0.5686			1.103	
Sample 11	0.5101			1.335	1.149
Sample 11	0.598	0.045	8.006	1.009	
Sample 12	1.2207			0.186	
Sample 12	1.1804			0.214	0.233
Sample 12	1.0669	0.080	6.899	0.300	
Sample 13	1.2134			0.191	
Sample 13	1.2091			0.194	0.187
Sample 13	1.2347	0.014	1.125	0.177	
Sample 14	0.3499			2.766	
Sample 14	0.274			5.425	4.229
Sample 14	0.29	0.040	13.134	4.496	
Sample 15	0.679			0.805	
Sample 15	0.642			0.890	0.869
Sample 15	0.6336	0.024	3.707	0.911	
Sample 16	0.3962			2.131	
Sample 16	0.434			1.788	2.122
Sample 16	0.3701	0.032	8.030	2.449	
Sample 17	0.7202			0.722	
Sample 17	0.6455			0.882	0.791
Sample 17	0.6965	0.038	5.553	0.769	
Sample 18	0.4691			1.550	
Sample 18	0.7883			0.608	0.911
Sample 18	0.8098	0.191	27.690	0.576	
Sample 19	0.8781			0.487	0.508
Sample 19	0.8748			0.491	

Figure B7. ELISA Plate 2 Raw Data Results (continued)

Sample 19	0.8319	0.026	2.991	0.546	
Sample 20	0.5754			1.080	1.170
Sample 20	0.5744			1.083	
Sample 20	0.5075	0.039	7.045	1.347	
Sample 21	0.2005			#NUM!	#NUM!
Sample 21	0.2104			189.473	
Sample 21	0.203	0.005	2.516	#NUM!	
Sample 22	0.3256			3.274	2.985
Sample 22	0.3351			3.055	
Sample 22	0.3581	0.017	4.921	2.628	
Sample 23	0.5337			1.232	1.197
Sample 23	0.5738			1.085	
Sample 23	0.524	0.026	4.855	1.273	
Sample 24	0.6735			0.817	0.884
Sample 24	0.6482			0.875	
Sample 24	0.6156	0.029	4.495	0.959	
Sample 25	0.1861			#NUM!	#NUM!
Sample 25	0.1676			#NUM!	
Sample 25	0.1934	0.013	7.292	#NUM!	

Figure B7. ELISA Plate 2 Raw Data Results (continued)

ELISA PLATE 3

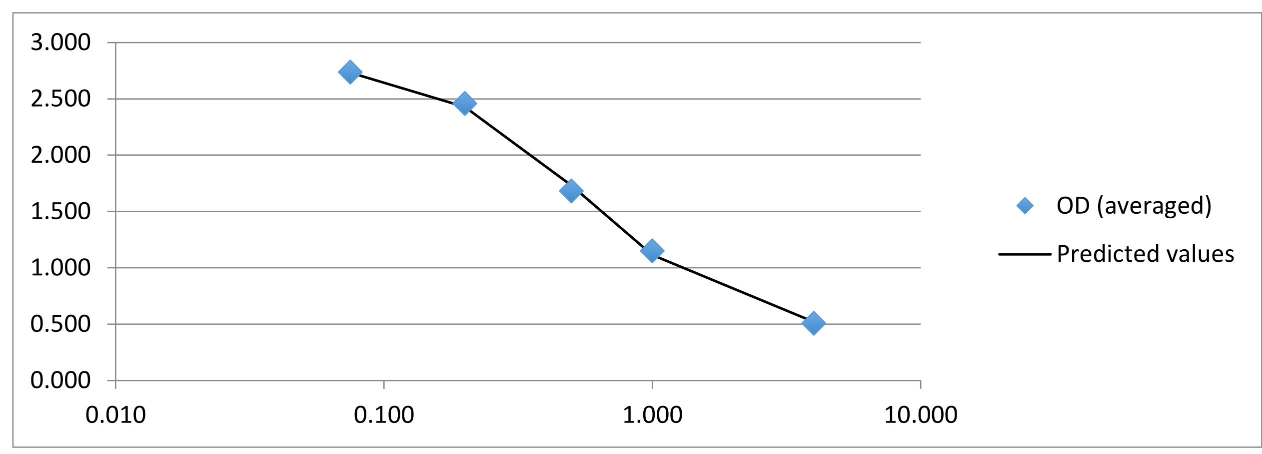
4-parametric logistic fitting with Excel Solver for GLYPHOSATE ELISA

**** USER MUST ONLY INPUT VALUES IN GREY-COLORED CELLS ****

Kit Lot # _______

Std 0	Std 1	Std 2	Std 3	Std 4	Std 5
Amax (0 ppb)	0.075 ppb	0.2 ppb	0.5 ppb	1 ppb	4 ppb
2.6654	2.7116	2.4313	1.5548	1.2292	0.4984
2.8836	2.6765	2.4738	1.7165	1.145	0.4985
2.9021	2.8271	2.4714	1.7781	1.0794	0.5262

Figure B8. ELISA Plate 3 Raw Data Results



GLYPHOSATE (ng/ml)	OD (averaged)	Predicted values	Residual Squares	B/Bo	Std Dev	%CV
0.000	2.817	2.833	2.70E-04		0.132	4.673
0.075	2.738	2.732	4.51E-05	0.972	0.079	2.877
0.200	2.459	2.428	9.59E-04	0.873	0.024	0.971
0.500	1.683	1.731	2.34E-03	0.597	0.115	6.852
1.000	1.151	1.113	1.47E-03	0.409	0.075	6.523
4.000	0.508	0.519	1.25E-04	0.180	0.016	3.156
Sum Of Squares			5.20E-03			

Parameter (Y=(A-D)/(1+(X/C)^B)+D)

A	2.83	(Max.)
B	1.55	(Slope)
C	0.56	(IC_{50})
D	0.41	(Min.)

R-Squared
0.99480

Figure B8. ELISA Plate 3 Raw Data Results (continued)

Sample	Absorbances	Std Dev	%CV	GLYPHOSATE (ng/ml)	GLYPHOSATE AVG (ng/ml)
Sample 1	0.515			4.097	4.109
Sample 1	0.5139			4.126	
Sample 1	0.5148	0.001	0.114	4.103	
Sample 2	0.7038			2.009	2.143
Sample 2	0.6596			2.260	
Sample 2	0.676	0.022	3.287	2.159	
Sample 3	1.1844			0.914	0.965
Sample 3	1.0518			1.084	
Sample 3	1.2016	0.082	7.153	0.895	
Sample 4	0.4691			5.974	5.544
Sample 4	0.4965			4.659	
Sample 4	0.4687	0.016	3.333	6.000	
Sample 5	1.1599			0.942	1.018
Sample 5	1.1048			1.011	
Sample 5	1.0399	0.060	5.453	1.102	
Sample 6	1.03			1.117	1.133
Sample 6	1.0132			1.144	
Sample 6	1.0176	0.009	0.854	1.137	
Sample 7	1.8292			0.450	0.471
Sample 7	1.7193			0.507	
Sample 7	1.8166	0.060	3.363	0.456	
Sample 8	2.5591			0.149	0.123
Sample 8	2.6203			0.125	
Sample 8	2.6878	0.064	2.455	0.096	
Sample 9	2.8115			0.027	#NUM!
Sample 9	2.928			#NUM!	
Sample 9	2.8064	0.069	2.415	0.031	
Sample 10	2.5765			0.142	0.148
Sample 10	2.5368			0.158	
Sample 10	2.5706	0.021	0.836	0.145	
Sample 11	1.8705			0.430	0.536
Sample 11	1.2975			0.800	
Sample 11	1.9866	0.369	21.472	0.377	

Figure B8. ELISA Plate 3 Raw Data Results (continued)

Sample 12	1.0033			1.160	1.228
Sample 12	0.9712			1.216	
Sample 12	0.9248	0.039	4.084	1.306	
Sample 13	0.6022			2.717	2.723
Sample 13	0.5995			2.744	
Sample 13	0.6032	0.002	0.318	2.708	
Sample 14	2.4356			0.197	0.165
Sample 14	2.4737			0.182	
Sample 14	2.6405	0.109	4.330	0.116	
Sample 15	0.6444			2.364	2.541
Sample 15	0.6342			2.439	
Sample 15	0.5923	0.028	4.427	2.819	
Sample 16	1.312			0.787	0.830
Sample 16	1.2625			0.833	
Sample 16	1.2262	0.043	3.400	0.869	
Sample 17	2.7409			0.070	0.118
Sample 17	2.5886			0.138	
Sample 17	2.5677	0.095	3.592	0.146	
Sample 18	0.4642			6.317	6.470
Sample 18	0.4504			7.624	
Sample 18	0.4777	0.014	2.941	5.470	
Sample 19	0.3102			#NUM!	#NUM!
Sample 19	0.287			#NUM!	
Sample 19	0.333	0.023	7.418	#NUM!	
Sample 20	1.3973			0.715	0.710
Sample 20	1.4471			0.678	
Sample 20	1.3717	0.038	2.728	0.736	
Sample 21	1.1933			0.904	0.960
Sample 21	1.1443			0.961	
Sample 21	1.1009	0.046	4.033	1.016	
Sample 22	0.7701			1.728	1.623
Sample 22	0.8131			1.585	
Sample 22	0.8231	0.028	3.511	1.556	
Sample 23	1.2137			0.882	0.875
Sample 23	1.1778			0.922	
Sample 23	1.2754	0.049	4.039	0.821	

Figure B8. ELISA Plate 3 Raw Data Results (continued)

Sample 24	1.2975			0.800	0.781
Sample 24	1.3458			0.757	
Sample 24	1.3149	0.024	1.854	0.784	
Sample 25	0.6035			2.705	3.693
Sample 25	0.483			5.207	
Sample 25	0.5638	0.061	11.163	3.168	

Figure B8. ELISA Plate 3 Raw Data Results (continued)

ELISA PLATE 4

4-parametric logistic fitting with Excel Solver for GLYPHOSATE ELISA

**** USER MUST ONLY INPUT VALUES IN GREY-COLORED CELLS ****

Kit Lot # ________

Std 0	Std 1	Std 2	Std 3	Std 4	Std 5
Amax (0 ppb)	0.075 ppb	0.2 ppb	0.5 ppb	1 ppb	4 ppb
1.9535	2.1702	2.1347	1.2756	0.6195	0.4158
2.1456	2.1554	1.9375	1.3232	0.8992	0.3976
2.1878	1.969	1.9249	1.2365	0.8745	0.3783

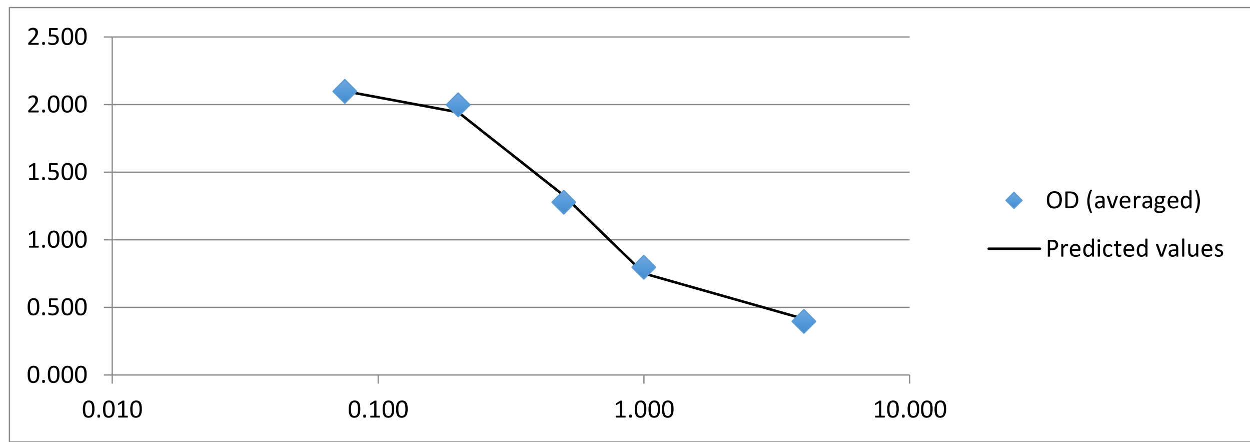


Figure B9. ELISA Plate 4 Raw Data Results

GLYPHOSATE (ng/ml)	OD (averaged)	Predicted values	Residual Squares	B/Bo	Std Dev	%CV
0.000	2.096	2.125	8.46E-04		0.125	5.959
0.075	2.098	2.101	7.71E-06	1.001	0.112	5.344
0.200	1.999	1.944	3.06E-03	0.954	0.118	5.886
0.500	1.278	1.328	2.48E-03	0.610	0.043	3.396
1.000	0.798	0.752	2.08E-03	0.381	0.155	19.411
4.000	0.397	0.416	3.69E-04	0.190	0.019	4.721
Sum Of Squares			8.84E-03			

Parameter (Y=(A-D)/(1+(X/C)^B)+D)

Parameter	Value	
A	2.12	(Max.)
B	2.17	(Slope)
C	0.54	(IC_{50})
D	0.39	(Min.)

R-Squared
0.99116

Sample	Absorbances	Std Dev	%CV	GLYPHOSATE (ng/ml)	GLYPHOSATE AVG (ng/ml)
Sample 1	2.1258			#NUM!	#NUM!
Sample 1	1.9331			0.206	
Sample 1	1.9529	0.106	5.290	0.195	
Sample 2	0.6086			1.326	1.169
Sample 2	0.7017			1.091	
Sample 2	0.702	0.054	8.026	1.090	
Sample 3	0.8754			0.835	0.835
Sample 3	0.9405			0.769	
Sample 3	0.8212	0.060	6.795	0.900	
Sample 4	2.2321			#NUM!	#NUM!
Sample 4	2.3743			#NUM!	
Sample 4	2.1273	0.124	5.523	#NUM!	
Sample 5	1.0003			0.716	0.741
Sample 5	0.9749			0.737	
Sample 5	0.9387	0.031	3.187	0.770	

Figure B9. ELISA Plate 4 Raw Data Results (continued)

Sample 6	0.8684			0.843	0.760
Sample 6	0.9847			0.729	
Sample 6	1.0092	0.075	7.884	0.708	
Sample 7	2.1024			0.073	#NUM!
Sample 7	2.1248			#NUM!	
Sample 7	2.0182	0.056	2.700	0.153	
Sample 8	0.3775			#NUM!	#NUM!
Sample 8	0.3658			#NUM!	
Sample 8	0.39	0.012	3.204	#NUM!	
Sample 9	1.4126			0.456	0.498
Sample 9	1.3518			0.488	
Sample 9	1.2365	0.089	6.707	0.551	
Sample 10	0.2784			#NUM!	#NUM!
Sample 10	0.3109			#NUM!	
Sample 10	0.2991	0.016	5.556	#NUM!	
Sample 11	0.6153			1.305	1.384
Sample 11	0.5881			1.398	
Sample 11	0.5747	0.021	3.490	1.451	
Sample 12	0.4454			2.695	2.918
Sample 12	0.4337			3.048	
Sample 12	0.4347	0.006	1.481	3.013	
Sample 13	0.2716			#NUM!	#NUM!
Sample 13	0.2387			#NUM!	
Sample 13	0.2418	0.018	7.246	#NUM!	
Sample 14	0.6663			1.167	1.169
Sample 14	0.6702			1.158	
Sample 14	0.6606	0.005	0.725	1.181	
Sample 15	0.2656			#NUM!	#NUM!
Sample 15	0.2557			#NUM!	
Sample 15	0.2764	0.010	3.894	#NUM!	

Figure B9. ELISA Plate 4 Raw Data Results (continued)