IMPACT OF NITROGEN AND WEEDS ON GLYPHOSATE-RESISTANT SUGARBEET YIELD AND QUALITY

By

Alicia Jeanne Spangler

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ABSTRACT

IMPACT OF NITROGEN AND WEEDS ON GLYPHOSATE-RESISTANT SUGARBEET YIELD AND QUALITY

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The commercialization of glyphosate-resistant sugarbeet has changed weed management in sugarbeet. Many growers are delaying their initial herbicide application because glyphosate can control larger weeds, which allows weeds to compete with crops for nutrients. Greenhouse and field experiments were conducted to examine the effect of nitrogen (N) rate and the competitive ability of weeds on glyphosate-resistant sugarbeet. In the greenhouse at a 1:1 sugarbeet: weed proportion, sugarbeet was able to assimilate more N and produced more biomass than common lambsquarters or Powell amaranth. Field experiments however, determined N assimilation by weeds was 3 to 4 times greater than sugarbeet on a per hectare basis early in the growing season. Higher weed densities in the field may have been responsible for decreased N assimilation by sugarbeet. Highest sugarbeet root yields were always achieved at the highest N rates (134 kg and 67:67 N ha^{-1}), and were greater when weeds were controlled early (< 2 cm tall). When weeds were not controlled prior to 8 cm, a 15% yield reduction was observed. Recoverable white sucrose per ha (RWSH) was highest at rates $\geq$ 100 kg N ha^{-1} in three of the four location by year combinations. RWSH was 8 to 16% lower if weeds were not controlled until they were 8 cm tall. Weeds prevent sugarbeet from utilizing available water, light, and nutrients, and should be controlled prior to 8-cm heights to avoid negative impacts on N assimilation, root yield and sucrose production.

Dedicated to my parents, Glenn and Patricia Spangler

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LITERATURE REVIEW

Introduction

Sugarbeet (*Beta vulgaris* L.) is a biennial crop grown annually for sucrose production (Elliot and Weston 1993). Commercial production of sugarbeet started in Europe in 1802 and successfully progressed to the United States in 1870 (Wilson 2001). Today, sugarbeet are grown in North and South America, Europe, Asia, and Northern Africa (Asadi 2006). They are grown in eleven states within the United States, including California, Colorado, Idaho, Michigan, Minnesota, Montana, Nebraska, North Dakota, Oregon, Washington, and Wyoming (NASS 2011b). In most temperate climates, sugarbeet seed is planted in early spring and roots are harvested in the fall (Asadi 2006). Prior to harvest, leaves are removed from the sugarbeet crown by a series of flails. Roots are then harvested mechanically, transported to sugar factories and processed for sucrose production (Smith 2001). Sugarbeet supplies 50 to 55% of the sucrose used within the United States and approximately 35% of the sucrose used worldwide (Wilson 2001; Harveson et al. 2009). Michigan is the fourth largest producer of sugarbeet in the United States behind Minnesota, North Dakota, and Idaho (NASS 2011b). Sugarbeet was planted on approximately 481,000 hectares each year in the United States from 2007 to 2011, with approximately 58,000 hectares grown in Michigan. In 2009, U.S. sugarbeet production was valued at almost 1.5 billion dollars, and Michigan contributed approximately 184 million dollars to that total (NASS 2011b; NASS 2011a).

Nitrogen and Sugarbeet

Nitrogen (N) is an important nutrient for sugarbeet, and it must be available soon after crop emergence (Draycott 1993). Sugarbeet rapidly assimilates N at the 4- to 5-leaf stage for canopy development (Draycott 1993). A well-developed sugarbeet canopy is important for shading late-emerging weeds and intercepting light for root growth and sucrose production (Draycott 1993; Scott and Jaggard 1993; Armstrong et al. 1986). Nitrogen must be available throughout the growing season to maintain the sugarbeet canopy and to promote root growth and sucrose production (Scott and Jaggard 1993). Adequate levels of N are also required to maintain a healthy crop which may prevent sugarbeet diseases from damaging the stand (Harveson et al. 2009).

Nitrogen application in sugarbeet however must be balanced to achieve high quality root yields because excess N reduces sucrose quality (Draycott 1993). Sugarbeet quality is defined as sucrose and non-sucrose concentrations in the root and clear juice purity (Dutton and Huijbregts 2006). Carter and Traveller (1981) conducted an experiment to determine the effect of N rate on sugarbeet yield and quality on irrigated silt loam soil. Increasing N rates improved root yield, but decreased sucrose concentrations. Sugarbeet root yields increased 25, 29 and 32% when N was applied at 112, 252, and 392 kg ha^{-1}compared with the 0 kg N ha^{-1} rate. Sucrose concentration however was reduced 1.3, 4.0 and 6.3% at the same N applications. The concentration of α-amino N, a non-sucrose parameter, increases as sugarbeet assimilates excessive levels of nitrate late in the growing season (Cariolle and Duval 2006). Sugarbeet root quality decreases as α-amino N concentration increases because the crystallization of sucrose is reduced (Cariolle and Duval 2006). In Germany, α- amino N concentrations in four varieties of sugarbeet increased linearly as preplant N application rates increased from 40 to 200 kg N ha^{-1}, and the α-amino N concentration at the 200 kg N ha^{-1} rate was approximately double the concentration value at the 40 kg N ha^{-1} rate (Hoffmann 2005).

Nitrogen recommendations for sugarbeet grown in Michigan are based on the previous crop grown within the rotation and results from soil tests (Michigan Sugar Company 2011). Following corn, the recommended rate of N for sugarbeet ranges from 135 to 168 kg N ha^{-1}; following all other crops the recommended rate of N ranges between 100 to 135 kg N ha^{-1} (Michigan Sugar Company 2011).

Glyphosate-Resistant Crops

Glyphosate has been widely adopted since its introduction in the early 1970's due to its ability to control a broad spectrum of weeds (Baylis 2000). The use of glyphosate continued to increase once glyphosate-resistant crops were introduced in 1996 because glyphosate made weed control easier and more effective, ultimately leading to a reduction in the number of tillage passes and herbicide applications (Gianessi 2008; Green 2009). Six agronomic crops have been engineered for glyphosate-resistance: soybean (*Glycine max* [L.] Merr), cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), canola (*Brassica napus* L. and *Brassica rapa* L.), alfalfa (*Medicago sativa* L.) and sugarbeet (Green 2009). Glyphosate-resistant sugarbeet were first commercially grown in the United States in 2008 (Armstrong and Sprague 2010). That year, glyphosate-resistant sugarbeet was planted on approximately half of Michigan's sugarbeet hectares (Armstrong 2009), and this increased to approximately 98% in 2011 (G. Clark, Agronomist, Michigan Sugar Company, Bay City, MI, personal communication).

Weed Control in Glyphosate-Resistant Sugarbeet

Traditional methods of weed control in conventional sugarbeet was challenging and expensive (Guza et al. 2002). Few conventional herbicides were available, and most controlled weeds poorly; therefore, growers relied on mechanical cultivation and hand-weeding to achieve effective weed control (Schweizer and May 1993). Conventional herbicides did not offer growers flexible application timings because weeds had to be controlled soon after emergence, and repeated applications were often required to maintain control (Dale and Renner 2005; Dexter 1994)

Glyphosate-resistant sugarbeet benefits growers because glyphosate is cheaper, and more effective at controlling a broad selection of weeds than conventional herbicides. This allows growers to achieve greater root and sucrose yields (Guza et al. 2002; Kniss et al. 2004; Wilson et al. 2002). Glyphosate is capable of controlling larger weeds than most postemergence (POST) herbicides, providing growers with flexible application timings (Kemp et al. 2009).

Several studies have been conducted to compare the effectiveness of glyphosate and conventional herbicides in glyphosate-resistant sugarbeet. Wilson et al. (2002) concluded that two applications of glyphosate (0.84 kg ai ha^{-1}) provided similar weed control results to three applications of the conventional herbicides desmedipham (0.18 kg ai ha^{-1}) + phenmedipham (0.18 kg ai ha^{-1}) + triflusulfuron (0.018 kg ai ha^{-1}) + clopyralid (0.1 kg ai ha^{-1}) beginning when weeds were 10 cm tall and 3 cm tall, respectively. Guza et al. (2002) determined that two or three applications of glyphosate (0.41, 0.63, and 0.84 kg ai ha^{-1}) controlled weeds more effectively than a conventional herbicide program. The results of these experiments imply that glyphosate is more effective at controlling weeds than conventional herbicides.

Glyphosate controls weeds very effectively. Dexter and Luecke (1999) and Kniss et al. (2004) concluded that two applications of glyphosate at 0.84 kg ai ha^{-1} controlled 96 and 95% of weeds, respectively. Dexter and Luecke (1999) applied glyphosate at the 2-leaf sugarbeet growth stage when weeds were very small; however, Kniss et al. (2004) did not apply glyphosate until weeds were 10 cm tall. In addition to excellent weed control, glyphosate reduces weed density and biomass more than conventional herbicides. Lowest weed density and biomass was achieved when glyphosate was applied to 5 cm tall weeds, followed by a second application to 10 cm tall weeds (Armstrong and Sprague 2010).

The appropriate time to control weeds to avoid reduction in glyphosate-resistant sugarbeet yield is dependent on crop-weed interactions. Kemp et al. (2009) determined that the critical time of weed removal for sugarbeet to avoid 10% yield loss ranged from 8 weeks after planting (WAP) to beyond 11 WAP. Lower weed densities may have extended the period of time for critical weed removal. Others have reported the critical weed removal time based on weed height. For example, Wilson et al. (2002) concluded that the optimum time of glyphosate application in glyphosate-resistant sugarbeet to avoid yield loss was when weeds were 10 cm tall. In a more recent study, Armstrong and Sprague (2010) also found over a range of row widths that waiting to control weeds until they were taller than 10 cm resulted in reduced sugarbeet yield.

In addition to achieving greater weed control at later application dates, glyphosate-resistant sugarbeet allows growers to alter their production practices. Greater weed control with glyphosate reduces growers' reliance on mechanical cultivation. Sugarbeet rows can be narrowed from 71- or 76-cm, to 38- or 51-cm rows because cultivation is no longer required for effective weed control. Narrow rows (38- and 51-cm) reduce weed density and biomass and increase sugarbeet root yield compared to 76-cm rows (Armstrong and Sprague 2010).

Nitrogen and Weed Interactions in Sugarbeet

Many studies have been conducted to determine the competitive interactions of crops and weeds. Nitrogen application rate and timing play a significant role in determining weed species' emergence, growth, and competitive ability (Sweeney et al. 2008; Blackshaw amd Brandt 2008). These interactions are important to understand because it allows growers to alter their weed management control programs, and managing N applications may reduce weed competition with crops (Di Tomaso 1995).

Nitrogen application timing affects sugarbeet-weed interactions. A study conducted by Paolini et al. (1999) determined that the response of common lambsquarters (*Chenopodium album* L.) and wild mustard (*Sinapis arvensis* L.) to N was dependent on the application timing. Common lambsquarters was more competitive with sugarbeet when 120 kg N ha^{-1} was applied at the 4- to 6-leaf growth stage, compared with later N applications at the 10- to 12-leaf growth stage. Wild mustard response was the opposite of common lambsquarters, and was more competitive when N was applied at the 10- to 12-leaf growth stage. Weeds' response to different application timings was influenced by emergence date and growth rate. When N was applied at the 4- to 6-leaf growth stage, wild mustard and sugarbeet were at identical growth stages. At the 10- to 12-leaf growth stage, wild mustard was near reproductive maturity, and competition with sugarbeet was most likely lower.

In addition to N application timing, N rate also influences the growth response of weeds. A study conducted by Blackshaw et al. (2003) examined the response of several weed species to increasing N application rates in a controlled environment. Root and shoot growth increased in all weeds as N rate increased, but the magnitude of response varied among species. Weeds, including common lambsquarters and redroot pigweed (*Amaranthus retroflexus* L.) were most responsive to increasing N because they are luxury consumers of N. A study conducted in sugarbeet also determined weeds respond to increasing applications of N. The biomass of common lambsquarters, ladysthumb (*Polygonum persicaria* L.), giant foxtail (*Setaria faberi* R. Herm.), redroot pigweed, and velvetleaf (*Abutilon theophrasti* Medik.) were greater when 168 kg N ha^{-1} were applied compared to no N application (Sweeney et al. 2008). Emergence of all these weeds, except redroot pigweed increased as higher N rates.

Increasing weed densities and the amount of time that weeds compete with sugarbeet negatively impacts sugarbeet root yield and sucrose production. At 6-, 12-, 18-, and 24- plants m^{-2}, Powell amaranth (*Amaranthus powellii* S. Watson) decreased sugarbeet root yields 8, 14, 24 and 25%, respectively, and sucrose yields were lowered 7, 13, 23 and 24%, respectively (Schweizer and Laurdison 1985). Common lambsquarters affected sugarbeet root and sucrose yields similarly to Powell amaranth (Schweizer 1983). The magnitude of sugarbeet response is dependent on the specific weed species. At equal densities, sunflower (*Helianthus annuus* L.) is three times more competitive than common lambsquarters and kochia (*Kochia scoparia* L.), and five times more competitive than velvetleaf (*Abutilon theophrasti* Medik.) (Schweizer 1983). Sunflower is the most competitive of these weed species due to its rapid growth in early spring and its large size by harvest (Schweizer 1983). The amount of time that weeds are present in the field also affects sugarbeet root yield. Uncontrolled kochia, common lambsquarters, and barnyardgrass that competed with sugarbeet all season reduced sugarbeet root yields by 95%, 94%, and 49%, respectively compared to season-long control of the weed species (Weatherspoon and Schweizer 1969; Dawson 1965).

Previous studies indicate high N application rates improve sugarbeet root yields but decreased sucrose quality. Competition from weeds also impacts sugarbeet production, specifically the density of weeds and the length of time the weeds are allowed to compete with sugarbeet. Additional studies should be conducted to determine how both N and weed competition influence sugarbeet yield and quality in order for growers to achieve greater production systems.

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CHAPTER 2

IMPACT OF NITROGEN AND WEEDS ON GLYPHOSATE-RESISTANT SUGARBEET YIELD AND QUALITY

Abstract

Field experiments were conducted in 2010 and 2011 at the Saginaw Valley Research and Extension Center near Richville, Michigan and at the Michigan State University Crop and Soil Sciences Research Farm in East Lansing, Michigan to determine the effects of nitrogen (N) application and weed removal timings on glyphosate-resistant sugarbeet yield and sugar quality. Nitrogen application rates were 0, 67, 100, 134 and 67:67 kg N ha^{-1} and weed removal timings were when weeds were < 2, 8, 15 and 30 cm tall. At the beginning of the growing season, weeds responded to N rates sooner than sugarbeet. Nitrogen assimilation by weeds was 3 times greater than sugarbeet at 0, 67, 100 and 134 kg N ha^{-1} and 4 times greater than sugarbeet with the split application (67:67 kg N ha^{-1}) averaged over weed removal timings. Higher N rates increased N sufficiency index values and sugarbeet canopy closure; weeds 30 cm tall reduced N sufficiency and impacted sugarbeet canopy closure. The effect of N on root yields varied, but the highest N rates (134 kg N ha^{-1} or 67:67 kg N ha^{-1}) was amongst the highest sugarbeet yields at all locations. Highest yields were achieved when weeds were controlled prior to reaching 2 cm tall at three of the four site-years. Waiting to control weeds until they were 8 or 15 cm tall resulted in up to 15% yield reductions, while 30 cm tall weeds reduced yields up to 21%. Recoverable white sucrose per ha (RWSH) followed the same trends as root yield, and values were 8 to 16% lower if weeds were not controlled until they were 8 cm tall. The results indicate weeds are highly competitive with sugarbeet and weeds assimilate large quantities of N early in the

growing season, especially at larger growth stages. Weed competition negatively impacts sugarbeet canopy development, root yield and sucrose production, and they should be controlled prior to 8-cm heights to avoid negative impacts.

Nomenclature: sugarbeet, *Beta vulgaris* L. 'Hilleshög 9042'

Key words: Glyphosate-resistant sugarbeet; nitrogen rate; weed removal timing; nitrogen assimilation; yield; sucrose production.

Introduction

The introduction of glyphosate-resistant sugarbeet in 2008 has improved weed management in sugarbeet fields. Traditional methods of controlling weeds in sugarbeet required growers to apply multiple postemergence (POST) applications of conventional herbicides beginning at 2.5 cm weed heights (Guza et al. 2002; Dale and Renner 2005). Glyphosate however, controls larger weeds, offering greater flexibility in application timing (Kemp et al. 2009). A single application of glyphosate can be as effective as multiple applications of conventional sugarbeet herbicides for weed control (Guza et al. 2002). Growers that utilize glyphosate weed control programs achieve higher sugarbeet root yields because weed control is more effective and sugarbeet injury is reduced in comparison to traditional conventional herbicide programs (Kniss et al. 2004; Kemp et al. 2009).

Although, growers have a greater period of time to initially control weeds with glyphosate, delaying the first application may negatively impact glyphosate-resistant sugarbeet root yield and sucrose production. Weeds compete with crops for water, nutrients and light (Schweizer and May 1993). The length of time that weeds compete with the crops and the amount of N available to the weeds influences their competitive ability. Researchers have shown that weeds can assimilate large amounts of nitrogen (N). Bast (2012) reported that common lambsquarters (*Chenopodium album* L.), common ragweed (*Ambrosia artemisiifolia* L.) and giant foxtail (*Setaria faberi* R. Herm.) assimilated at least twelve times more N than corn across N rates ranging from 0 to 202 kg N ha^{-1}. Additionally, as weed height increased, N assimilation by weeds also increased. Effective and timely weed control in sugarbeet is necessary because an adequate quantity of N is required early in the growing season. At the 4- to 5-leaf growth stage, sugarbeet rapidly assimilates N to promote canopy development, and N should be available throughout the growing season to maintain the canopy and assist root growth (Scott and Jaggard 1993).

The appropriate time to control weeds to avoid reduction in yield is dependent on crop-weed interactions. Kemp et al. (2009) determined that the critical time of weed removal for sugarbeet to avoid 10% yield loss ranged from 8 weeks after planting (WAP) to beyond 11 WAP. Others have reported the critical weed removal time based on weed height. For example, Wilson et al. (2002) concluded that the optimum time of glyphosate application in glyphosate-resistant sugarbeet to avoid yield loss was when weeds were 10 cm tall. In a more recent study, Armstrong and Sprague (2010) also found over a range of row widths that waiting to control weeds until they were taller than 10 cm resulted in reduced sugarbeet yield. Many of these recommendations have been derived from research conducted with optimum N fertilization. However, due to increases in N fertilizer prices growers are examining ways to optimize N in sugarbeet production systems.

Understanding the influence of weed control timing and N fertilization on glyphosate-resistant sugarbeet yield and quality will allow growers to achieve greater production systems. Therefore, the objectives of this research were to 1) determine the amount of N assimilated by weeds and sugarbeet early in the growing season, 2) determine how N rate and weed height influence sugarbeet growth and 3) determine how N rate and weed height affect sugarbeet yield and quality.

Materials and Methods

Field experiments were conducted in 2010 and 2011 at the Saginaw Valley Research and Extension Center near Richville, Michigan and at the Michigan State University Crop and Soil Sciences Research Farm in East Lansing, Michigan. The soil at Richville was a Tappan-Londo loam complex (fine-loamy, mixed, active, calcareous, mesic Typic Endoaquolls and fine-loamy, mixed, semiactive, mesic Aeric Glossaqualfs) and the soil at East Lansing was a Capac loam (fine-loamy, mixed, active, mesic Aquic Glossudalfs). Soil samples were taken prior to preplant N fertilization and planting to determine soil pH, organic matter, total N, and nitrate-N (NO_3-N) and ammonium-N (NH_4-N) concentrations (Table 2.1). The crops prior to these experiments were soybean (*Glycine max* [L.] Merr) at Richville and East Lansing in 2010, and corn (*Zea mays* L.) at Richville in 2011. The field was fallow prior to the 2011 experiment at the East Lansing location. All fields were chisel plowed in the fall and leveled with a field cultivator in the spring.

The experiment was setup as a two factor strip plot design with four replications. The first factor was N application at five levels: 0, 67, 100, 134 kg N ha^{-1} applied preplant and a split application totaling 134 kg N ha^{-1}, where 67 kg N ha^{-1} was applied preplant and an additional 67 kg N ha^{-1} was applied to sugarbeet at the 4- to 6-leaf growth stage. The second factor was weed removal timing at four levels: weeds < 2, 8, 15 and 30 cm in height totaling 20 treatments. Plots were 4 rows wide (3 m) and 9.1 m long. Prior to planting (2-21 d), the preplant N treatments were applied as urea (46-0-0) and incorporated with a soil finisher. Urea at 67 kg N ha^{-1} was broadcast to 4- to 6-leaf sugarbeet prior to a rainfall event for the split application treatment (67:67 kg N ha^{-1}).

The glyphosate-resistant sugarbeet variety 'Hilleshög 9042' (Syngenta Seeds Inc., 1020 Sugarmill Rd., Longmont, CO 80501) was planted at a depth of 2.5 cm in 76 cm wide rows at a population of 122,000 seeds ha^{-1}. Planting dates were March 31, 2010 and May 4, 2011 at Richville and April 2, 2010 and May 5, 2011 at East Lansing. Sugarbeet was replanted on May 19 in 2010 at East Lansing, due to poor sugarbeet emergence from soil crusting.

To achieve the four weed removal timings, weeds were controlled by applying glyphosate (Roundup PowerMAX, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167) at 0.84 kg ae ha^{-1} + ammonium sulfate (Actamaster, Loveland Products, Inc., 3005 Rocky Mountain Avenue, Loveland, CO 80538) at 2% v/v. Application timings were based on average weed canopy heights of < 2, 8, 15 and 30 cm tall. Table 2.2 lists the number of days after planting and the sugarbeet growth stages for each application time. All plots were maintained weed-free after the initial weed removal timing by periodic applications of glyphosate. Fungicide treatments were applied to prevent Rhizoctonia crown and root rot (*Rhizoctonia solani* Kühn) and Cercospera leaf spot (*Cercospora beticola* Sacc.) disease damage according to local standard practices for sugarbeet grown in Michigan.

Precipitation data was recorded throughout the growing season and obtained from the Michigan *Automated Weather Network (*http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, MI).

Nitrogen Assimilation. Weeds were sampled for N analysis at the 8, 15 and 30 cm weed removal timings prior to the initial glyphosate application. Roots and shoots for all weeds from two 0.25 m^2 sampling areas were collected in each plot between the two center sugarbeet rows. The number of weeds was recorded at the time of collection (Table 2.3). Predominant weed species at Richville were common lambsquarters and Pennsylvania smartweed (*Polygonum pensylvanicum* L.) with some *Amaranthus* species (Powell amaranth (*Amaranthus powellii* S. Watson) and redroot pigweed (*Amaranthus retroflexus* L.)) (< 5 m^{-2}). At East Lansing, the weeds were much more diverse. In 2010, common lambsquarters, Powell amaranth, giant foxtail (*Setaria faberi* R. Herm.), velvetleaf (*Abutilon theophrasti* Medik.), and common purslane (*Portulaca oleracea* L.) contributed to the majority of the weed biomass. In 2011, common ragweed and wild mustard (*Sinapis arvensis* L.) were added to this mix. At East Lansing in 2011 there were several winter annual seedlings (henbit (*Lamium amplexicaule* L.), shepherd's-purse (*Capsella bursa-pastoris* L. Medik.), and field pennycress (*Thlaspi arvense* L.)) that were present but did not contribute substantially to the weed biomass and therefore are not included in the weed density counts. In addition, six sugarbeets from each plot were collected for N analysis. Weed and sugarbeet samples were oven-dried at 66°C in a forced air oven for 7 d and weighed.

Prior to harvest, four sugarbeets were hand-harvested for N analysis from each treatment. Sugarbeet leaves were removed from the root, oven-dried at 66°C for 7 d, and weighed. Weights were determined for sugarbeet roots. In 2010, sugarbeets were cut by hand into small pieces and dried in a forced air oven until no further weight loss occurred. In 2011, subsamples were taken from each sugarbeet with a brei saw. Brei samples were frozen at -80°C for 2 to 14 d, and then transferred to a freeze dryer (VirTis Genesis 12 EL Lyophilizer, VirTis, 815 Route 208, Gardiner, New York, 12525) for 7 d.

All weed and sugarbeet samples were mechanically ground using a Wiley Mill (Arthur H. Thomas Wiley Cutting Mill, Thomas Scientific, 1654 High Hill Road, Swedesboro, NJ 08085), Cyclone Mill (Cyclone Sample Mill, Thomas Scientific, 1654 High Hill Road, Swedesboro, NJ 08085), or Christy Mill (Christy & Norris Lab Mill, Christy Turner Limited, Knightsdale Road, Ipwich, Suffolk, IP1 4LE, United Kingdom) depending on the dry biomass weight and passed through sieves $\leq$ 2 mm. Weeds with low dry biomass were ground with a mortar and pestle to ensure adequate sample size for analysis. The 2011 sugarbeet samples that were freeze-dried were pulverized in a shaker (Geno Grinder 2000, Spex SamplePrep, LLC, 203 Norcross Avenue, Metuchen, NJ 00840) for 20 min at 1700 strokes min^{-1}. Total N content for the weed and sugarbeet samples was determined by the micro-Kjeldahl digestion method (Bremner 1996; Jung et al. 2003) and colorimetric analysis through a Lachat rapid flow injector autoanalyzer (Lachat Instruments, Milwaukee, WI). Nitrogen assimilation was calculated by multiplying the plant biomass by nitrogen concentration.

In-Season N Sufficiency Index. Chlorophyll readings were taken with a Minolta SPAD (Soil-Plant Analysis Development) 502 chlorophyll meter to determine sugarbeet leaf greenness (Spectrum Technologies, Inc., 12360 S. Industrial Dr. E., Plainfield, IL 6058). SPAD meter readings are positively correlated with leaf N and leaf chlorophyll concentrations (Schepers et al. 1992) and the meter may be used to monitor a crop’s N content. Low chlorophyll meter readings may indicate N deficiency in crops, and additional applications of N may be applied early in the growing season to correct the deficiency (Shapiro et al. 2006). Ten sugarbeet plants $plot^{-1}$ were randomly selected for non-destructive SPAD meter readings. The meter was placed halfway between the leaf tip and petiole and between the leaf margin and midvein of the newest fully emerged sugarbeet leaves. Readings were taken when sugarbeet had approximately 8-true leaves (late-June), 16-true leaves (mid-July), and 26-true leaves (mid-August). Chlorophyll readings were used to calculate N sufficiency index (Schepers et al. 1992; Shapiro et al. 2006) with equation 1.

N sufficiency index = [average treatment reading /

average non-limiting N treatment (i.e., < 2-cm, 134 kg ha^{-1} treatment)]*100 [1]

Canopy Development. Light interception by the sugarbeet canopy was measured using a 1 m by 13 mm wand (SunScan Canopy Analysis System, Dynamax Inc., 10808 Fallstone Road #350, Houston, TX 77099) at three random locations above the canopy (96 cm above the ground) and three corresponding locations below the canopy (5 cm above the ground). The wand was placed perpendicular to the sugarbeet rows. Data was collected using a hand-held computer (Recon, Trimble Navigation Limited, 935 Stewart Drive, Sunnyvale, CA 94085). Light interception measurements were used to determine canopy closure using equation 2. Canopy closure measurements were taken at two different times, early- and late-July.

Canopy closure (%) = [(above canopy measurement - below canopy measurement) /

(above canopy measurement)]*100 [2]

Yield and Quality. Sugarbeet roots from the two center rows of each plot were mechanically harvested and weighed from all treatments. Harvest dates were September 21, 2010 and

September 16, 2011 at Richville and October 7, 2010 and November 2, 2011 at East Lansing. Sucrose concentration was determined from samples of 10 roots plot^{-1} at the Michigan Sugar Company laboratory (Michigan Sugar Company, 2600 S. Euclid Avenue, Bay City, MI 48706).

Statistical Analysis. Data were analyzed using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc, 100 SAS Campus Drive, Cary, NC 27513) to determine if locations and years could be combined. Data was combined over locations and/or years when there was no significant treatment by location and/or year interaction. Data were presented as main effects when there was not a significant N by weed removal timing interaction. Nitrogen assimilation by weeds and sugarbeet was modeled over weed removal height for each N rate using regression with PROC REG in SAS 9.3. Sugarbeet root yield was subjected to regression analysis. However, the data did not fit the logistic equation commonly used to model yield loss by weed removal timings (Kemp et al. 2009; Everman et al. 2008; Knezevic et al. 2002). Sugarbeet root yield data and all remaining data were analyzed using the PROC MIXED procedure in SAS 9.3. Normality of the residuals was evaluated by examination of normal probability and stem-and-leaf plots. Homogeneity of the variances was evaluated by the Levene's test ($p \leq 0.05$) and they were grouped to improve the model. An analysis of variance was performed and treatment means were compared using Fisher's Protected LSD at the $p \leq 0.05$ significance level.

Results and Discussion

Growing Conditions. Monthly precipitation was recorded at each location and is listed in Table 2.4 along with the 30-yr precipitation averages. Growing seasons varied at all locations due to differences in precipitation. Growers in Michigan strive to plant sugarbeet as soon as soil conditions are conducive in the spring. To prevent any sugarbeet seed germination issues from N application, the intent was to allow a few days between preplant N application and sugarbeet planting. The 2010 growing season started early and preplant N application and planting was completed by April 2. However, due to poor emergence from soil crusting at East Lansing sugarbeet were replanted 7 wk after the initial N application, and 17 cm of precipitation was recorded between the preplant N application and replanting. In 2011, precipitation events were high early in the season, delaying planting for 3 wk after preplant N application at both locations. Throughout the growing season monthly precipitation was well below the 30-yr average for July and August at Richville in 2010, July at Richville in 2011, and August for East Lansing in 2010.

Soil samples taken prior to preplant N fertilization and planting indicated residual NO_3-N and NH_4-N levels were higher in 2010 at both locations because the crop prior to these experiments were soybean (Table 2.1) (Hoeft et al. 2000). Higher levels of N were available to sugarbeet early in the growing season in 2010 at both locations than at either location in 2011.

Biomass and Tissue N Concentration. Due to a lack of significant interactions, weed biomass data could be combined across locations and years (Table 2.5). Nitrogen application, regardless of rate, did not affect weed biomass at the 8 cm weed removal timing. However, when weeds were harvested at the 15 or 30 cm weed removal timing, any addition of N increased biomass over the 0 kg N ha^{-1} treatment. At the lowest N rate (67 kg N ha^{-1}) weed biomass was 40% or more higher at the 15 and 30 cm weed removal timing when compared with the 0 kg N ha^{-1}. Nitrogen rates higher than 67 kg N ha^{-1} did not significantly increase weed biomass.

The N concentration found in weeds was affected by N rate and weed removal timing. Combined over all locations and years, N concentration was higher with the addition of preplant N (Table 2.5). The N concentration for weeds harvested from the 0 kg N ha^{-1} treatment was 3.1%, while N concentration was 3.4 to 3.6% when N was applied. Similar to the weed biomass results, increases in N rate did not influence the N concentration in weeds. Although not consistent across all locations, increases in weed size decreased weed N concentration (Table 2.6). Exceptions were observed at the 15 cm weed removal timing at the combined Richville locations and at East Lansing 2010, where N concentration was either similar to or greater than weeds that were sampled at the 8 cm weed removal timing.

Within each weed removal timing N only influenced sugarbeet biomass at the 30 cm weed height (Table 2.7). The addition of N led to at least a 30% increase in sugarbeet biomass. Sugarbeet biomass was also higher at the 100 and 134 kg N ha^{-1} rates compared with the split-application that totaled 134 kg N ha^{-1}, suggesting that weeds reduced the amount of available N to sugarbeet from the second N application. This is contrary to what was observed at the 30 cm weed removal timing for weed biomass, where weed biomass at the split N application was similar to the preplant applications (Table 2.5). Differences in N effects on weed and sugarbeet biomass at the split application may have been caused by the N application method; the second application of N was broadcasted, not banded near the row. Nitrogen application methods effect weed-crop interactions (Di Tomaso 1995), and proper N placement is especially important in sugarbeet fields due to the early season assimilation of N by sugarbeet (Draycott 1993). Nitrogen may have been less available to sugarbeet because the broadcast application did not place the N close to the root (Stevens et al. 2007). A study conducted by Blackhaw et al. (2002)

determined the importance of N placement in spring wheat (*Triticum aestivum* L. 'Katepwa') grown in competition with green foxtail (*Setaria viridis* L.) and wild mustard. Both green foxtail and wild mustard had greater biomass when preplant N was broadcasted on to the soil surface compared to preplant N applications that were banded on the soil surface or injected into the soil profile.

Similar to weed N concentration, sugarbeet N concentration was around 3.5% when N was applied and was higher than the 0 kg N ha^{-1} rate combined over all weed removal timings (Table 2.7). Nitrogen concentration in sugarbeet was also affected by weed height. At three of the four location by year combinations, sugarbeet N concentration was lowest at the 30 cm weed removal timing (Table 2.6). Low sugarbeet and weed N concentrations at the 30 cm weed removal timing might be attributed to a "dilution effect" because of the greater plant biomass at this weed removal timing (Blackshaw et al. 2003).

Nitrogen Assimilation. Weed height at the time of removal and N rate greatly affected N assimilation by weeds. Combined over all locations and years, N assimilation by weeds increased linearly with increasing weed growth at all N rates (Figure 2.1a and Table 2.8). At the 8 cm weed removal timing, weed N assimilation was low (< 5.5 kg N ha^{-1}) and was not affected by N rate. At the 15 and 30 cm weed removal timings, weed N assimilation ranged from 8 to 16 and 19 to 40 kg N ha^{-1}, respectively. Nitrogen application rate had a greater influence on weed N assimilation at the later weed removal timings. Any application of N, regardless of rate, resulted in higher N assimilation by weeds compared with the 0 kg N ha^{-1} and differences between N rates only occurred at the 30 cm weed removal timing. Weeds 30 cm tall assimilated the greatest amount of N (40 kg N ha^{-1}) when N was applied as a split application (67:67 kg N ha^{-1}). Several weed species in study, including common lambsquarters, redroot pigweed and wild mustard, have been reported to be highly responsive to increasing doses of N and are categorized as luxury consumers (Blackshaw and Brandt 2008). The split-application of N provided a greater chance for these weeds to assimilate higher amounts of N as weeds increased in size.

Nitrogen assimilation by sugarbeet also increased linearly with the later weed removal timings at all N rates (Figure 2.1b and Table 2.8). However, N assimilation by sugarbeet was considerably lower than weeds and was not affected by N rate until the 30 cm weed removal timing. Sugarbeet may have had lower N assimilations than weeds due to high weed density. In corn, N assimilation by weeds was 12 times greater than the crop as a result of high weed density pressure and luxury consumption of N by weeds (Bast 2012). Nitrogen assimilation by sugarbeet was less than 1 and less than 4 kg N ha^{-1} at the 8 and 15 cm weed removal timings, respectively. Sugarbeet was able to assimilate 8.3 to 13.2 kg N ha^{-1} at the 30 cm weed removal timing. Nitrogen assimilation was higher at the 67, 100 and 134 kg N ha^{-1} rates, and lower at the split application (67:67 kg N ha^{-1}). Similar to sugarbeet biomass, N assimilation by sugarbeet at the split application may have also been influenced by the method of N application. In a banded or point-injected N application, N is more accessible to sugarbeet for assimilation because it is placed closer to the root (Stevens et al. 2007). In competition with green foxtail and wild mustard, N assimilation by spring wheat was greater when N was injected into the soil profile compared to banded or broadcasted on top of the soil surface, and higher N assimilations improved spring wheat's competitive ability with the weed species (Blackshaw et al. 2002).

In-Season N Sufficiency Index. The main effects of N and weed removal timing are presented for the in-season N sufficiency index data for sugarbeet. There was a significant effect of N rate for sugarbeet N sufficiency in late-June when sugarbeet had approximately 8-true leaves (Table 2.9). All applications of N resulted in higher sugarbeet N sufficiency index values than the 0 kg N ha^{-1}, most likely as a result of N assimilation by sugarbeet early in the growing season for canopy development (Scott and Jaggard 1993). Nitrogen rates of 100 and 134 kg N ha^{-1} resulted in higher N sufficiency indices than the 67 kg N ha^{-1} rate because N deficient sugarbeet has lower chlorophyll concentrations (Draycott and Christenson 2003). Sugarbeet N sufficiency index values for the split application of N (67:67 kg N ha^{-1}) were similar to the 67 and 100 kg N ha^{-1} but lower than the 134 kg N ha^{-1} rate. This was most likely due to the second application of N only being applied approximately 2.5 wk prior to SPAD chlorophyll meter readings and sugarbeet may not have fully utilized the total available N. The main effect of weed removal timing also affected sugarbeet N sufficiency in late-June. Sugarbeet N sufficiency was lowest when weeds were not controlled by the 30 cm weed removal timing. At this timing, weeds were able to assimilate up to 40 kg N ha^{-1} (Figure 2.1a) that would not have been available to sugarbeet.

As the season progressed and sugarbeet was approaching 16-true leaves in mid-July, sugarbeet N sufficiency index values differed by year and location for the main effect of N rate and weed removal timing. At Richville in 2010 as long as N was applied, sugarbeet N sufficiency index values were similar (Table 2.9). The lack of differences between N rates at this timing may have been due to below average precipitation throughout the month of July (Table 2.4), resulting in lower N availability to sugarbeet. At Richville in 2011, differences in N rate for sugarbeet N sufficiency index values were similar to late-June measurements, with the exception that the highest sugarbeet N sufficiency index values were from the preplant and the split applications of N that totaled 134 kg N ha^{-1}. A high N sufficiency index value at the split application indicates that sugarbeet was able to utilize all available N from the second N application. The main effect of weed removal timing at Richville combined over 2010 and 2011, showed similar results as the late-June measurements, indicating that weeds not removed prior to 30 cm resulted in significant N assimilation by weeds that was not available to sugarbeet. At East Lansing in 2010 and 2011, there was no difference in sugarbeet N sufficiency index values for the main effects of N rate or weed removal timing. At this location for both years, excessive precipitation between early season N applications and planting may have exhausted available N for sugarbeet earlier in the season.

Near the end of the growing season in mid-August when sugarbeet had approximately 26-true leaves, there were no differences in sugarbeet N sufficiency index values for the main effects of N rate or weed removal timing. Sugarbeet may not have been actively assimilating N in mid-August due to below average precipitation throughout the month of August (Table 2.4) (Armstrong et al. 1986). Additionally, N sufficiency index values may not have been significant in mid-August because N was redistributed from the sugarbeet leaves to the roots to assist with growth (Armstrong et al. 1986; Scott and Jaggard 1993).

Canopy Development. The main effects of N and weed removal timing are presented for sugarbeet canopy closure. The main effect of N rate did not affect early-July sugarbeet canopy closure in Richville 2010 or for the combined 2010 and 2011 East Lansing data (Table 2.10). At Richville in 2011, the higher rates of N increased sugarbeet canopy closure compared with the 0 kg N ha^{-1}. Sugarbeet canopy closure was at least 49 percentage points higher at the 100, 134 or the 67:67 kg N ha^{-1} rates compared with the 0 kg N ha^{-1} rate because higher N rates enhanced sugarbeet leaf growth (Scott and Jaggard 1993). Early sugarbeet canopy closure is important for preventing weed emergence later in the growing season and enhancing light interception for sucrose production (Kemp et al. 2009; Scott and Jaggard 1993; Briscoe et al.1980). The main effect of weed removal timing had little effect on sugarbeet canopy closure at East Lansing in 2010. However, weed removal timing has more of an impact on sugarbeet canopy closure at Richville in 2010 and 2011 and at East Lansing 2011. Weeds needed to be controlled by 15 and 30 cm tall at East Lansing 2011 and Richville (2010 and 2011), respectively, to maximize sugarbeet canopy closure. Weed competition impacted sugarbeet canopy closure at East Lansing earlier than Richville most likely because weed density was higher (Table 2.3). Sugarbeet canopy closure was lower when weeds were not removed by the time they were 30 cm tall.

Similar to the early-July sugarbeet canopy closure measurements canopy closure in late-July was not significant for the main effect of N for Richville in 2010 (Table 2.10). However, N rate had a significant influence on sugarbeet canopy closure for Richville in 2011 and for the combined 2010 and 2011 East Lansing data. At Richville in 2011, any application of N led to greater sugarbeet canopy closure. At East Lansing, sugarbeet canopy closure was similar at the higher N rates of 100, 134, 67:67 kg N ha^{-1}. The main effect of weed removal timing on sugarbeet canopy closure in late-July was similar between all years and locations. Canopy closure was greater when weeds were controlled prior to 2-cm.

Nitrogen Assimilation of Sugarbeet Prior to Harvest. The main effect of N affected sugarbeet N assimilation prior to harvest. Combined over all locations and years, sugarbeet assimilated more N at the highest N rates of 100, 134 or the 67:67 kg N ha^{-1} (Table 2.11). Preplant N applications of 0, 67, 134 and 202 kg N ha^{-1} in an irrigated field study found incremental increases in sugarbeet N assimilation as N rate increased (Draycott and Durrant 1971); however, our data suggests that sugarbeet N assimilation plateaued at 100 kg N ha^{-1}. Differences in N assimilation by sugarbeet at the higher N application rates may not have been observed due to the large accumulations of precipitation early in the growing season in 2010 at East Lansing (17 cm) and in 2011 at Richville (10 cm) and East Lansing (8.9 cm). In clay loam soils, 2.54 cm of rainfall moves NO_3 2.54 cm downward in the soil profile (Nelson and Huber 1992). In our experiment, N may have been less available to sugarbeet at the higher application rates due to the downward movement of NO_3. Sugarbeet N assimilation prior to harvest was also affected by weed removal timing. At the later weed removal timings, sugarbeet N assimilation was lower (Table 2.11). At these later weed removal timings weeds were able to assimilate greater quantities of N that would not be available for sugarbeet to utilize (Figure 2.1a).

Sugarbeet Root Yield and Quality. The main effects of N and weed removal timing are presented for sugarbeet root yield and quality. Overall sugarbeet root yield was the highest at Richville in 2010 (Table 2.12). Shorter growing seasons at Richville 2011 and the combined

2010 and 2011 East Lansing locations, from delayed planting due to excessive early season precipitation, resulted in sugarbeet root yields averaging 25 and 43% lower, respectively, compared with the Richville 2011 location. Even though data could not be combined across all locations and years, the treatment with the highest N rate (134 kg N ha^{-1}) whether applied preplant or as split application was always amongst the highest sugarbeet yields. How the lower N rates affected sugarbeet yield varied for the different locations. Sugarbeet yield was lower at Richville 2010, and similar at Richville 201l and the combined 2010 and 2011 East Lansing data for the 100 kg N ha^{-1} rate compared with highest rate of N. At the 67 kg N ha^{-1} rate, sugarbeet root yield was similar to the highest N rate (134 kg N ha^{-1} or 67:67 kg N ha^{-1}) at Richville in 2010 and the combined 2010 and 2011 East Lansing data. High residual N levels in the soil at both locations in 2010 may have provided an additional source of N to sugarbeet which may have increased sugarbeet root yield at the lowest N application rate (Table 2.1) (Hoeft et al. 2000; Winter 1984).

The main effect of weed removal timing influenced sugarbeet root yield. In three of the four location by year combinations early-season weed control was extremely important (Table 2.12). At Richville 2010 and the combined 2010 and 2011 East Lansing, sugarbeet root yields were highest when weeds were controlled prior to reaching 2 cm tall. The effects of the different weed removal timings on sugarbeet yield were much more critical at Richville 2010 with the longer growing season than the other locations. Averaged across all N rates, waiting to control weeds until they were 8 or 15 cm tall resulted in a 15% reduction in yield and waiting until weeds were 30 cm tall resulted in a 21% reduction in yield compared with the < 2 cm weed removal timing at Richville 2010. Sugarbeet root yield was 8% lower and similar for the 8, 15, and 30 cm weed removal timings compared with the < 2 cm weed removal timing for the combined 2010 and 2011 East Lansing data. Regardless if weeds were removed at < 2 or 30 cm tall, sugarbeet yield was similar at Richville 2011. Others have shown that the critical time of weed removal can vary between years and locations. For example, Kemp et al. (2009) determined that the critical time of weed removal to avoid 10% sugarbeet root yield loss occurred 8 weeks after planting during the first year of the study, to beyond 11 weeks after planting the following study year. Lower weed densities may have extended the critical time of weed removal during the second year of the study. At the Richville 2011 location where there was little effect of weed removal timing on sugarbeet yield, weed populations were much lower than any of the other year by location combinations (Table 2.3), even though these weeds were able to remove significant amount of N at the later weed removal timings (Figure 2.1a).

Recoverable white sucrose per Mg of sugarbeet root (RWSMg) was affected by the main effect of N, but not the main effect of weed removal timing. The main effect of N on RWSMg was only significant for the combined 2010 and 2011 East Lansing data (Table 2.12). At East Lansing, RWSMg was 8% lower at the highest N rate (134 kg N ha^{-1}) applied preplant or as a split application and 4% lower at the 100 kg N ha^{-1} compared with the 0 kg N ha^{-1} rate. Sugarbeet may have not been able to utilize all available N available because of the shorter growing seasons. Well-balanced N applications are important in sugarbeet because N is needed to increase yield; however, too much N can decrease sucrose concentration (Anderson and Peterson 1988; Carter et al. 1976; and Carter and Traveller 1981). In an irrigated field study on silt loam soil, Carter and Traveller (1981) determined sucrose concentration was reduced 1.3, 4.0

and 6.3% when N was applied preplant at 112, 252, and 392 kg ha^{-1}, respectively, compared to 0 kg N ha^{-1}.

In addition to RWSMg, α-amino N provides an indication of sugarbeet quality because increasing α-amino N concentrations reduce crystallization of sucrose (Cariolle and Duval 2006). The concentration of α-amino N increases as sugarbeet assimilates excessive levels of nitrate late in the growing season (Cariolle and Duval 2006). The main effect of N influenced α-amino N concentration for the combined 2010 and 2011 Richville data (data not shown). Concentration values ranged from 7.8 to 12.9, with the lowest value observed at the 0 kg ha^{-1} rate and the highest values recorded at the highest N application rate (134 and 67:67 kg ha^{-1}). Any application of N, regardless of rate, resulted in higher α-amino N concentrations compared with the 0 kg N ha^{-1} rate. Averaged across weed removal timings, the highest N rate (134 kg N ha^{-1} and 67:67 kg N ha^{-1}) increased α-amino N concentrations 64% compared to 0 kg N ha^{-1}. At 100 and 67 N ha^{-1}, α-amino N concentrations increased 29% compared to 0 kg N ha^{-1}. Similar trends were observed for the combined 2010 and 2011 East Lansing data. In Germany, N application rate also influenced α-amino N concentrations. In four varieties of sugarbeet, α-amino N concentrations increased linearly as preplant N application rates increased from 40 to 200 kg N ha^{-1}, and the α-amino N concentration at the 200 kg N ha^{-1} rate was approximately double the concentration value at the 40 kg N ha^{-1} rate (Hoffmann 2005).

The main effect of weed removal timing was significant for the combined 2010 and 2011 Richville α-amino N concentration data (data not shown). Averaged across N rates, α-amino N concentrations were 18% higher at < 2-, 8- and 15-cm weed removal timings compared to the 30-cm weed removal timing. High N assimilation by 30 cm tall weeds reduced the available NO_3 to sugarbeet, lowering the α-amino N concentration.

The main effect of N on recoverable white sucrose ha^{-1} (RWSH) was only significant at Richville in 2010 and 2011 (Table 2.12). The higher RWSMg that was observed at East Lansing compensated for the reductions in root yield observed at the lower N rates, therefore there were no differences in RWSH for any of the N rates. However at Richville, regardless of year, any application rate of N improved RWSH over the 0 kg N ha^{-1} rate. In 2010, all applications of N led to similar RWSH results; however, RWSH at the 100 kg N ha^{-1} rate was also similar to RWSH at the 0 kg N ha^{-1} rate. Lower RWSH at 100 kg N ha^{-1} was the result of lower sugarbeet root yield (Table 2.12). In 2011, N applications needed to be $\geq$100 kg N ha^{-1} to maximized RWSH.

Similar to sugarbeet root yields, the main effect of weed removal timing affected RWSH at Richville 2010 and the combined 2010 and 2011 East Lansing data (Table 2.12). Weeds needed to be controlled prior to 2 cm to maximize RWSH at Richville 2010 and at East Lansing (2010 and 2011). RWSH was 16 and 8% lower at Richville 2010 and East Lansing (2010 and 2011) if weeds were controlled when they were 8 cm tall compared to 2 cm.

Overall, individual N application rates had less effect on sugarbeet root yield and quality than weed removal timing, and the competitive ability of the weeds may have been influenced by light quality. The ratio of far to far-red (F: FR) light reflected from the surface of weeds’ leaves can alter crop growth. Low F: FR ratios (shade conditions) reflected from redroot pigweed and common lambsquarters onto corn seedlings decreased the rate of corn leaf appearance and shoot dry weight compared to corn seedlings not grown in the presence of weeds (Liu et al. 2009; Mahoney and Swanton 2008). The results of these studies indicate weeds in corn, as well as other crops, should be controlled early to avoid adverse impacts on crop growth due to changes in the light quality environment.

Study Implications. Weeds 15 or 30 cm tall had higher biomass at any application of N; however, sugarbeet biomass were not affected by N until the 30 cm weed growth stage. N assimilation by weeds was considerably higher than sugarbeet most likely due to luxury consumption of N (Blackshaw and Brandt 2008) and high weed density populations. Weeds 30 cm tall assimilated 19 to 40 kg N ha^{-1}, while sugarbeet assimilated 8.3 to 11 kg N ha^{-1}.

In-season measurements indicate N rate and weed removal timing impacted sugarbeet canopy development. In-season N sufficiency index values were higher at 100 and 134 kg N ha^{-1} in late-June and at 134 and 67:67 kg N ha^{-1} in mid-July. Sugarbeet canopy closure was higher at the 100, 134 and 67:67 kg N ha^{-1} rates in early- and late-July. In general, weeds 30 cm tall reduced N sufficiency index values, while various weed growth stages negatively sugarbeet canopy closure.

The effect of N on root yields varied, but the highest N rate (134 kg N ha^{-1} or 67:67 kg N ha^{-1}) was amongst the highest sugarbeet yields at all locations. Weed removal timing greatly impacted sugarbeet root yield. Highest yields were achieved when weeds were controlled prior to reaching 2 cm tall at three of the four site-years. Waiting to control weeds until they were 8 or

15 cm tall resulted in up to 15% yield reductions, while 30 cm tall weeds reduced yields up to 21%. RWSH followed the same trends as root yield, and values were 8 to 16% lower if weeds were not controlled until they were 8 cm tall.

The results indicate weeds are highly competitive with sugarbeet. Weeds assimilate large quantities of N early in the growing season, especially at larger growth stages. Weed competition negatively impacts sugarbeet canopy development, root yield and sucrose production, and they should be controlled prior to 8-cm heights to avoid negative effects on sugarbeet growth and production.

Table 2.1. Soil characteristics of fields in Richville, MI and East Lansing, MI in 2010 and 2011 determined from soil samples taken prior to preplant N fertilization and planting.

	Richville		East Lansing	
	2010	2011	2010	2011
pH	7.3	7.8	6.1	6.8
Organic matter (%)	3.0	2.6	3.4	3.2
Total nitrogen (g kg^{-1})	1.4	1.3	1.7	1.1
Nitrate-nitrogen (mg kg^{-1})	20.2	2.4	13.5	4.7
Ammonium-nitrogen (mg kg^{-1})	4.4	1.6	6.3	1.5

Table 2.2. The number of days after planting and the stage of sugarbeet growth at the different weed removal timings at Richville, MI and East Lansing, MI in 2010 and 2011.

Weed removal	Richville		East Lansing	
timing [a]	2010	2011	2010	2011
	d after planting		d after planting	
< 2 cm weeds	36 (2 leaf)[b]	16 (cotyledon)	16 (2 leaf)	26 (2 leaf)
8 cm weeds	46 (4 leaf)	29 (4 leaf)	21 (4 leaf)	34 (4-7 leaf)
15 cm weeds	54 (6 leaf)	34 (6 leaf)	27 (8 leaf)	40 (7-8 leaf)
30 cm weeds	62 (8-12 leaf)	47 (8-9 leaf)	33 (9-10 leaf)	48 (8-9 leaf)

[a] Weeds were removed by applications of glyphosate at 0.84 kg ae ha^{-1} + ammonium sulfate at 2% v/v.

[b] Sugarbeet growth stage is shown in parenthesis.

Table 2.3. Average weed densities at each of the weed removal timings at Richville, MI and East Lansing, MI in 2010 and 2011.

Weed removal timing[a]	Richville 2010	Richville 2011	East Lansing 2010	East Lansing 2011
	weeds m^{-2}		weeds m^{-2}	
8 cm weeds	55 ± 6[b]	19 ± 2	30 ± 3	206 ± 14
15 cm weeds	46 ± 3	23 ± 2	51 ± 3	174 ± 12
30 cm weeds	44 ± 3	14 ± 2	91 ± 5	190 ± 12

[a] Weeds were removed by applications of glyphosate at 0.84 kg ae ha^{-1} + ammonium sulfate at 2% v/v.

[b] Standard error of the means.

Table 2.4. Monthly and 30-yr average precipitation for Richville, MI and East Lansing, MI in 2010 and 2011.[a]

Month	Richville 2010	Richville 2011[c]	Richville 30-yr ave.	East Lansing 2010[c]	East Lansing 2011[c]	East Lansing 30-yr ave.
	cm					
March	(0)[b]	-	5.0	-	-	4.3
April	5.5	-	8.0	-	-	7.3
May	8.5	(9.8)	7.6	(3.1)	(14.6)	8.4
June	6.9	3.8	9.3	10.6	4.0	8.4
July	2.3	3.4	7.6	6.4	13.0	8.2
August	3.2	7.6	8.0	3.4	7.8	8.3
September	(6.7)	(1.6)	10.8	9.2	6.7	9.2
October	-	-	7.8	(0.8)	7.5	6.9
November	-	-	7.4	-	(0)	6.9
Total	33.1	26.2	71.4	33.5	53.6	67.9

[a] Precipitation data was collected from Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/).

[b] Numbers in parenthesis indicate monthly precipitation data that occurred during the experiment.

[c] There was 10-, 17-, and 8.9-cm of precipitation between N application and planting at Richville 2011, East Lansing 2010, and East Lansing 2011, respectively.

Table 2.5. Effect of N rate and weed removal timing on weed biomass and weed N concentration for the combined Richville and East Lansing 2010 and 2011 experiments.[a]

N rate[b]	Weed biomass			N concentration in weeds[d]
	8 cm weeds[c]	15 cm weeds	30 cm weeds	
	$g\ m^{-2}$			% N
0 $kg\ ha^{-1}$	8.5 e	27.7 d	82.1 b	3.1 b
67 $kg\ ha^{-1}$	12.1 e	41.6 c	115.3 a	3.4 a
100 $kg\ ha^{-1}$	12.5 e	36.8 cd	124.7 a	3.5 a
134 $kg\ ha^{-1}$	12.8 e	36.5 cd	118.7 a	3.6 a
67:67 $kg\ ha^{-1}$ [e]	10.5 e	41.3 c	138.7 a	3.5 a

[a] Means followed by the same letter within a section are not statistically different at the $p \leq 0.05$ level of significance according to Fisher's Protected LSD.

[b] Nitrogen was applied as preplant urea, with the exception of the 67:67 $kg\ ha^{-1}$ split treatment where the second application was applied at the 4- to 6-leaf sugarbeet stage.

[c] Weeds were removed by applications of glyphosate at 0.84 kg ae ha^{-1} + ammonium sulfate at 2% v/v.

[d] Main effect of N concentration since there was not a significant interaction with weed removal timing.

[e] Weeds removed at 8- and 15-cm weeds had not yet received the second application of N.

Table 2.6. Main effect of weed removal timing on weed and sugarbeet N concentration for Richville and East Lansing in 2010 and 2011. Data is combined over N rates.[a]

Weed removal timing[b]	Weed N concentration			Sugarbeet N concentration		
	Richville[c]	East Lansing		Richville		East Lansing
	2010 & 2011	2010	2011	2010	2011	2010 & 2011
	% N	% N		% N		% N
8 cm weeds	3.5 b	3.7 ab	3.6 a	3.7 b	3.4 a	3.8 a
15 cm weeds	4.1 a	3.8 a	3.2 b	4.0 a	3.2 a	3.8 a
30 cm weeds	2.8 c	3.4 b	2.4 c	3.3 c	3.4 a	2.9 b

[a] Means followed by the same letter within a column are not statistically different at the $p \leq 0.05$ level of significance according to Fisher's Protected LSD.

[b] Weeds were removed by applications of glyphosate at 0.84 kg ae ha^{-1} + ammonium sulfate at 2% v/v.

[c] Data was combined over years when there was not a significant treatment by year interaction.

Table 2.7. Effect of N rate and weed removal timing on sugarbeet biomass and sugarbeet N concentration for the combined Richville and East Lansing 2010 and 2011 experiments.[a]

N rate[b]	Sugarbeet biomass			N concentration in sugarbeet[d]
	8 cm weeds[c]	15 cm weeds	30 cm weeds	
	——— $g\ m^{-2}$ ———			——— % N ———
0 $kg\ ha^{-1}$	1.5 f	6.4 de	26.4 c	3.3 b
67 $kg\ ha^{-1}$	2.1 ef	9.7 d	38.6 ab	3.5 a
100 $kg\ ha^{-1}$	2.3 ef	9.9 d	40.5 a	3.5 a
134 $kg\ ha^{-1}$	1.9 ef	10.0 d	41.5 a	3.6 a
67:67 $kg\ ha^{-1}$ [e]	2.1 ef	9.5 d	34.3 b	3.6 a

[a] Means followed by the same letter within a section are not statistically different at the $p \leq 0.05$ level of significance according to Fisher's Protected LSD.

[b] Nitrogen was applied as preplant urea, with the exception of the 67:67 $kg\ ha^{-1}$ split treatment where the second application was applied at the 4- to 6-leaf sugarbeet stage.

[c] Weeds were removed by applications of glyphosate at 0.84 kg ae ha^{-1} + ammonium sulfate at 2% v/v.

[d] Main effect of N concentration since there was not a significant interaction with weed removal timing.

[e] Weeds removed at 8- and 15-cm weeds had not yet received the second application of N.

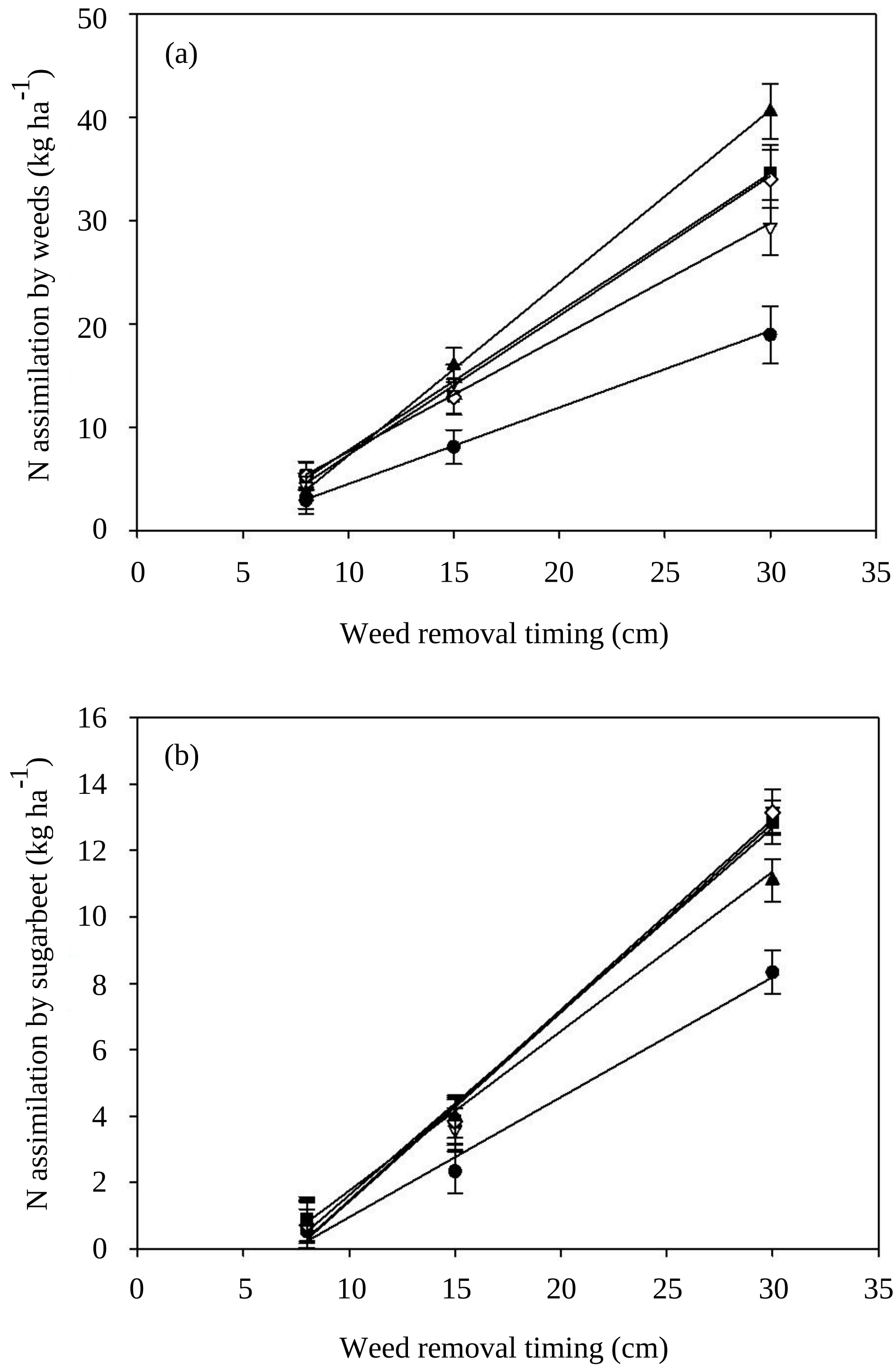

Figure 2.1. Nitrogen assimilation by (a) weeds and (b) glyphosate-resistant sugarbeet at 0 (●), 67 (▽), 100 (■), 134 (◊) and 67:67 kg ha^{-1} (▲) for the combined data in Richville, MI and East Lansing, MI in 2010 and 2011. Data are presented as the interaction between nitrogen rate and weed height at time of removal. Vertical bars for each point represent standard error of the mean. Equations for the data are presented in Table 2.8.

Table 2.8. Linear regression equation, P-value, and R^2 value for the N assimilation by weeds and sugarbeet for the combined data in Richville, MI and East Lansing, MI in 2010 and 2011 displayed in Figure 2.1.

	N Rate	Linear Regression equation	p-value	R^2 value
	kg ha^{-1}			
N assimilation by weeds	0	y = 0.742x – 2.93	< 0.0001	0.32
	67	y = 1.107x – 3.45	< 0.0001	0.43
	134	y = 1.351x – 6.23	< 0.0001	0.50
	100	y = 1.343x – 5.68	< 0.0001	0.46
	67:67	y = 1.674x – 9. 48	< 0.0001	0.52
N assimilation by sugarbeet	0	y = 0.362x – 2.67	< 0.0001	0.60
	67	y = 0.574x – 4.28	< 0.0001	0.62
	134	y = 0.553x – 3.91	< 0.0001	0.61
	100	y = 0.570x – 4.28	< 0.0001	0.56
	67:67	y = 0.481x – 3.05	< 0.0001	0.57

Table 2.9. Main effects of nitrogen and weed removal timing on the N sufficiency index of sugarbeet in late-June, mid-July, and mid-August in Richville, MI and East Lansing, MI in 2010 and 2011.[a]

	Late-June[b]	Mid-July			Mid-August
	Richville & East Lansing	Richville		East Lansing	Richville & East Lansing
Nitrogen rate[c]	2010 & 2011	2010	2011	2010 & 2011	2010 & 2011
	N sufficiency index[e]				
0 kg ha^{-1}	90 d	93 b	82 d	97 a	98 a
67 kg ha^{-1}	95 c	100 a	89 c	99 a	100 a
100 kg ha^{-1}	97 ab	100 a	92 bc	100 a	98 a
134 kg ha^{-1}	99 a	100 a	98 a	100 a	100 a
67:67 kg ha^{-1}	96 bc	100 a	93 a	100 a	100 a
Weed removal timing[d]		2010 & 2011		2010 & 2011	
< 2 cm weeds	97 a	96 a		100 a	99 a
8 cm weeds	96 a	96 a		99 a	100 a
15 cm weeds	95 ab	95 ab		99 a	100 a
30 cm weeds	93 b	93 b		100 a	98 a

[a] Means followed by the same letter within a column are not statistically different at the $p \leq 0.05$ significance level according to Fisher's Protected LSD.

[b] Sugarbeet had approximately 8-true leaves in late-June, 16-true leaves in mid-July, and 26-true leaves in mid-August.

[c] Nitrogen was applied as preplant urea, with the exception of the 67:67 kg ha^{-1} split treatment where the second application was applied at the 4- to 6-leaf sugarbeet stage.

[d] Weeds were removed by applications of glyphosate at 0.84 kg ae ha^{-1} + ammonium sulfate at 2% v/v.

[e] N sufficiency index was calculated based on the SPAD chlorophyll meter readings from the < 2 cm weed removal timing with 134 kg ha^{-1} of N.

Table 2.10. Main effects of nitrogen and weed removal timing on canopy closure of sugarbeet in early- and late-July in Richville and East Lansing in 2010 and 2011.[a]

	Early July			Late July		
	Richville		East Lansing	Richville		East Lansing
Nitrogen rate[b]	2010	2011	2010 & 2011	2010	2011	2010 & 2011
	canopy closure (%)					
0 kg ha^{-1}	57 a	42 c	47 a	56 a	50 b	62 b
67 kg ha^{-1}	65 a	59 b	47 a	59 a	68 a	62 b
100 kg ha^{-1}	64 a	63 ab	46 a	58 a	73 a	70 a
134 kg ha^{-1}	67 a	65 ab	50 a	61 a	76 a	70 a
67:67 kg ha^{-1}	67 a	68 a	48 a	65 a	73 a	66 ab

Weed removal timing[c]	Richville 2010 & 2011	East Lansing 2010	East Lansing 2011	Richville & East Lansing 2010 & 2011
< 2 cm weeds	65 a	64 a	42 a	69 a
8 cm weeds	62 a	64 a	32 b	65 b
15 cm weeds	63 a	65 a	26 c	64 b
30 cm weeds	57 b	63 a	25 c	62 b

[a] Means followed by the same letter within a column are not statistically different at the $p \leq 0.05$ significance level according to Fisher's Protected LSD.

[b] Nitrogen was applied as preplant urea, with the exception of the 67:67 kg ha^{-1} split treatment where the second application was applied at the 4- to 6-leaf sugarbeet stage.

[c] Weeds were removed by applications of glyphosate at 0.84 kg ae ha^{-1} + ammonium sulfate at 2% v/v.

Table 2.11. Main effects of nitrogen and weed removal timing on and N assimilation of sugarbeet prior to harvest. Data are presented as the combined Richville and East Lansing locations in 2010 and 2011. [a]

Nitrogen rate[b]	Sugarbeet N assimilation
	—— $kg\ ha^{-1}$ ——
0 $kg\ ha^{-1}$	185 b
67 $kg\ ha^{-1}$	199 b
100 $kg\ ha^{-1}$	239 a
134 $kg\ ha^{-1}$	242 a
67:67 $kg\ ha^{-1}$	244 a
Weed removal timing[c]	
< 2 cm weeds	235 ab
8 cm weeds	238 a
15 cm weeds	211 bc
30 cm weeds	205 c

[a] Means followed by the same letter within a column are not statistically different at the $p \leq 0.05$ significance level according to Fisher's Protected LSD.

[b] Nitrogen was applied as preplant urea, with the exception of the 67:67 $kg\ ha^{-1}$ split treatment where the second application was applied at the 4-to 6-leaf sugarbeet stage.

[c] Weeds were removed by applications of glyphosate at 0.84 kg ae ha^{-1} + ammonium sulfate at 2% v/v.

Table 2.12. Main effects of nitrogen and weed removal timing on sugarbeet root yield, recoverable white sucrose per Mg of root (RWSMg), and recoverable white sucrose per ha (RWSH) in Richville, MI and East Lansing, MI in 2010 and 2011.[a]

	Yield			RWSMg			RWSH		
	Richville		East Lansing	Richville		East Lansing	Richville		East Lansing
Nitrogen rate[b]	2010	2011	2010 & 2011	2010	2011	2010 & 2011	2010	2011	2010 & 2011
	— $Mg\ ha^{-1}$ —		— $Mg\ ha^{-1}$ —	— $kg\ Mg^{-1}$ —		— $kg\ Mg^{-1}$ —	— $kg\ ha^{-1}$ —		— $kg\ ha^{-1}$ —
0 $kg\ ha^{-1}$	49.7 c	31.6 c	30.1 b	132 a	139 a	135 a	6548 b	4408 c	4031 a
67 $kg\ ha^{-1}$	57.0 ab	41.0 b	32.2 ab	130 a	142 a	132 ab	7404 a	5817 b	4248 a
100 $kg\ ha^{-1}$	55.4 b	45.3 a	32.7 a	128 a	141 a	129 b	7072 ab	6413 a	4216 a
134 $kg\ ha^{-1}$	59.5 a	47.9 a	32.9 a	125 a	141 a	125 c	7412 a	6744 a	4115 a
67:67 $kg\ ha^{-1}$	60.3 a	48.9 a	33.1 a	125 a	138 a	124 c	7536 a	6729 a	4133 a

Weed removal timing[c]	Yield Richville 2010	Yield Richville 2011	Yield East Lansing 2010 & 2011	RWSMg Richville 2010	RWSMg Richville 2011	RWSMg East Lansing 2010	RWSMg East Lansing 2011	RWSH Richville 2010	RWSH Richville 2011	RWSH East Lansing 2010 & 2011
< 2 cm weeds	64.3 a	41.8 a	34.3 a	128 a	139 a	128 a	132 a	8244 a	5807 a	4447 a
8 cm weeds	55.3 b	42.3 a	31.5 b	126 a	142 a	129 a	132 a	6963 b	6013 a	4079 b
15 cm weeds	55.2 b	45.7 a	31.6 b	127 a	141 a	127 a	130 a	6986 b	6438 a	4050 b
30 cm weeds	50.7 c	41.9 a	31.4 b	130 a	139 a	130 a	125 a	6584 b	5831 a	3400 b

[a] Means followed by the same letter within a column are not statistically different at the $p \leq 0.05$ significance level according to Fisher's Protected LSD.

[b] Nitrogen was applied as preplant urea, with the exception of the 67:67 $kg\ ha^{-1}$ split treatment where the second application was applied at the 4-to 6-leaf sugarbeet stage.

[c] Weeds were removed by applications of glyphosate at 0.84 $kg\ ae\ ha^{-1}$ + ammonium sulfate at 2% v/v.

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CHAPTER 3

SUGARBEET IS MORE COMPETITIVE FOR NITROGEN THAN COMMON LAMBSQUARTERS AND POWELL AMARANTH

Abstract

Nitrogen (N) is an important nutrient to maximize sugarbeet yield, yet improper N application timings or rates may negatively impact sugarbeet grown in competition with weeds. A greenhouse study was conducted to examine the effect of three N rates on the competitive ability of common lambsquarters and Powell amaranth grown with glyphosate-resistant sugarbeet. Sugarbeet and each weed species were grown in replacement series design with eight plants pot^{-1} at proportions of 100:0 (sugarbeet:weed), 75:25, 50:50, 25:75 and 0:100 at three N application rates at 0, 67, and 134 kg ha^{-1}. All plants responded positively to N applications. Sugarbeet and Powell amaranth had incrementally higher N concentrations as N rate increased, while common lambsquarters had N concentrations that were not significantly different at 67 and 134 kg ha^{-1} rates. Sugarbeet had greater N concentrations than common lambsquarters at all N rates and higher N concentrations than Powell amaranth at 67 and 134 kg ha^{-1}. Overall, sugarbeet was more competitive than either weed species, especially at the 134 kg N ha^{-1} rate. Sugarbeet had higher relative N assimilations and greater relative yields at all N rates than both weed species. Although sugarbeet was more competitive than common lambsquarters and Powell amaranth, uncontrolled weeds reduce available water, light and nutrients to sugarbeet, and weed-crop interactions in field settings may produce different results than controlled environments. Therefore, timely weed control strategies and appropriate N management should

be implemented to avoid negative effects of competition with common lambsquarters and Powell amaranth on sugarbeet growth and production.

Nomenclature: Common lambsquarters, *Chenopodium album* L. CHEAL; Powell amaranth, *Amaranthus powellii* S. Watson AMAPO; sugarbeet, *Beta vulgaris* L. 'Crystal 827RR'

Key words: Glyphosate-resistant sugarbeet; common lambsquarters; Powell amaranth; weed density; nitrogen rate; nitrogen assimilation; competition

Introduction

Effective and appropriately timed weed control strategies are critical in cropping systems because weeds compete with crops for water, nutrients, and light (Schweizer and May 1993). Nitrogen is an important nutrient applied to crops to obtain high yields, and it is the most significant nutrient for sugarbeet (Draycott 1993). Sugarbeet rapidly assimilates N at the 4- to 5-leaf stage (Scott and Jaggard 1993). An adequate quantity of N is required early in the growing season to promote canopy development, and N should be available throughout the growing season to maintain the canopy and assist with root growth (Draycott 1993; Armstrong et al. 1986).

Weeds are capable of assimilating large amounts of N, especially at high N rates. Both common lambsquarters (*Chenopodium album* L.) and redroot pigweed (*Amaranthus retroflexus* L.) are highly responsive to N (Blackshaw et al. 2003; Bast 2012). At 120 mg N kg^{-1} soil, a total of 17 out of 23 weed species assimilated levels of N that were similar to the levels of N assimilated by common lambsquarters and redroot pigweed. At 240 mg N kg^{-1} soil however, only three weed species, hairy nightshade (*Solanum sarrachoides* Sendtner), redstem filaree (*Erodium cicutarium* (L.) L'Her. ex Air) and round-leaved mallow (*Malva pusilla* Sm.), assimilated levels of N that were similar to the levels of N assimilated by common lambsquarters and redroot pigweed, indicating that common lambsquarters and redroot pigweed were two of the most responsive weed species to increasing levels of N in the study (Blackshaw et al. 2003).

How weeds respond to increasing N rates is dependent on the particular species and the time of the N application. Redroot pigweed shoot N concentration increased 31 and 107% as N rate increased from 60 to 120 and 60 to 240 mg kg^{-1} soil, respectively, when grown in competition with spring wheat (*Triticum aestivum* L. 'AC Barrie') (Blackshaw and Brandt 2008). However, shoot N concentrations for Persian darnel (*Lolium persicum* Boiss. & Hohen. ex Boiss), a weed species that is characterized as having a low response to N, only increased 23 and 51% for the same increases in N rates. Other studies have shown that common lambsquarters was more competitive with sugarbeet when 120 kg N ha^{-1} was applied at the 4- to 6-leaf sugarbeet stage, compared with later N applications at the 10- to 12-leaf sugarbeet stage (Paolini et al. 1999).

The commercialization of glyphosate-resistant sugarbeet has changed how growers approach weed management strategies in sugarbeet. The use of glyphosate for weed control provides growers with greater flexibility in application timings than traditional methods for controlling weeds in conventional sugarbeet (Kniss et al. 2004). Since glyphosate can control larger weeds many growers are delaying their initial herbicide application, which allows weeds to compete with crops for valuable resources, such as N. Understanding how weeds compete with sugarbeet for N will help provide growers information that would be useful in devising appropriate weed and fertilizer management strategies. Experiments examining the effects of N rate on common lambsquarters and Powell amaranth in competition with sugarbeet would be beneficial because those weed species are most problematic in Michigan sugarbeet fields (G.

Clark, Agronomist, Michigan Sugar Company, Bay City, MI, personal communication). Therefore, the objective of this research was to determine the effect of N application rate on the competitive ability of two problematic weeds, common lambsquarters and Powell amaranth, grown alone and with sugarbeet.

Materials and Methods

A greenhouse study was conducted in 2011 at Michigan State University in East Lansing, Michigan. The experiment was arranged in a randomized complete block design, replicated five times and repeated in time. Treatments included glyphosate-resistant sugarbeet competing with either common lambsquarters or Powell amaranth at three N fertilizer rates. Sugarbeet and each weed species were grown in a replacement series design at proportions of 100:0, 75:25, 50:50, 25:75 and 0:100 with a total of 8 plants pot^{-1}. Nitrogen fertilizer rates were 0, 67, and 134 kg N ha^{-1}.

Glyphosate-resistant sugarbeet 'Crystal RR 827' (ACH Seeds Inc., PMB 305, 574 Prairie Center Drive #135, Eden Prairie, MN 55344), common lambsquarters, and Powell amaranth seeds were initially planted into flats (Landmark Plastic, 1331 Kelly Avenue Akron, OH 44306) filled with potting media (Michigan Grower Products, Inc., 251 McCollum, Galesburg, MI 49053) and placed in the greenhouse. Greenhouse temperatures were maintained at 25 ± 5°C with a 16-h photoperiod of natural sunlight and supplemental lighting was provided at 1,000 µmol/m^2/s photosynthetic photon flux. Individual 3.1 L pots (ITML Horticultural Products, 75 Plant Farm Blvd., Brantford, Ontario, N3T 5M8) were filled with 2.6 kg of steam sterilized Spinks loamy sand soil (sandy, mixed, mesic Lamellic Hapludalfs) with a pH of 6.7, 2.2%

organic matter, and total N content of 0.5 g kg^{-1} for the first experiment, and a pH 7.3, 2.1% organic matter, and total N content of 0.6 g kg^{-1} for the second experiment. Each pot was watered to bring the soil water content to field capacity. Aqueous solutions of urea (46-0-0) containing the desired amount of N were uniformly pipetted onto the soil surface and incorporated with approximately 150 mL of water. When sugarbeet, common lambsquarters and Powell amaranth were at the cotyledon stage (approximately 10 d after planting and 2 to 3 d after N application) they were transplanted into the N amended soils. Eight plants pot^{-1} at the 100:0, 75:25, 50:50, 25:75, and 0:100 (sugarbeet:weeds) proportions were transplanted at an equidistant spacing around the pots' circumference, keeping spacing between species equal. Pots were watered daily using individual sub-irrigation units and rotated once a week to avoid possible differences in lighting and temperature among the treatments.

Glyphosate-resistant sugarbeet, common lambsquarters, and Powell amaranth plants were harvested 28 d after transplanting when sugarbeet were at the 6-8 leaf stage. Rapid N assimilation by sugarbeet occurs at the 4- to 5-leaf growth stage (Scott and Jaggard 1993). Common lambsquarters were 8-10 cm tall and exhibited 20-30 leaves, while Powell amaranth was 10-12 cm tall and exhibited10-20 leaves. Excess soil was removed from the roots with water, and roots were blotted dry with paper towel. The entire plant (shoots and roots) was oven-dried at 66°C for 7 d and weighed. Plant samples were ground to pass through a sieve $\leq$ 0.5. Total N content was determined by the micro-Kjeldahl digestion method (Bremner 1996; Jung et al. 2003) and colorimetric analysis through a Lachat rapid flow injector autoanalyzer (Lachat Instruments, Milwaukee, WI). Nitrogen assimilation by each species was calculated by multiplying the plant biomass by nitrogen concentration.

Data were analyzed using the PROC MIXED procedure in SAS 9.3 (SAS® 9.3 Software, SAS Institute Inc, 100 SAS Campus Drive, Cary, NC 27513). Normality of the residuals was evaluated by examination of normal probability and stem-and-leaf plots. Homogeneity of the variances was evaluated by the Levene's test ($p \leq 0.05$) and they were grouped to improve the model. An analysis of variance was performed and treatment means were compared using Fisher's Protected LSD at the $p \leq 0.05$ significance level. Data were combined over trials because there was not a significant treatment by trial interaction, and the data was presented for main effects when interactions were not significant. Nitrogen assimilation and biomass data of sugarbeet, common lambsquarters and Powell amaranth were converted to relative N assimilation (RN) or relative yield (RY) to produce replacement-series diagrams (Blackshaw and Brandt 2008; Cousens 1991; Weigelt and Jolliffe 2003) to determine the competitiveness of each species in mixture compared with a monoculture at each of the three N fertilizer rates using equation 1.

RN or RY = N assimilation or yield in mixture/N assimilation or yield in monoculture [1]

Similar to Blackshaw and Brandt (2008), weed aggressivity values (AI) were calculated as an additional measurement of species competitiveness when grown in a mixture. Weed AI values that are above zero indicated that the weed is more competitive than sugarbeet and weed AI values that are less than zero indicated that the weed is less competitive than sugarbeet. Weed AI values are calculated for nitrogen assimilation and biomass accumulation for each species using equation 2.

$$AI = (\text{Yield of weed species in mixture/Yield of weed species in monoculture}) - (\text{Yield of sugarbeet in mixture/Yield of weed species in mixture}) \quad [2]$$

Results and Discussion

Relative Nitrogen Assimilation. Sugarbeet was more competitive than common lambsquarters in terms of relative N assimilation at all N rates (Figure 3.1). As N rate increased it appeared that sugarbeet was more competitive, especially at the 134 kg N ha^{-1} rate. However, there was no difference in the magnitude of sugarbeet competitiveness with increasing N rates, since the common lambsquarters aggressivity index (AI) values were not significant (Table 3.1).

Sugarbeet N assimilation was superior to Powell amaranth when grown in mixture (Figure 3.1). As N rate increased sugarbeet became more competitive with Powell amaranth. Powell amaranth AI values were -0.30, -1.12, and -2.32 at 0, 67 and 134 kg N ha^{-1}, respectively. The values indicate sugarbeet was able to progressively capture more soil N than Powell amaranth as N fertilizer rate increased.

Common lambsquarters and redroot pigweed have been reported to be luxury consumers of N at high N rates (Blackshaw et al. 2003). It is expected that Powell amaranth would also be responsive to N at higher N rates since it is in the same family as redroot pigweed (*Amaranthus spp.*). Blackshaw and Brandt (2008) determined that N assimilation of redroot pigweed increased as N rate increased from 60 to 240 mg N kg^{-1}soil when grown in competition with spring wheat. At 60 and 120 mg N kg^{-1}soil, N assimilation by redroot pigweed was inferior to spring wheat, but at the 240 mg N kg^{-1}rate, N assimilation by redroot pigweed was slightly superior, indicating the weed was highly responsive to the increasing N rates.

Unlike Blackshaw and Brandt's (2008) results, the relative N assimilation by sugarbeet was always superior to both common lambsquarters and Powell amaranth, and sugarbeet became more competitive than both weed species as N rate increased. Sugarbeet may have had higher relative N assimilation than both weed species because sugarbeet rapidly assimilates N at the 4- to 5-leaf stage for canopy development (Scott and Jaggard 1993; Draycott 1993). In this study, individual sugarbeet plants assimilated greater quantities of N than both weed species (data not shown), which most likely contributed to higher relative N assimilations by sugarbeet. Nitrogen assimilation by sugarbeet may have also been greater than both weed species due to the placement and proportion of weed species in the pots. In field settings, broadcast applications of N often favor weed N assimilation and growth because the N is not placed close to the sugarbeet plant (Blackshaw et al. 2002; Stevens et al. 2007), and the density of weeds can be much higher than crop density (Bast 2012). In our study however, N availability for sugarbeet and weeds was similar because the plants were transplanted at an equidistant spacing between species and the proportion of weeds to sugarbeet was very low.

Nitrogen concentrations of sugarbeet were higher than common lambsquarters at all N rates and greater than Powell amaranth at 67 and 134 kg N ha^{-1} (Table 3.2). Increases in the magnitude of N concentrations in both weed species were noted when N rate was increased from 0 to 67 kg N ha^{-1} and only for Powell amaranth when N rate increased from 67 to 134 kg N ha^{-1}. Blackshaw and Brandt (2008) noted that redroot pigweed shoot N concentration increased 107% when N rate was increased from 60 to 240 mg N kg^{-1} soil, while spring wheat shoot N concentration only increased 71%. In our study, the percentage increase of N concentration from 0 to 134 kg N ha^{-1} in sugarbeet was greater than common lambsquarters and Powell amaranth.

Sugarbeet and common lambsquarters N concentrations increased 27 and 21%, respectively, while sugarbeet and Powell amaranth N concentrations increased 50 and 36%, respectively.

Relative Yield. The relative yield of sugarbeet, common lambsquarters and Powell amaranth biomass was similar to relative N assimilation for each of these species (Figure 3.2). Sugarbeet was more competitive than common lambsquarters in terms of relative yield, and there were no differences in competition across all N rates. Common lambsquarters AI values indicate N rate did not influence the competitive ability of the weed (Table 3.3).

The relative yield of sugarbeet was superior to Powell amaranth at all N rates, with the competitive ability of sugarbeet increasing with increasing N rates. Powell amaranth AI values were -0.41, -0.96, and -2.21 at 0, 67 and 134 kg N ha^{-1}, respectively. These values indicate that Powell amaranth was less competitive with sugarbeet at higher N rates, which is reflected in Figure 3.2. Many Powell amaranth plants showed injury symptoms to N at the 134 kg N ha^{-1} rate, which may have impacted Powell amaranth's competitive ability with sugarbeet (personal observation). Injured plants were stunted and had slower rates of growth than healthy plants for a period of time after transplanting. Neither sugarbeet nor common lambsquarters were negatively affected by the highest N application rate.

Common lambsquarters and Powell amaranth were inferior to sugarbeet at all N rates and had lower relative yields; however, Powell amaranth may be able to compete with sugarbeet more successfully than common lambsquarters. At 0 and 134 kg N ha^{-1}, the relative yield of Powell amaranth was higher than expected at the 75:25 proportion, while common lambsquarters relative yield was lower than expected at all N rates and proportions. Powell amaranth may have been more competitive than common lambsquarters in terms of relative yield due to its morphology and greater above-ground competition. Powell amaranth was slightly taller than common lambsquarters and its leaf area was greater (data not shown). Tall weeds shade the sugarbeet canopy, reducing light interception and sugarbeet biomass production (Dawson 1965; Weatherspoon and Schweizer 1969; Scott and Jaggard 1993). Bast (2012) noted that redroot pigweed shoot biomass was greater than common lambsquarters 3 weeks after emergence at 0 and 67 kg N ha^{-1}, suggesting that redroot pigweed exhibits more above-ground competition than common lambsquarters early in the growing season. It is expected that Powell amaranth would also exhibit greater shoot biomass than common lambsquarters early in the growing since it is in the same family as redroot pigweed.

Study Implications. The results of study indicate that sugarbeet had increased competitive ability as N rate increased, and the crop was superior to common lambsquarters and Powell amaranth in terms of relative N assimilation and yield. Sugarbeet relative N assimilation may have been greater than both weed species due to its rapid assimilation of N at the 4- to 5-leaf stage for canopy development (Scott and Jaggard 1993; Draycott 1993). Powell amaranth competed with sugarbeet more effectively than common lambsquarters in terms of relative yield, possibly because its tall height and large leaf area shaded the sugarbeet canopy, reducing light interception and sugarbeet biomass production and due to greater above-ground competition than common lambsquarters (Dawson 1965; Weatherspoon and Schweizer 1969; Scott and Jaggard 1993; Bast 2012).

Although sugarbeet was more competitive than common lambsquarters and Powell amaranth, uncontrolled weeds reduce available water, light and nutrients to sugarbeet. Weed-crop interactions in field settings may produce different results than controlled environments. Weed density in fields can be very high, causing weeds to assimilate high quantities of N (Bast 2012). Therefore, timely weed control strategies and appropriate N management should be implemented to avoid negative effects of competition with common lambsquarters and Powell amaranth on sugarbeet growth and production.

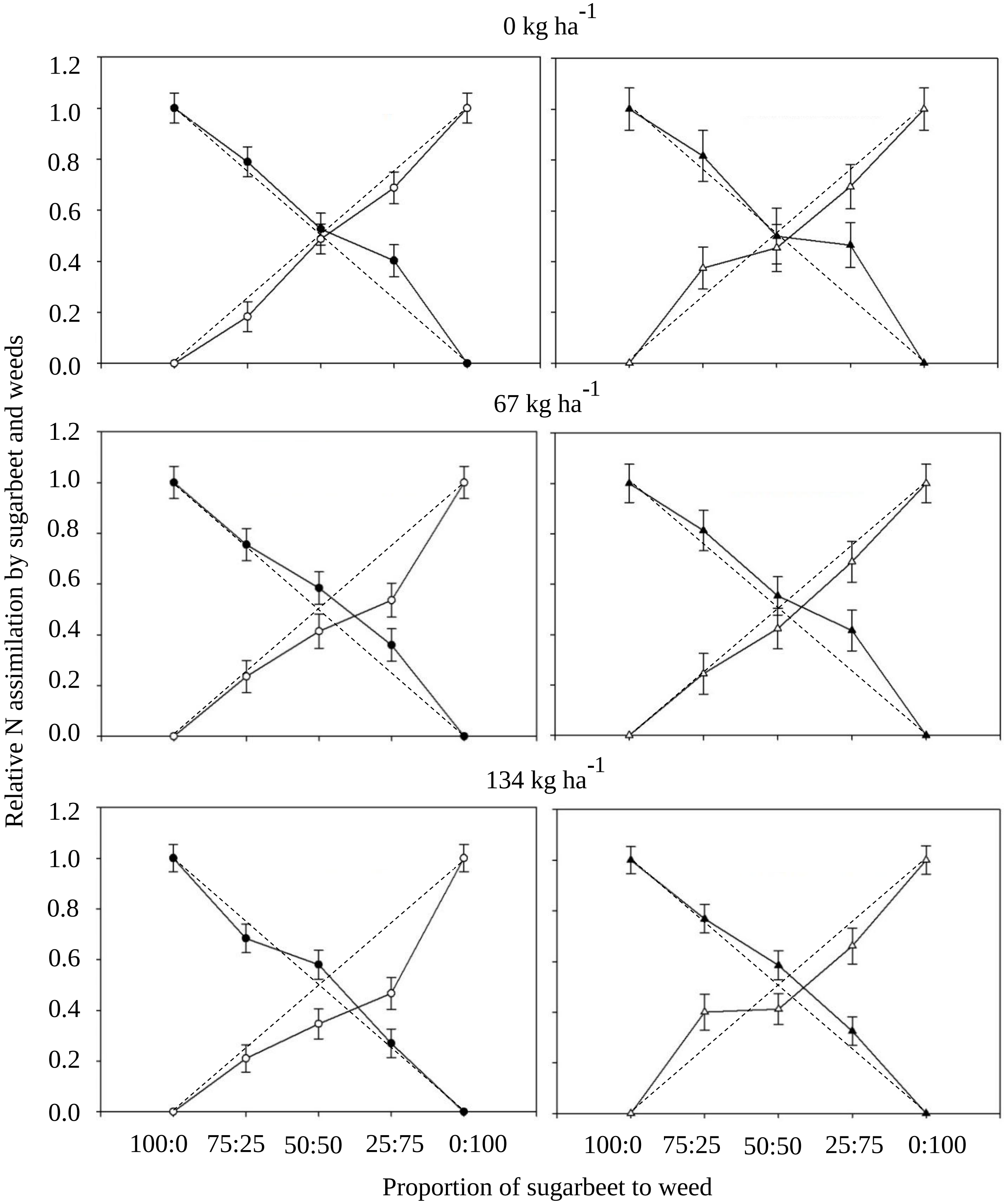

Figure 3.1. Relative nitrogen assimilation by sugarbeet (●) competing with common lambsquarters (○) and sugarbeet (▲) competing with Powell amaranth (△) at 0, 67, and 134 kg N ha^{-1}. Vertical bars represent standard error of the mean.

Table 3.1. Effect of nitrogen rate on common lambsquarters and Powell amaranth aggressivity index (AI) values[a] determined from sugarbeet and weed relative N assimilation data.[b]

Nitrogen Rate	Common lambsquarters	Powell amaranth
0 kg ha^{-1}	- 0.74 a	- 0.30 a
67 kg ha^{-1}	- 0.92 a	- 1.13 ab
134 kg ha^{-1}	- 1.74 a	- 2.32 b

[a] Weed AI values that are above zero indicated that the weed is more competitive than sugarbeet and weed AI values that are less than zero indicated that the weed is less competitive than sugarbeet.

[b] Means followed by the same letter within a column are not statistically different at the $p \leq 0.05$ level of significance according to Fisher's Protected LSD.

Table 3.2. Effect of nitrogen rate on nitrogen concentrations of sugarbeet, common lambsquarters, and Powell amaranth.[a]

Nitrogen rate	Sugarbeet	Common lambsquarters	Sugarbeet	Powell amaranth
	% N		% N	
0 kg ha^{-1}	1.5 d	1.4 e	1.4 cd	1.3 d
67 kg ha^{-1}	1.7 b	1.6 cd	1.7 b	1.5 c
134 kg ha^{-1}	1.9 a	1.7 bc	2.1 a	1.8 b

[a] Means followed by the same letter within a section are not statistically different at the $p \leq 0.05$ significance level according to Fisher's Protected LSD.

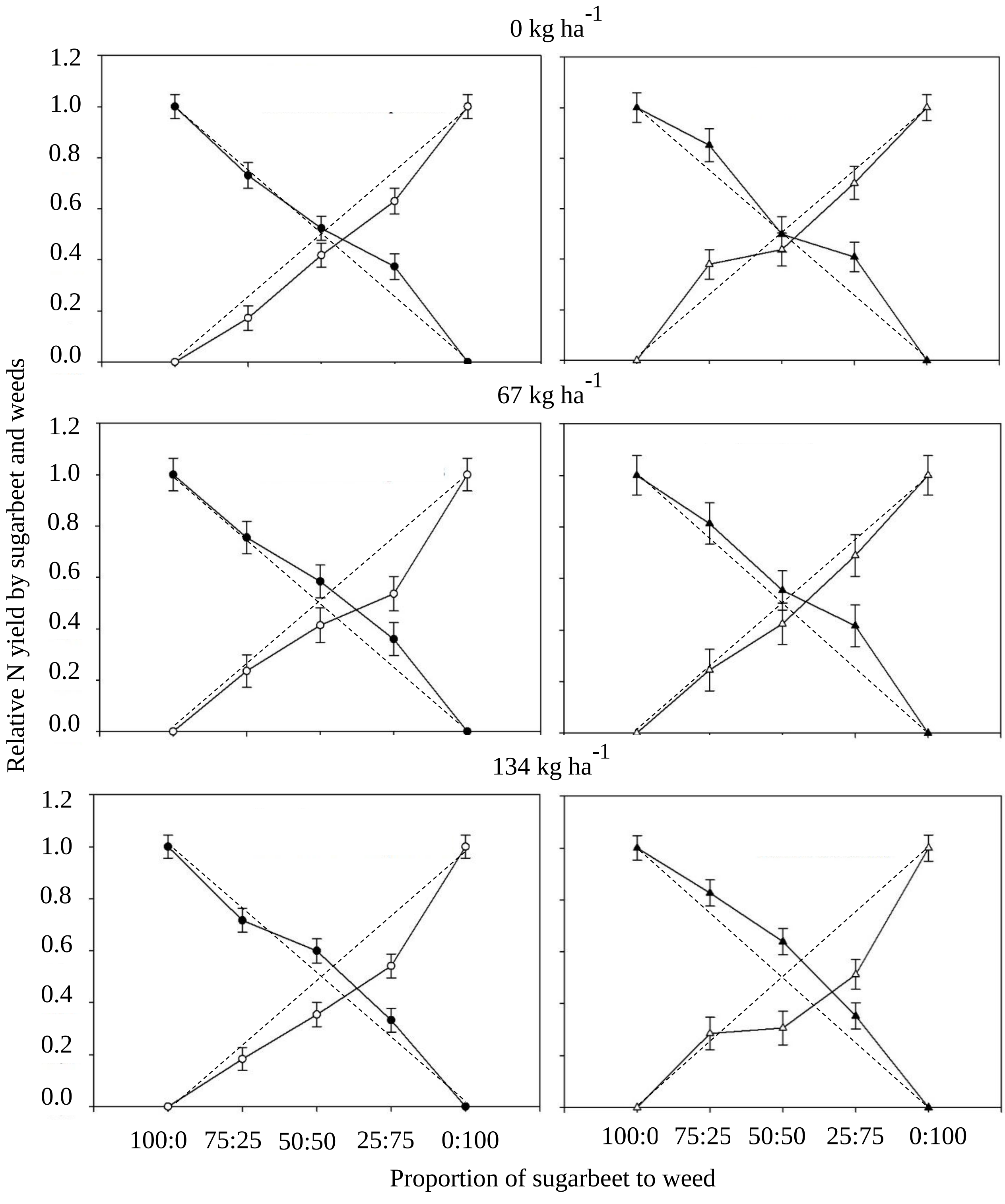

Figure 3.2. Relative yield of sugarbeet (●) competing with common lambsquarters (○) and sugarbeet (▲) competing with Powell amaranth (△) at 0, 67, and 134 kg N ha^{-1}. Vertical bars represent standard error of the mean.

Table 3.3. Effect of nitrogen rate on common lambsquarters and Powell amaranth aggressivity index (AI) values[a] determined from sugarbeet and weed relative yield data.[b]

Nitrogen Rate	Common lambsquarters	Powell amaranth
0 kg ha^{-1}	- 0.79 a	- 0.42 a
67 kg ha^{-1}	- 0.94 a	- 0.96 ab
134 kg ha^{-1}	- 1.21 a	- 2.21 b

[a] Weed AI values that are above zero indicated that the weed is more competitive than sugarbeet and weed AI values that are less than zero indicated that the weed is less competitive than sugarbeet.

[b] Means followed by the same letter within a column are not statistically different at the $p \leq 0.05$ level of significance according to Fisher's Protected LSD.

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APPENDICES

APPENDIX A

SOIL SAMPLES COLLECTED AFTER PREPLANT NITROGEN APPLICATION IN 2011

In 2011, soil cores (20-24 samples) were taken in plots that received no nitrogen (N) application (0 kg N ha^{-1}) and plots that received the highest N application rate (134 kg N ha^{-1}) to a depth of 20 cm. The samples were taken 6- to 7-wk after the preplant N application to determine if N was still available to sugarbeet after large accumulations of rainfall were recorded. There was 10- and 8.9-cm of precipitation between N application and planting at Richville in 2011 and East Lansing in 2011, respectively.

Soil samples were oven-dried at 38°C for 7 d and ground to pass through a 2 mm sieve. Ten grams of soil and 50 mL of 1 *M* KCl were added to 125 mL Erlenmeyer flasks. Nitrate-N (NO_3-N) and ammonium-N (NH_4-N) concentrations were determined by the ammonia-salicylate method and cadmium reduction method, respectively, with a Lachat rapid flow injector autoanalyzer following extraction with 1 *M* KCl (Mulvaney, R. L. 1996. Nitrogen-inorganic forms. p. 1123-1184 *in* D. L. Sparks, ed. Methods of Soil Analysis. Part 3, Chemical Methods. Book Series 5. Madison, WI: Soil Science Society of America). Data are presented as the main effects of N and weed removal timing on NO_3-N and NH_4-N concentrations at Richville and East Lansing in 2011.

Table A.1. Nitrate-nitrogen and ammonium-nitrogen concentrations in soil collected after nitrogen application and planting in Richville, MI and East Lansing, MI in 2011.

	Nitrate-Nitrogen		Ammonium-Nitrogen	
	Richville	East Lansing	Richville	East Lansing
	mg kg^{-1}			
0 kg ha^{-1}	2.5	6.1	1.4	1.9
134 kg ha^{-1}	17.7	16.0	1.6	1.9

APPENDIX B

SOIL SAMPLES COLLECTED AFTER SUGARBEET HARVEST

In 2010 and 2011, soil cores (6-10 samples) were taken in each plot after sugarbeet harvest to a depth of 20 cm to determine nitrate-nitrogen (NO_3-N) and ammonium-nitrogen (NH_4-N) concentrations. Samples were oven-dried at 38°C for 7 d and ground to pass through a 2 mm sieve. Ten grams of soil and 50 mL of 1 *M* KCl were added to 125 mL Erlenmeyer flasks. NO_3-N and NH_4-N concentrations were determined by the ammonia-salicylate method and cadmium reduction method, respectively, with a Lachat rapid flow injector autoanalyzer following extraction with 1 *M* KCl (Mulvaney, R. L. 1996. Nitrogen-inorganic forms. p. 1123-1184 *in* D. L. Sparks, ed. Methods of Soil Analysis. Part 3, Chemical Methods. Book Series 5. Madison, WI: Soil Science Society of America). Data for soil samples collected after sugarbeet harvest were combined over sites and/or years when there were no significant treatments by site or year interactions. Data are presented as the main effect of N and weed removal timing on NO_3-N and NH_4-N concentrations at Richville and East Lansing in 2010 and 2011.

Table B.1. Main effects of nitrogen and weed removal timing on nitrate-nitrogen and ammonium-nitrogen concentrations from soils collected from all plots in Richville, MI and East Lansing, MI after sugarbeet harvest [a].

	Nitrate-Nitrogen			Ammonium-Nitrogen		
	Richville & East Lansing			Richville		East Lansing
Nitrogen rate [b]	2010 & 2011			2010 & 2011		2010 & 2011
	mg kg^{-1}					
0 kg ha^{-1}	2.6 b			2.3 a		2.3 a
67 kg ha^{-1}	2.8 b			2.3 a		2.2 a
100 kg ha^{-1}	3.0 b			2.5 a		2.5 a
134 kg ha^{-1}	3.0 b			2.6 a		2.5 a
67:67 kg ha^{-1}	3.8 a			2.5 a		2.5 a
	Richville		East Lansing			
Weed removal timing [c]	2010	2011	2010 & 2011	2010	2011	
< 2 cm weeds	2.9 bc	2.3 a	3.5 a	2.9 b	2.1 a	2.4 a
8 cm weeds	3.6 a	2.1 a	3.5 a	3.4 a	1.5 b	2.5 a
15 cm weeds	3.4 ab	2.2 a	3.2 a	3.4 a	1.9 ab	2.3 a
30 cm weeds	2.8 c	2.3 a	3.2 a	2.7 b	1.6 ab	2.3 a

[a] Means followed by the same letter within a column are not statistically different at the $p \leq 0.05$ significance level according to Fisher's Protected LSD.

[b] Nitrogen was applied as pre-plant urea, with the exception of the 67:67 kg ha^{-1} split treatment where the second application was applied at the 4- to 6-leaf sugarbeet stage.

[c] Weeds were removed by applications of glyphosate at 0.84 kg ae ha^{-1} + ammonium sulfate at 2% v/v.

APPENDIX C

NITROGEN CONCENTRATION OF SUGARBEET PRIOR TO HARVEST

Prior to harvest, four sugarbeets were hand-harvested for nitrogen (N) analysis from each treatment. Sugarbeet leaves were removed from the root, oven-dried at 66°C for 7 d, and weighed. Weights were determined for sugarbeet roots. In 2010, sugarbeets were cut by hand into small pieces and dried until no further weight loss occurred. They were mechanically ground using a Wiley Mill (Arthur H. Thomas Wiley Cutting Mill, Thomas Scientific, 1654 High Hill Road, Swedesboro, NJ 08085) and passed through a sieve. In 2011, subsamples were taken from each sugarbeet with a brei saw. Brei samples were frozen at -80°C for 2 to 14 d, and then transferred to a freeze dryer (VirTis Genesis 12 EL Lyophilizer, VirTis, 815 Route 208, Gardiner, New York, 12525) for 7 d. Sugarbeet were pulverized in a shaker (Geno Grinder 2000, Spex SamplePrep, LLC, 203 Norcross Avenue, Metuchen, NJ 00840) for 20 min at 1700 strokes min^{-1}. Total N content for sugarbeet samples was determined by the micro-Kjeldahl digestion method (Bremner, J. M. 1996. Nitrogen-total. p. 1085-1121 *in* D. L. Sparks, ed. Methods of Soil Analysis. Part 3, Chemical Methods. Book Series 5. Madison, WI: Soil Science Society of America; Jung, S., D. A. Rickert, N. A. Deak, E. D. Aldin, J. Recknor, L. A. Johnson and P. A. Murphy. 2003. Comparison of Kjeldahl and Dumas Methods for Determining Protein Contents of Soybean Products. J. Am. Oil Chem. Soc. 80:1169-1173.) and colorimetric analysis through a Lachat rapid flow injector autoanalyzer (Lachat Instruments, Milwaukee, WI). Data are presented as the main effect of N and weed removal timing at Richville and East Lansing in 2010 and 2011

Table C.1. Main effects of nitrogen and weed removal timing on nitrogen concentration of sugarbeet prior to harvest for Richville and East Lansing in 2010 and 2011.[a]

	Nitrogen concentration of sugarbeet root				Nitrogen concentration of sugarbeet leaves
	Richville		East Lansing		Richville & East Lansing
Nitrogen rate[b]	2010	2011	2010	2011	2010 & 2011
	% N				
0 kg ha^{-1}	0.70 b	0.35 b	0.79 b	0.50 b	1.87 d
67 kg ha^{-1}	0.86 c	0.37 b	0.96 a	0.54 b	1.94 cd
100 kg ha^{-1}	0.92 bc	0.48 a	0.93 a	0.60 a	2.07 bc
134 kg ha^{-1}	1.04 a	0.53 a	1.00 a	0.63 a	2.17 ab
67:67 kg ha^{-1}	1.01 ab	0.56 a	0.96 a	0.65 a	2.28 a
Weed removal timing[c]	2010 & 2011				
< 2 cm	0.69 a		0.87 b	0.65 a	2.09 a
8 cm	0.71 a		0.98 a	0.63 a	2.08 a
15 cm	0.68 ab		0.93 ab	0.55 b	2.07 a
30 cm	0.65 b		0.94 a	0.53 b	2.04 a

[a] Means followed by the same letter within a column are not statistically different at the $p \leq 0.05$ significance level according to Fisher's Protected LSD.

[b] Nitrogen was applied as preplant urea, with the exception of the 67:67 kg ha^{-1} split treatment where the second application was applied at the 4- to 6-leaf sugarbeet stage.

[c] Weeds were removed by applications of glyphosate at 0.84 kg ae ha^{-1} + ammonium sulfate at 2% v/v.

APPENDIX D

SOIL SAMPLES COLLECTED AFTER SUGARBEET, COMMON LAMBSQUARTERS AND POWELL AMARANTH HARVEST

Soil samples were collected from each pot after sugarbeet, common lambsquarters and Powell amaranth harvest to determine nitrate-nitrogen (NO_3-N) and ammonium-nitrogen (NH_4-N) concentrations. Samples were oven-dried at 38°C for 7 d and ground to pass through a 2 mm sieve. NO_3-N and NH_4-N concentrations were determined by the ammonia-salicylate method and cadmium reduction method, respectively, with a Lachat rapid flow injector autoanalyzer following extraction with 1 *M* KCl (Mulvaney, R. L. 1996. Nitrogen-inorganic forms. p. 1123-1184 *in* D. L. Sparks, ed. Methods of Soil Analysis. Part 3, Chemical Methods. Book Series 5. Madison, WI: Soil Science Society of America). Data were combined between trials because there was not a significant treatment by trial interaction, and the data are presented for main effect of N rate.

Table D.1. Effect of nitrogen on nitrate-nitrogen and ammonium-nitrogen concentration of soils after plant were destructively harvested for nitrogen analysis.[a]

Nitrogen rate	Nitrate-Nitrogen		Ammonium-Nitrogen	
	Sugarbeet and Common lambsquarters	Sugarbeet and Powell amaranth	Sugarbeet and Common lambsquarters	Sugarbeet and Powell amaranth
	mg kg^{-1}			
0 kg ha^{-1}	1.2 c	1.4 c	1.4 b	1.3 b
67 kg ha^{-1}	1.5 c	2.2 b	1.4 b	1.3 b
134 kg ha^{-1}	2.7 ab	3.0 a	1.8 a	1.9 a

[a] Means followed by the same letter within a column are not statistically different at the $p \leq 0.05$ level of significance according to Fisher's Protected LSD.

APPENDIX E

PROTOCOL FOR THE TOTAL NITROGEN ANALYSIS OF PLANT TISSUE USING THE MICRO-KJELDAHL BLOCK DIGESTION METHOD

1. Assign 38 ground plant tissue samples to their respective 38 digestion tubes.

2. Weigh out 0.150 grams of a ground plant tissue sample onto Zig-Zag original cigarette papers.

    a. Fold the cigarette paper in half, followed by the sides and the top of the paper to contain the sample in the cigarette paper. Roll the paper into a ball and drop it into the correct digestion tube. Fold the paper tight enough so the plant sample does not fall onto the sides of the tube, but do so carefully because the paper is very fragile.

    b. Add an empty piece of cigarette paper to digestion tube 39.

    c. Weigh 0.150 grams of HSRM onto cigarette paper, fold and drop it into digestion tube 40. This is a plant standard and it can be obtained from the MSU Soil and Plant Nutrient Laboratory.

3. Add 1 catalyst tablet to each digestion tube (ST AUTO 1.5 grams K_2SO_4 and 0.015 grams of Se, Alfie Packers, Inc., 8901 J. Street, Suite 10, Omaha, NE 68127).

4. Under a hood, add 7 mL of concentrated sulfuric acid to each digestion tube.

5. Set the digestion tubes in the digestion block. Turn the controller on and set it to digest the samples for 2 hours at 375°C. It will take approximately 1 hour for the digestion block to heat to 375°C.

6. After the digestion is completed, let the digestions tubes cool until they can be handled. It will take approximately 1 hour or longer for the digestion tubes to cool. To speed up this process, elevate the digestion tubes above the digestion block.

7. Dilute each sample to 75 mL using deionized water.

    a. While vortexing, slowly pour about 50 mL of room temperature deionized water from a beaker into each digestion tube. Make sure any precipitate that may have formed on the sides of the tube or in the solution is re-dissolved.

    b. After the solution has cooled to near room temperature, bring the volume up to 75 mL with a pipette. Adding the final volume of water after the solution has cooled will prevent it from evaporating.

8. Label plastic scintillation vials with the digestion tube number (1-40), their corresponding sample identification number, your name, the date, your primary investigator and the digestion type (plant tissue total nitrogen).

9. Place a rubber stop on top of a digestion tube, and invert the tube at least 7 times to re-dissolve the solution.

10. Filter about 20 mL of the solution through Whatman No. 2 filter paper and into the corresponding labeled plastic scintillation vial. The vial should not be filled to the brim.

11. Repeat the inversion and filtering for each digestion tube until all 40 vials are filled.

12. Cap the vials and place them in the vial box. Label the outside of the box with the range of sample identification numbers, your name, the date, your primary investigator and the digestion type (plant tissue total nitrogen).

13. Take the samples to the MSU Soil and Plant Nutrient Laboratory to determine the total nitrogen content in the diluted digest solutions.

14. Dispose of the remaining solution in the digestion tubes into a hazardous waste container. Place used filter papers and gloves into a sealed plastic bag and dispose in a trashcan.

15. Wash the digestion tubes, funnels, and any other dirty dishes using an acid wash procedure.

16. After receiving the results from the MSU Soil and Plant Nutrient Laboratory, multiply the total nitrogen content of each plant tissue sample by 0.05. The factor 0.05 comes from (μg/mL)x(75 mL/0.15 g)/(10,000 μg/g per % N).

APPENDIX F

PROTOCOL FOR THE TOTAL NITROGEN ANALYSIS OF SUGARBEET BREI USING THE VIRTIS GENESIS 12 EL LYOPHILIZER AND MICRO-KJELDAHL BLOCK DIGESTION METHOD

1. Remove the sugarbeet leaves from the root and place them in a paper bag. Set the bag in an air-forced oven for 7 days to dry and record the dry biomass of the leaves.

2. After removing the leaves, record the weight of the sugarbeet roots from each plot. Saw the sugarbeet roots and collect the brei from each plot.

3. Add approximately 30 mL of the sugarbeet brei to a plastic, 50 mL conical tube and record the fresh weight of the brei.

4. After collecting the brei from all plots, place the conical tubes in a -80°C freezer or a -20°C freezer if the -80°C cooler is not available. The brei must be transferred to a -80°C freezer prior to freeze drying.

5. Prior to freeze drying, remove the conical tubes from the -80°C freezer. Unscrew the caps from each conical tube almost completely, or until the last thread. The caps must be unscrewed in order for the water to evaporate. If they are unscrewed just slightly, they may re-seal in the freeze dryer, and the water will not be removed from the samples.

6. Stack the tubes on metal trays. Approximately 100 tubes will fit on two trays.

7. Place the trays containing the conical tubes into the -80°C freezer. This will cool the trays and prevent condensation from accumulating in the freeze dryer.

8. Turn on the VirTis Genesis 12 EL Lyophilizer. Detailed instructions are posted on the machine and should be followed accordingly. Employees from the USDA-ARS SBRU (Sugarbeet and Bean Research Unit) will provide supervision when using the freeze dryer.

9. Approximately three hours after turning on the freeze dryer, the temperature will be at the correct level to place the metal trays containing the conical tubes into the freeze dryer. Periodically check the freeze dryer during the first hour to confirm that it is operating properly.

10. Remove the samples from the freeze dryer once the vacuum has reached a steady state. This is when the vacuum level increases and decreases slightly for about 1 day. One hundred sugarbeet brei samples require approximately 7 days of drying.

11. Turn off the freeze dryer, following the instructions that are posted on the machine.

12. Tighten the caps on all of the conical tubes.

13. Pulverize the dry sugarbeet brei using the Geno Grinder 2000 for 20 minutes at 1700 strokes min^{-1}. Employees from the USDA-ARS SBRU will provide instruction on how to operate the grinder. A total of 20 conical tubes can be ground at one time if they are 50 mL in size. After 20 minutes, there may be a ball of brei in the center of the tube. Re-pulverize the samples if desired.

14. Record the dry weight of the brei. The fresh weight of the total sugarbeet root, the fresh weight of the brei, and the dry weight of the brei can be used to calculate the total root dry weight. The total root dry weight for each plot is used for nitrogen calculations.

15. Determine the total nitrogen concentration of the sugarbeet brei and leaves using the micro-Kjeldahl block digestion method.