A COLLECTION OF THREE INDEPENDENT STUDIES: INVESTIGATING THE IMPACT OF STARTER FERTILIZER ON MAIZE GROWTH & DEVELOPMENT, VALIDATING AN ALTERNATIVE ROOT STUDY METHOD, AND TESTING THE EFFICACY OF BIOSTIMULANTS IN MAIZE PRODUCTION

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Dedicated to my parents

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ABSTRACT

Starter fertilizer applied with or near the seed at planting often enhances early season maize growth (*Zea mays* L.) but does not always result in higher grain yield. Other responses to starter fertilizer, such as reduced thermal time to reach silking, which suggests accelerated plant development, have been documented. The objective of this study was to examine the relationship between dry matter production and accelerated plant development with respect to 5x5 cm starter (ST) and in-furrow popup (PU) fertilizer. A field experiment was conducted in 2016 with three at-planting treatments consisting of one single rate and formulation of ST (53 N and 21 P kg ha^{-1}) or PU (4 N and 6 P kg ha^{-1}), and an untreated control. In 2018, the study included four additional site-years with treatments consisting of an intermediate (ST) or high (STH) starter fertilizer rate, and an untreated control. For ST treatments, depending on location, nitrogen (N) and phosphorus (P) fertilizer rates ranged between 26-28 and 6-10 kg ha^{-1}, respectively, and for STH treatments N and P fertilizer rates ranged between 47-56 and 12-20 kg ha^{-1}, respectively. In 2016, as new leaf collars appeared, dry matter increased exponentially, but at an equal number of leaf collars ST and PU had similar dry matter as the control. In 2018, dry matter for ST, STH, and control was also similar when normalized for leaf collar number at each site. Overall, these results suggest that enhanced dry matter at a given point in time from ST, STH, or PU was a function of accelerated leaf development as opposed to physically more robust plants of the same leaf collar number. Grain yield was unaffected by ST, STH, or PU treatments at any site-year.

Methods used to study roots in crop fields have included extracting soil cores, excavating entire root systems, using radioactive and non-radioactive chemical tracers, or using mini-rhizotrons. However, due to the intensive nature, level of difficulty, and cost associated with these methods, their use in crop fields has been minimal. We developed an alternative method to quantify

maize rooting density over time. The method involved perforated cylinders installed vertically into the soil at different distances from the row, which made roots growing into the cylinder voids visible from the soil surface and possible to count [root number density (RND)] at different depths using a video recording device (1m-long borescope). The objective of this study was to determine if the cylinder method could quantify rooting density throughout the growing season (V3, ~V7, and R2-R3) similar to the more intensive soil core method, compared in two starter fertilizer trials [continuous maize (M/M) and maize/soybean (M/S) rotation]. Cylinders were constructed with perforated (49% voids) polypropylene resin to an inside diameter of 2.58 cm and a length of 30 cm. Cylinders were painted with red and green alternating markings (5 cm) on the outside and inside walls to visually aid in identifying depth from the soil surface. After plants emerged, cylinders were inserted vertically into the soil after drilling a 3.5 cm diameter borehole. Ten perforated cylinders were installed in a parallel line 13 or 25 cm away from, and on both sides of, the planted row. Soil cores were also collected at the same relative locations for conducting root extractions and subsequent calculation of length density (RLD). At V3, methods frequently resulted in the same significant ($p \leq 0.10$) or insignificant ($p > 0.10$) main and interaction effects in both fields, whereas at ~V7 and R2-R3, there were several instances where the cylinder method failed to detect the same effects as the soil core method. At times both the cylinder method and the soil core method detected significant main or interaction effects, but the direction of the effect was opposite.

In-furrow biological (BIO) and plant growth regulator (PGR) products, otherwise known as biostimulants, are becoming increasingly available in the commercial maize market. The objective of this study was to compare the effects of several commercially available in-furrow biostimulant products on maize growth and development, nutrient uptake, and grain yield to starter

fertilizer in large-plot field trials. The study was conducted across five locations in 2016, and three locations each in 2017 and 2018 at Purdue University research farms. At each location, treatments consisted of four different BIO or PGR products plus starter fertilizer, starter fertilizer only, and an untreated control. Compared to the control, starter-only increased grain yield at 7 of 8 site-years in 2016 and 2018 ranging from 125 to 753 kg ha^{-1}, depending on location, but no increase was found at any of the 3 locations in 2017. Grain yield was increased (3 of 11 site-years) or decreased (2 of 11 site-years) by some of the BIO or PGR products, but in 6 of 11 site-years none of the products affected yield compared to starter-only.

CHAPTER 1. INTRODUCTION

1.1 Introduction

Nearly 90 years ago, Millar (1930) determined that placing fertilizer near the side and below maize (*Zea mays* L.) seeds would precisely align with developing roots and avoid the dangers of placing the fertilizer with the seed, which was a practice at that time. To our knowledge, this study was the advent of banded starter fertilizer. Nowadays, most planters can apply starter fertilizer 5 cm below and 5 cm to one side of the seed (ST) or directly in the planting furrow (popup). The motive for applying ST or popup is to stimulate early season growth and ultimately increase grain yield. However, several studies suggest that even though ST or popup frequently stimulate early season growth, increased grain yield does not always ensue (Bundy and Andraski, 1999; Bermudez and Mallarino, 2002; Kaiser et al., 2005; Kim et al., 2013). Despite the multitude of ST and popup fertilizer related studies, none have offered a clear explanation for why enhanced early season growth does not necessarily result in higher grain yield.

In addition to increasing early season growth, which is determined on whole plant dry matter or height, multiple studies have also shown that ST and popup reduced the number of days between planting and silking (Mascagni and Boquet, 1996; Cromley et al., 2003, 2006; Kaiser et al., 2016). Bullock et al. (1993) also found that ST accelerated crop growth rates. Consequently, at a given point in time, plants treated with ST or popup may appear larger simply due to being further along in their development. This would then explain why Bullock et al. (1993) found that plants with and without starter fertilizer produced the same total dry matter and grain yield in the end. Perhaps this phenomenon is why enhanced growth, or the illusion of enhanced growth for that matter, does not consistently result in increased grain yield. However, more work is certainly warranted to support this explanation.

The effects of ST or popup on aboveground growth parameters, such as plant height, dry matter, days to silking, and growth rates are well documented. However, little is known about the effects of ST or popup belowground on root development. In fact, our overall understanding of root development in maize cropping systems in general is limited relative to our knowledge of aboveground growth. In field trials, roots are difficult to study. Crop root systems in the field have been studied with several methodologies, including extraction of soil cores, excavation of entire root systems, use of radioactive and non-radioactive chemical tracers, and mini-rhizotrons. Unfortunately, the intensive nature, high level of difficulty, and cost of these methods has limited their extensive use in field experiments. Alternative methods that are easier and less expensive are needed to advance our understanding of rooting patterns in cropping systems.

In a preliminary field experiment, an alternative to the minirhizotron or soil core method was developed by our research group to measure the effect of ST on rooting behaviors in maize. The method consisted of installing perforated cylinders at various distances from the row that allowed roots to grow into the cylinder voids at their normal trajectory. The roots were then easily visible from the soil surface by looking down into the cylinders. By monitoring the cylinders every other day, we were able to detect the extent of the total root system at 13 and 25 cm from the row over a period of time based on the change in percentage of cylinders with new visible roots with respect to starter fertilizer.

This new method was an effective way to quantify the rate of root movement over time with minimal effort and low cost. In retrospect, counting the number of roots at different depths on each day would have provided a means for estimating root density. Currently, in order to measure root density over time, researchers are primarily limited to collecting soil cores on multiple dates or monitoring them with minirhizotrons. The perforated cylinder method could be

a viable alternative that is less labor intensive than collecting soil cores and much cheaper than minirhizotrons. But first, we need to determine if the perforated cylinder method is an accurate measure of rooting density at different distances and depth from the maize row over time.

In recent years, other early season amendments, such as in-furrow biologicals or plant growth regulator products have begun to appear in the commercial maize market to apply with ST or popup fertilizers. Biological and PGR products are collectively referred to as biostimulants. Biostimulants are commonly marketed as amendments that will stimulate early season growth, enhance nutrient uptake, and ultimately increase grain yield. A limited number of independent field trials have evaluated the responses of maize to biostimulants. A recent study conducted in 11 Wisconsin maize fields reported that a PGR product containing cytokinin, gibberellic acid, and indole butyric acid marketed for maize resulted in no yield advantage over untreated checks (Lauer, 2017). Nevertheless, the marketing claims behind biostimulants can be appealing to farmers, especially considering their low-cost relative to other inputs. As farmers continuously look for new ways to increase yield, coupled with public demands for higher food production, biostimulants will likely gain in popularity as crop production inputs. Evaluating the efficacy of commercially available biostimulants by independent field trials is important to determine if marketing claims are valid.

In summary, this dissertation includes three separate chapters that focus on the three main objectives previously discussed. The first chapter, titled “Starter and Popup Fertilizer Effects on Plant Dry Matter, Leaf Appearance Rate, and Grain Yield in Maize” studies the relationship between dry matter production and accelerated plant growth as affected by ST and popup fertilizer. The second chapter, titled “Visual Estimation of Rooting Profiles and Density in the top 30 cm of Soil Using Perforated Cylinders and a Long Borescope Equipped with a Video Recording Device”

evaluates the accuracy of an alternative root study method developed by our lab to measure rooting patterns over time in a field setting. The third chapter, titled “Corn Response to Starter Fertilizer and In-Furrow Biological and Plant Growth Regulators” evaluates the effects of several commercially available in-furrow biostimulant products on maize growth and development, nutrient uptake, and grain yield under multiple growing environments in field-scale trials. Chapters may slightly differ in their delivery and units of measure depending on the requirements for the intended journal of publication.

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CHAPTER 2. STARTER AND POPUP FERTILIZER EFFECTS ON PLANT DRY MATTER, LEAF APPEARANCE RATE, AND GRAIN YIELD IN MAIZE

2.1 Abstract

Starter fertilizer applied with or near the seed at planting often enhances early season maize growth (*Zea mays* L.) but does not always result in higher grain yield. Other responses to starter fertilizer, such as reduced thermal time to reach silking, which suggests accelerated plant development, have been documented. A better understanding of how starter affects plant development may provide insight to better explain the lack of consistent yield responses. The primary objective of this study was to examine the relationship between dry matter production and accelerated plant development with respect to starter (ST) and in-furrow popup (PU) fertilizer applications at planting. A field experiment was conducted in 2016 with three at-planting treatments consisting of one single rate and formulation of ST (53 N and 21 P kg ha^{-1}) or PU (4 N and 6 P kg ha^{-1}), and an untreated control. Early season dry weight and leaf collar stage were measured every 3-5 days between 8 and 28 days after planting, along with grain yield after maturity. In 2018, the study included four additional site-years with treatments consisting of an intermediate (ST) or high (STH) starter fertilizer rate, and an untreated control. One of the four locations only contained the STH and control treatments. Nitrogen and phosphorus (P) supplied through fertilizer treatments in 2018 varied by location. For ST treatments, N and P rates ranged between 26-28 and 6-10 kg ha^{-1}, respectively. For STH treatments, N and P rates ranged between 47-56 and 12-20 kg ha^{-1}, respectively. At all site-years, ST and STH were applied 5 cm below and 5 cm to one side of the seed, and PU was applied in-furrow. Dry weight and leaf collar stage were measured throughout the entire vegetative cycle every 1-2 weeks between V1 and silking. Additional

measurements included total leaf number around silking, final dry weight shortly after physiological maturity (R6), and grain yield. At one location, all measurements except final dry weight at maturity and grain yield were terminated after the third sampling date due to an early June hailstorm. On any given sampling date, dry weight and average leaf collar number were generally enhanced by STH, ST, and PU at each site-year. In 2016, as new leaf collars appeared, dry matter increased exponentially. When normalized for leaf collar number, ST and PU had similar dry matter as the control. In 2018, dry matter for ST, STH, and control was also similar when compared at the same leaf collar number at each of the three sites. Overall, these results suggest that enhanced dry matter at a given point in time from ST, STH, or PU was a function of accelerated leaf development as opposed to physically more robust plants of the same leaf collar number. Consequently, dry matter at maturity was similar among all treatments at each location in 2018. Average total leaf number was increased by ST or STH at two of three sites compared to the control. At one responsive site, ST or STH treatments reduced the number of plants with 17 leaves and increased the number of plants with 18 and 19 leaves, compared to the control. At the other responsive site, ST or STH treatments had more plants with 19 leaves and fewer with 18 leaves, compared to the control. Grain yield was unaffected by ST, STH, or PU treatments at any site-year. Despite no effects on yield, grain moisture was reduced by ST, STH or PU (0.1 to 0.9 g kg^{-1}), compared to the control, at 4 of 5 locations.

2.2 Introduction

Early season maize growth (*Zea mays* L.) is often stimulated by starter fertilizer placed with or near the seed at planting (Mascagni and Boquet, 1996; Gordon et al., 1997; Vetsch and Randall, 2002; Niehues et al., 2004). However, grain yield is often inconsistently affected by starter fertilizer (Bermudez and Mallarino, 2002; Kaiser et al., 2005; Kim et al., 2013). Averaged across

100 on-farm starter fertilizer trials in Wisconsin (Bundy and Andraski, 1999), starter fertilizer increased plant height 4% irrespective of a significant yield increase over no-starter. The overall relationship between the effects of starter fertilizer on plant growth and yield response is poorly understood. Enhanced growth from starter is usually quantified by total dry matter or height estimates made at a single point in time. In fact, all the previously mentioned studies, and almost every study reported in this literature review for that matter, only measured total dry matter or height at a single point in time, which was frequently around the 6^{th} leaf collar stage. The issue is that growth estimates only measured once at a single point in time fail to account for the possibility that treatments may not be at the exact same leaf collar stage. Therefore, reports of stimulated dry matter production or plant height may be artificial if treatments were not at the exact same growth stage.

Starter fertilizer can be applied in various different positions relative to the seed, but in this discussion, "starter" will refer to 5 cm below and 5 cm to one side of the seed and "popup" will refer to placement directly with the seed in the planting furrow. Unless otherwise noted, growth estimates from starter or popup mentioned from now on refer to measurements made only at a single point in time during the growing season. Several field studies have documented that starter or popup fertilizers increase early season growth in conventional and reduced tillage systems (Vetsch and Randall, 2002; Cromley et al., 2003), low and high soil P environments (Bermudez and Mallarino, 2004), and irrigated and rainfed systems (Wortmann et al., 2006). Limited evidence suggests that later maturity hybrids exhibit a greater growth response to starter fertilizer than earlier maturity hybrids when planted early (Cromley et al., 2006). However, most field studies generally conclude that starter fertilizer stimulates plant growth regardless of hybrid (Gordon et al., 1997; Buah et al., 1999; Cromley et al., 2003; Wortmann et al., 2006).

Nitrogen (N) and phosphorus (P) are common nutrient sources for starter or popup fertilizer (Mascagni and Boquet, 1996; Gordon et al., 1997; Cromley et al., 2003; Kaiser et al., 2016), but additions of potassium (K) (Kaiser et al., 2005; Mallarino et al., 2011) or sulfur (S) (Wortmann et al., 2006; Kim et al., 2013), and occasionally both (Niehues et al., 2004), are often used as well. In general, early season growth is optimized by starter fertilizer when N is applied alone or in combination with P (Touchton and Karim, 1986; Kim et al., 2013). However, N-only starter fertilizer applications may have little influence on early season growth if at least half of the total N requirement is applied pre-plant rather than all at sidedress (Roth et al., 2003). Fixen and Lohry (1993) summarized the results from multiple starter fertilizer trials and concluded that a 1:0.44 ratio of N and P outperformed greater N:P ratios for stimulating early season growth; however, it is important to note that N-only solutions were not included in the analysis. A study conducted in Kansas also found that starter fertilizer applied at 34 kg N ha^{-1} produced similar levels of dry matter around the 6^{th} leaf collar stage as 67, 101, and 134 kg N ha^{-1}, when P and K were held constant at 15 and 9 kg ha^{-1}, respectively (Niehues et al., 2004). In similar studies, starter or popup fertilizers applied with just P (Touchton and Karim, 1986) or K (Mallarino et al., 2011) generally did not alter early season growth. Adding S to N and P starter fertilizer formulations has also shown to benefit early season plant growth, but only under specific situations that were not well identified (Niehues et al., 2004; Wortmann et al., 2006; Kim et al., 2013).

The magnitude of the increases in plant dry matter or height with starter or popup fertilizers is difficult to predict, considering the variations in fertilizer rates, management practices, planting dates, soil chemical and physical properties, and weather conditions across studies. However, according to a study conducted in Iowa, the magnitude of dry matter increase from starter or popup fertilizers was poorly correlated with the magnitude of the yield increase (Bermudez and Mallarino,

2002). Therefore, dry matter or plant height measurements appear to be poor predictors for increased grain yield from starter or popup fertilizers.

Plants treated with starter fertilizer often require fewer days to reach silking than plants without starter fertilizer. Ammonium polyphosphate (APP, 10-34-0) applied in-furrow reduced the number of days to silking in three consecutive seasons in Louisiana, but more so when maize was planted in mid-March (e.g. ~ 4.6 d) versus early May (e.g. ~ 1.7 d) (Mascagni and Boquet, 1996). A study conducted in Missouri also found that starter fertilizer applied at 34 kg N and 15 kg P ha^{-1} reduced the number of days to silking for multiple hybrids when planted in April (avg. ~ 1.8 d) and to a lesser extent when planted in May (avg. ~ 0.5 d) (Cromley et al., 2006). In a tillage study from Missouri, starter fertilizer (34 kg N and 15 kg P ha^{-1}) reduced the number of days to silking (avg. ~ 0.8 d) across three hybrids in 1 of the 2 years, but the response was similar in both conventional and no-till systems (Cromley et al., 2003). In Minnesota, APP applied in-furrow reduced the number of days to silking by about one day, irrespective of planting date (Kaiser et al., 2016).

In an Illinois study, and to our knowledge the only study where growth response from starter fertilizer was measured at several time points throughout the growing season, APP applied as starter fertilizer, in comparison to no starter fertilizer, increased above-ground plant dry matter between 450 and 1200 GDD (°C) after planting (Bullock et al., 1993). However, calculated crop growth rates, which were expressed as g m^{-2} $(100\ GDD)^{-1}$, indicated that plants with starter fertilizer simply just reached maximum growth rate sooner than untreated plants, and that the overall amount of total dry matter by the end of the season was similar between treatments.

Based on the previous results of accelerated crop growth rate (Bullock et al., 1993), coupled with reduced days to silking (Mascagni and Boquet, 1996; Cromley et al., 2003, 2006; Kaiser et

al., 2016), plants treated with starter or popup at a given point in time may appear larger simply due to being further developed. Studies that document stimulated growth from starter or popup fertilizers from a single observation during the growing season may have captured an apparent difference that is more of a function of advanced development in contrast to greater dry matter production at identical leaf stages. This phenomenon might explain why studies often find that enhanced growth does not consistently translate to increased grain yield. More work is certainly warranted to support these claims. Our objective was to study the relationship between dry matter production and accelerated plant growth as affected by starter and popup fertilizer applications.

2.3 Materials & Methods

2.3.1 Field Description & Trial Design

A field experiment was conducted in 2016 at the Throckmorton Purdue Agricultural Center (TPAC: 40.268010 lat., -86.882712 long.) to evaluate starter (ST) and in-furrow or "popup" (PU) fertilizer effects on early season plant growth and yield of maize. The field had been managed without tillage for more than 10 years and the previous crop was maize. Additional field experiments were established in 2018 at the Southeast (SEPAC: 39.041513 lat., -85.523897 long.), Northeast (NEPAC: 41.114404 lat., -85.448944 long.), Pinney (PPAC: 41.451088 lat., -86.941363 long.) and Throckmorton (same field as 2016) Purdue Agricultural Centers. All fields had been managed without tillage for several years except PPAC, which was tilled once in the fall with a disc chisel and twice in the spring with a disc and then a field cultivator. The previous crop at each field was maize. Soil characteristics and predominant soil types are described in Table 2-1.

Table 2-1. Soil characteristics and predominant soil types for field experiments conducted at the Southeast (SEPAC), Northeast (NEPAC), and Pinney (PPAC) Purdue Ag. Centers in 2018 and Throckmorton Purdue Ag. Center (TPAC) in 2016 and 2018.

Location	Bray-P1 (mg kg^{-1})	K (mg kg^{-1})	O.M (g kg^{-1})	pH	Series†	Type	Classification
SEPAC	18	88	21	6.2	Avonburg Nabb	Silt loam Silt loam	fine-silty, mixed, active, mesic Aeric Fragic Glossaqualfs fine-silty, mixed, active, mesic Aquic Fragiudalfs
NEPAC	27	110	20	6.1	Haskin Rawson	Loam Sandy loam	fine-loamy, mixed, active, mesic Aeric Epiaqualfs fine-loamy, mixed, active, mesic Oxyaquic Hapludalfs
TPAC-2016	25	112	25	6.6	Toronto-Millbrook	Silt loam-silt loam	fine-silty, mixed, superactive, mesic Udollic Epiaqualfs fine-silty, mixed, superactive, mesic Udollic Endoaqualfs
TPAC-2018	17	80	23	6.3	Lauramie	Silt loam	fine-loamy, mixed, active, mesic Mollic Hapludalfs
PPAC	38	128	32	5.9	Sebewa	Loam	fine-loamy, over sandy, mixed, superactive, mesic Typic Argiaquolls

†Combined >50% of entire plot area

In 2016, a hybrid adapted to central Indiana [RM 109; GDD (°C) to maturity 1540] was planted on May 24 at 81,510 seeds ha^{-1} using a 6-row commercial planter. Each row unit on the planter was equipped with 0.43 mm plastic hoses allowing liquid fertilizer to be placed in-furrow (PU) and with separate coulters capable of positioning liquid fertilizer 5 cm to one side and 5 cm below the seed (ST). Three treatments were established at planting and consisted of one single rate and formulation of ST or PU (Table 2-2), and an untreated control.

Table 2-2. Fertilizer rates and formulation descriptions for starter (ST) and popup (PU) treatments at TPAC in 2016 and for intermediate (ST) and high (STH) starter rate treatments at SEPAC, NEPAC, PPAC, and TPAC in 2018. Starter was applied 5 cm below and 5 cm to one side of the seed, and popup was applied in-furrow. At all locations, control treatments had no N or P applied except NEPAC, which had 3 kg N ha^{-1} applied as ammonium thiosulfate.

Location	Treatments	N-P-K-S (%)	kg N ha^{-1}	kg P ha^{-1}
TPAC 2016	Control	0-0-0	0	0
	PU	10-15-0	4	6
	ST	19-7-0	53	21
SEPAC 2018	Control	0-0-0	0	0
	ST	22-5-0	28	6
	STH	22-5-0	56	12
NEPAC† 2018	Control	2-0-0-4	3	0
	ST	21-4-0-4	28	6
	STH	24-2-0-2	56	6
PPAC 2018	Control	0-0-0	0	0
	ST	19-7-0	26	10
	STH	19-7-0	52	20
TPAC 2018	Control	0-0-0	0	0
	STH	19-7-0	47	18

†All treatments were supplied with 5 kg S ha^{-1} as ammonium thiosulfate

Fertilizer sources at TPAC 2016 were a 1:1 mixture of urea ammonium nitrate (UAN) and APP (ST), or APP alone (PU). Treatments were replicated 3 times in a randomized complete block design. Individual plots were 12 rows wide (9 m) and approximately 370 m long. Surface application of UAN plus a urease inhibitor NBPT [(N-(n-butyl)-thiophosphoric triamide) N-

methyl-2-pyrrolidone applied 1.2 L ha^{-1}] occurred 48 days after planting using a high clearance sprayer equipped with drop-down nozzles. Applied N rates at planting and sidedress varied by treatment (281 – 310 kg ha^{-1}), but the total N rate exceeded the agronomic optimum recommendation at this location (Camberato and Nielsen, 2019) by 65 kg ha^{-1}.

In 2018, experiments were planted May 7, 8, 9, and 29 at NEPAC, SEPAC, TPAC, and PPAC, respectively. A common, adapted, hybrid [RM 108; 1478 GDD (°C) to maturity] was planted at all locations except at SEPAC [RM 111; 1529 GDD (°C) to maturity]. Fields were planted with 6-row commercial planters at 74,100 seeds ha^{-1} with row units equipped with separate coulters capable of positioning liquid fertilizer 5 cm to one side and 5 cm below the seed (ST). Treatments were established at planting and consisted of intermediate (ST) or high (STH) starter fertilizer rates (Table 2-2), plus an untreated control. Starter treatments at all locations contained UAN and APP. In addition, all treatments at NEPAC, including the control, received 5 kg S ha^{-1} and 3 kg N ha^{-1} applied at planting as ammonium thiosulfate because that location was known to be sulfur deficient. Treatments were replicated 4 to 5 times in a randomized complete block design depending on location. Individual plots were 12 rows wide (9 m) and varied in length depending on field size (128 – 640 m). Total N rate was equalized across treatments at sidedress based on N rates applied at planting. Total optimum N rates for continuous maize cropping, specific to each location, were 291, 241, 247, and 241 kg ha^{-1} at NEPAC, SEPAC, PPAC, and TPAC, respectively. Sidedress N (UAN) was coulter injected in between rows around the 6^{th} leaf collar stage at all locations. Sidedress application at the NEPAC location also included ammonium thiosulfate to supply 11 kg S ha^{-1} to all treatments.

2.3.2 Field Measurements & Sample Processing

The frequency and intensity of sampling differed between the two years of study. In 2016, early season plant growth was measured by excavating 30 evenly spaced plants from the control, PU, and ST plots at 8, 12, and 16 days after planting (DAP). The growth stage of each sampled plant was expressed in terms of V#, where # represents the total number of visible leaf collars (Abendroth et al., 2011). Shoots were separated from the root system directly above the coleoptilar node. Shoot tissue was carefully cleaned with forced air to remove any soil. Roots were soaked and cleaned with running water. Seeds and roots were hand separated. Dry weights were recorded for each plant part on a per plant basis following several days of drying in a forced air oven at 60˚C until sample weights stabilized. Subsequent above-ground shoot samples were collected from the same plots at 20, 23, and 28 DAP by cutting 30 evenly spaced plants directly at the soil surface. A composite dry weight was obtained for all 30 plants following several days of drying in a forced air oven at 60˚C. An average dry weight plant^{-1} was estimated by dividing the composite dry weight by the total number of plants collected within each plot. The average growth stage (V#) for each plot for each of the final three sampling dates was calculated as the mathematical mean of 30 evenly spaced plants. Shoot tissues from each sampling date were composited by plot and then ground until passing a 2 mm mesh screen. Laboratory procedures and nutrient analyses were performed by A&L Great Lakes Laboratories, Inc. (Fort Wayne, IN). Total concentration of P, K, Mg, Ca, S, Na, Fe, Al, Mn, B, Cu, and Zn were measured with inductively coupled plasma atomic emission spectroscopy (ICP-AES) after microwave acid digestion. Total N was determined by dry combustion (Horneck and Miller, 1998).

For root systems collected from the first three sampling dates, it was difficult to remove all soil aggregates with running water. Therefore, to remove soil as a confounding factor, an “ash-free organic dry weight” (Oliveira et al., 2000) was determined for each sample by subtracting an ash

weight from the original dry weight. Ash weights were obtained by placing roots in a muffle furnace at 650˚C for 5 hr to remove all organic material leaving only the inorganic ash.

In 2018, plant measurements and sampling procedures were similar across all four locations. Seven groups of 10 consecutive plants, uniform for emergence, were predetermined for sampling within each plot around the first leaf collar stage. Multiple samples were collected in two or three areas of each plot per sampling date to increase sample size and to account for field variability. Growth stage (V#) was determined in all targeted sampling areas for each plant and plant tissue was sampled by cutting each plant directly at the soil surface. The first sample was taken when >50% of the plants exhibited at least one visible leaf collar. The process was repeated approximately every 1-2 weeks until all targeted sampling areas were collected. The tip of the 5^{th} and 10^{th} leaves of each plant targeted for subsequent sampling were torn to serve as marker leaves to keep track of the total number of visible leaf collars after the lower leaves senesced. At the onset of the reproductive growth stages, the number of plants exhibiting a fully developed tassel and/or silk protruding from the ear shoot was recorded. Total number of leaves $plant^{-1}$ was determined at the final sampling date. In addition to the seven targeted sampling dates, a final above-ground dry matter sample was collected shortly after kernel black layer occurred (physiological maturity). Ten consecutive plants from two or three areas within each plot were cut directly at the soil surface. Ears were separated from the plant, and the grain was then mechanically shelled. All plant samples after each sampling date were then dried in a forced air oven at 60˚C to stop all physiological activity in the tissue. Plants were dried again for several days until constant weight prior to determining dry matter. Cobs and ear husks from the samples collected at maturity were included in the dry matter weight, whereas the shelled grain was dried and weighed separately. Shoot tissues

from each sampling date prior to reproduction were analyzed for total nutrient concentration by the same laboratory and using similar procedures previously described for the 2016 experiment.

2.3.3 Grain Harvest Procedures

Grain harvest procedures were similar in both the 2016 and 2018 field trials. Grain yield and moisture were estimated from the middle six rows of each 12-row plot using commercial combines equipped with GPS-enabled yield monitors. Yield monitors (impact sensors) were calibrated at each location on the day of harvest based on the recommended guidelines provided by the manufacturer. Data processing and cleaning procedures with GIS software included removing data points 1) within 15-23 m from end-rows, 2) from anomalous poor areas of the field (i.e. gullies, wet spots, extreme slopes, etc.), and 3) equal to or greater than ±two standard deviations from each treatment mean. All remaining data points within each plot were then averaged to obtain a single yield and moisture value for each plot.

2.3.4 Statistical Procedures

Grain yield and moisture, shoot dry weight, and growth stage measurements from the 2016 and 2018 field experiments were analyzed by location and sampling date. An analysis of variance was performed with a mixed model where treatment was considered fixed and replication (block) was considered random. A similar analysis was performed for total leaf number plant^{-1} measurements from 2018 field experiments.

For early season root and shoot dry weight measurements that were collected across three sampling dates in 2016 on a per plant basis, the average coefficient of variation [$(CV) = \frac{\sigma}{\mu} * 100$ where σ = standard deviation and μ = population mean] was calculated for each plot along with the average of root dry weight plant^{-1}. The ratio between shoot and root dry weight was also

calculated for each plot and by sampling date. An analysis of variance with a mixed model was performed where sampling date and treatment were considered fixed, and replication (block) was considered random.

All mixed models were analyzed in SAS version 9.4 using the PROC MIXED command (SAS Institute, 2015). Least-square means were computed using the LSMEANS statement to compare treatment and date (when applicable) differences or treatment*date interactions (when applicable), but only if the overall main effect or interaction effect from the mixed model was significant ($p \leq 0.10$).

The relationship between shoot dry matter production and leaf collar stage estimates from the 2016 and 2018 field experiments was analyzed by fitting a best-fit regression equation to the data based on the highest R^2 value. Differences in regression lines between treatments were determined based on the overlapping of vertical (shoot dry matter) and horizontal (growth stage) error bars. Error bars were calculated based on the standard error of the mean SE = $\frac{\sigma}{\sqrt{n}}$ where σ equals standard deviation of the treatment mean across replications and *n* equals the number of replications.

Nutrient content in the shoot tissue was expressed based on growth stage rather than sampling date. The relationship between nutrient content and growth stage development was analyzed by fitting a best-fit regression equation to the data based on the highest R^2 value. Differences in regression lines between treatments were determined based on the overlapping of vertical (nutrient content) and horizontal (growth stage) error bars.

2.3.5 Weather Data

Daily temperature and precipitation data were recorded with automated weather stations located <8 km from each field. Missing temperature and precipitation data for a specific day were substituted by data recorded from the next closest weather station. A 30-year monthly average temperature and total precipitation were acquired for each location's respective climate division number for Indiana. All data were acquired through the national weather service (NWS) Cooperative Observer Program (US-COOP) obtained from the Midwestern Regional Climate Center, cli-MATE (MRCC Application Tools Environment). Weather data are presented as the average temperature and total precipitation for each month from April through November. Growing degree day [GDD] values based on degrees Celsius were calculated for each day [(T_{max} + T_{min})]/2 – T_{base} where T_{max} and T_{min} denotes the maximum (≤30°C) and minimum (≥10°C) daily air temperature thresholds, respectively, and T_{base} is constant at 10°C (Gilmore and Rogers, 1958). Sampling dates are expressed as the total number of accumulated GDDs between the day of planting and the day of sampling.

2.4 Results & Discussion

2.4.1 Weather: 2016 & 2018

In April and May of 2016, total precipitation and average daily temperature were below the 30-year average (Fig. 2-1). Temperatures in June were near normal but frequent rainfall events later in the month resulted in total precipitation 40 mm above the 30-year average. Following average weather patterns in July, total precipitation and average daily temperatures throughout August and September were well above the 30-year average.

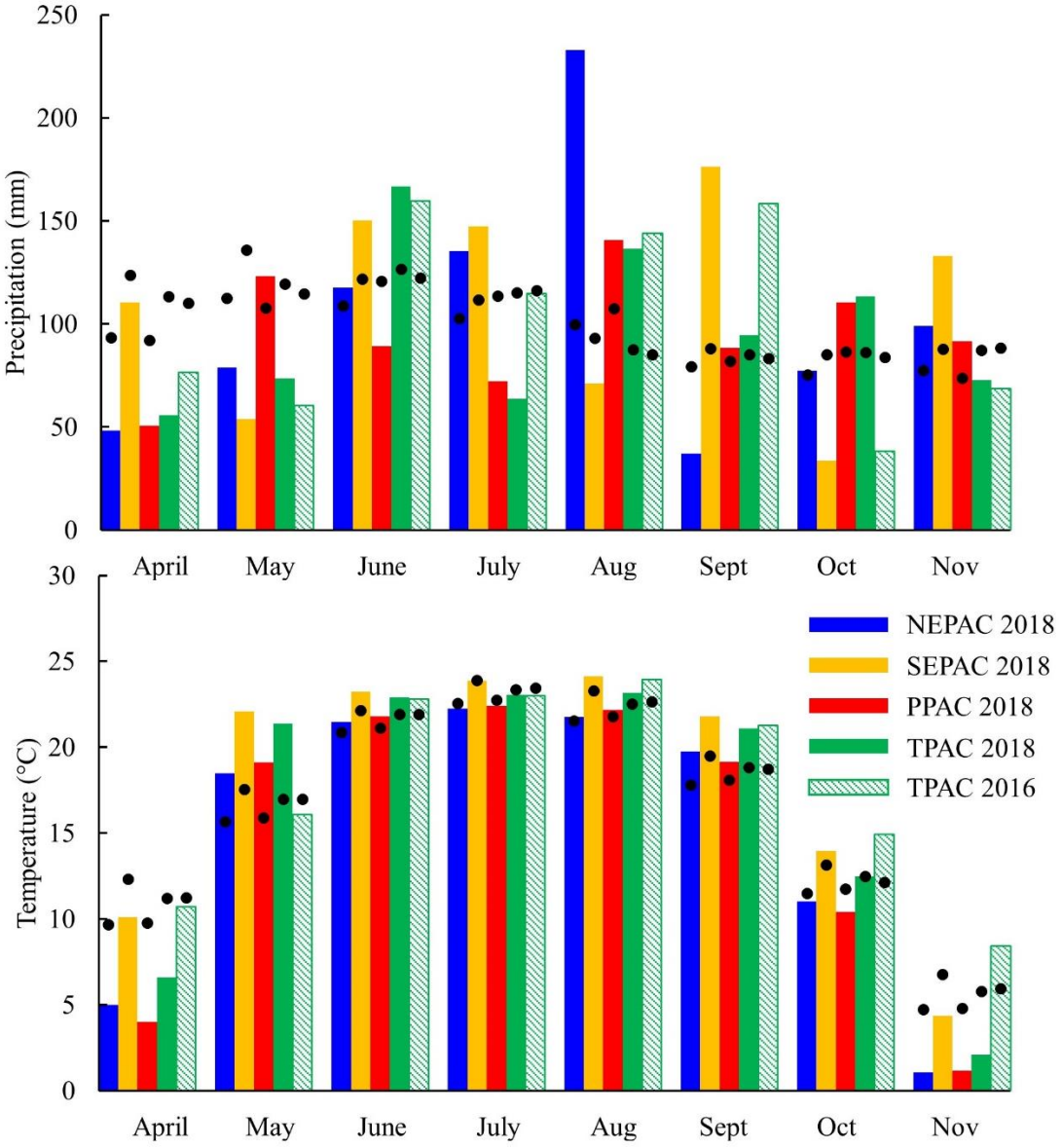

Figure 2-1. Monthly total precipitation (top) and average temperature (bottom) data collected from automated weather stations located <8 km from each field. Black circles associated with each bar represents the monthly, 30-year average temperature or total precipitation for each location's respective climate division # for Indiana. All data were acquired through NWS Cooperative Observer Program (US-COOP) obtained from the Midwestern Regional Climate Center, cli-MATE (MRCC Application Tools Environment).

In 2018, the growing season began in April with statewide total precipitation and average daily temperatures well below the 30-year average (Fig. 2-1). A warm and dry period in May allowed for timely planting at all locations except PPAC, in which planting was delayed due to above average rainfall. Temperatures throughout June, July, and August for all locations were near the 30-year average. The NEPAC location received a significant amount of rainfall throughout the summer months, most noticeably in August, where total precipitation was 133 mm more than the 30-year average. During the same time period, total precipitation at the remaining locations fluctuated above and below the 30-year average depending on month, but no visible signs of crop stress were noted.

2.4.2 Shoot Dry Matter & Leaf Collar Stage: 2016

In 2016, seedlings from each treatment were first sampled between VE and V1 at 120 GDD, or 8 days after planting. Plant development was unaffected by ST and PU at 3 sampling dates within the first 199 GDD, as all treatments had similar number of total leaf collars and shoot dry matter (Table 2-3). Young seedlings rely primarily on stored seed reserves for nourishment (Nadeem et al., 2011) until roughly the V3 stage (Cooper and MacDonald, 1970). Thus, ST or PU may have little impact on seedling development until seed resources are nearly depleted.

Table 2-3. Effects of starter (ST) (53 kg N and 21 kg P ha^{-1}) applied 5 cm to one side and 5 cm below the seed and in-furrow popup (PU) (4 kg N and 6 kg P ha^{-1}) fertilizer on the number of visible leaf collars and shoot dry matter $plant^{-1}$ in maize across multiple early-season sampling dates corresponding to different growing degree days (GDD) at TPAC in 2016. Per observation date, shoot dry matter comparisons among the different fertilizer treatments were always measured at a single point in time.

		Visible leaf collars $plant^{-1}$			Shoot dry matter (g $plant^{-1}$)		
Date	**GDD†**	**Control**	**PU**	**ST**	**Control**	**PU**	**ST**
6/1	120	0.73	0.77	0.87	0.03	0.04	0.04
6/5	164	1.40	1.62	1.80	0.08	0.12	0.12
6/9	199	2.09	2.46	2.40	0.18	0.29	0.28
6/13	255‡	-	-	-	0.42 b	0.72 ab	0.83 a
6/16	296	4.02 c	4.57 b	4.79 a	0.88 b	1.51 a	1.58 a
6/21	365	5.00 c	5.50 b	5.72 a	2.23 b	3.69 a	4.32 a

†Cumulative GDD (°C) since planting, May 24.
‡Leaf collars were not determined at this date.
Treatment means within a sampling date and variable followed by a different letter are different ($p \leq 0.10$).

At later sampling dates, 255, 296, and 365 GDD after planting, shoot dry matter values with the ST treatment were 98, 80, and 94% greater compared to the control. The PU treatment, in comparison to the control, also increased shoot dry matter 72 and 65% at 296 and 365 GDD after planting, respectively. At each sampling date, PU and ST had similar shoot dry matter values.

The treatment differences observed at specific GDD after planting were confounded with treatment effects on the rate of leaf stage progression. Even though no differences were detected for shoot dry matter between the two fertilizer treatments at 296 and 365 GDD (Table 2-3), average leaf collar number within the sampled plants was slightly greater in the ST treatments than the PU treatments at both sampling dates. Leaf collar development of both fertilizer treatments was more advanced than that of the control. The relationship between leaf collar number and shoot dry matter was not influenced by fertilizer treatment (Fig. 2-2). As new leaf collars appeared, dry matter increased exponentially, but when normalized for number of leaf collars, the control, PU, and ST had similar dry matter.

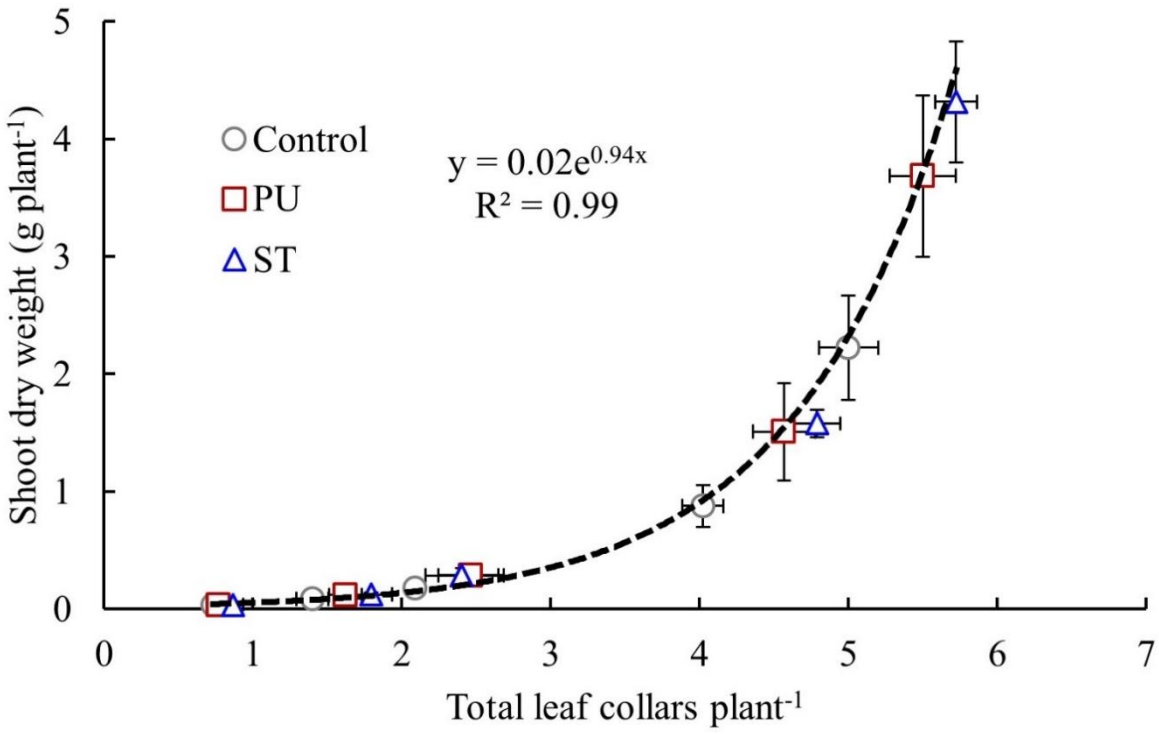

Figure 2-2. Effects of starter (ST) (53 kg N and 21 kg P ha^{-1}) applied 5 cm to one side and 5 cm below the seed and in-furrow popup (PU) (4 kg N and 6 kg P ha^{-1}) fertilizer on the relationship between shoot dry matter production and leaf collar number in maize at TPAC in 2016. Data points are means of three replications of each treatment. Horizontal and vertical error bars represent the standard error of the mean. The dotted line represents the exponential equation across all treatments.

Between 120 and 199 GDD (VE to V2), dry matter was recorded on an individual plant basis to determine plant-to-plant variability. The average CV (data not shown) for shoot dry matter was higher (treatment; p=0.09) in the PU (28%) treatment than in the ST (22%) or control (24%), regardless of sampling date (treatment*date; p=0.87). Across treatments, the average CV for shoot dry matter was 24%, regardless of sampling date (date; p=0.13). For comparison, Edmeades and Daynard (1979) reported CV values of 20 to 30% for shoot dry matter measurements across multiple plant densities (50,000 to 200,000 plants ha^{-1}) measured at the time of tassel differentiation (presumably about the V6 stage; Abendroth et al., 2011).

2.4.3 Shoot Dry Matter & Leaf Collar Stage: 2018

In 2018, starter effects on shoot dry matter and leaf stage progression were evaluated across multiple sampling dates throughout the vegetative cycle and up to the early reproductive stages. Fertilizer treatments consisted of a control (no starter), an intermediate (ST) and high (STH) starter rate, but no popup treatment. Neither dry matter nor leaf collar stage data were collected at TPAC after the third sampling date due to an early June hailstorm that damaged the leaf canopy.

Shoot dry matter on a given sampling date was greater for the starter fertilizer treatments versus the control across all locations at least at some time during the growing season (Table 2-4). Greater dry matter in starter treatments versus the control were usually detected within the first 200 GDD, or approximately V2. Starter effects on shoot dry matter, compared to the control, were less pronounced at PPAC than at the other three locations. At PPAC, dry matter was enhanced 50% at 166 GDD but then decreased to 13 and 8% at 363 and 509 GDD, respectively. Regardless of fertilizer rate, starter had no effect on dry matter at sampling dates after 509 GDD at PPAC. At the SEPAC and NEPAC locations, shoot dry matter was greater with either starter treatment compared to the control throughout most of the vegetative cycle and through the early stages of reproduction. Compared to the control, the largest percentage increase in shoot dry matter from either starter treatment was observed at 319 (43%) and 419 (57%) GDD at SEPAC and NEPAC, respectively. However, the starter treatment effect on shoot dry matter gradually decreased to only a 10% increase from either starter treatments by 867 or 786 GDD at SEPAC and NEPAC, respectively. At the same locations, STH increased shoot dry matter more than ST at later sampling dates, but in either case both starter treatments produced similar dry matter once nearly 100% of plants were in reproductive growth stages. At TPAC, STH increased early season shoot dry matter 100, 67, and 82% at 174, 263, and 324 GDD, respectively, compared to the control.

Table 2-4. Effects of intermediate (ST) and high (STH) starter fertilizer rates applied 5 cm to one side and 5 cm below the seed on the average number of visible leaf collars, % of plants tasseling and/or silking, and shoot dry matter in maize across four locations in 2018 at multiple sampling dates and corresponding growing degree days (GDD). Plants were considered tasseling when the final tassel branch was fully exposed from the whorl and considered silking when fully visible silks (approx. >6 mm) were protruding from the ear shoot. Per observation date, shoot dry matter comparisons among the different fertilizer treatments were always measured at a single point in time.

			Visible leaf collars plant^{-1}			Shoot dry matter (g plant^{-1})		
Location	**Date**	**GDD**[†]	**Control**	**ST**	**STH**	**Control**	**ST**	**STH**
SEPAC	5/23	198	2.2	2.2	2.2	0.2 b	0.2 b	0.3 a
	6/1	319	4.7 b	5.0 a	5.0 a	1.5 b	2.1 a	2.2 a
	6/14	473	7.8 b	8.0 a	8.0 a	14.3 b	19.1 a	20.2 a
	6/29	679	12.6 b	13.5 a	13.6 a	61.5 c	68.9 b	75.8 a
			% plants tasseling and/or silking					
	7/5	774	0.4 b	6.3 a	7.1 a	88.7 c	99.3 b	106.2 a
	7/12	867	99.2	98.3	99.2	128.5 b	141.7 a	140.2 a
NEPAC	5/25	137	1.5	1.5	1.4	0.1	0.1	0.1
	6/6	279	4.0 b	4.1 a	4.1 a	2.5 b	3.7 a	3.9 a
	6/18	419	6.1 c	6.4 b	6.5 a	11.8 c	18.1 b	19.0 a
	6/28	529	8.8 c	9.5 b	9.6 a	31.2 c	42.9 b	49.7 a
	7/3	604	10.8 c	11.7 b	11.9 a	53.9 b	66.2 a	68.4 a
			% plants tasseling and/or silking					
	7/11	706	4.0 b	42.4 a	37.0 a	87.9 b	100.2 a	105.7 a
	7/17	786	91.0 b	99.0 a	100.0 a	114.6 b	125.2 a	129.9 a
PPAC	6/12	166	2.4 c	2.6 a	2.6 b	0.2 b	0.3 a	0.3 a
	6/28	363	6.0	6.0	6.0	9.2 b	10.1 a	10.6 a
	7/9	509	9.4 c	9.7 a	9.6 b	45.0 b	48.7 a	48.7 a
	7/13	558	10.8 b	11.0 a	10.9 a	59.3	63.4	62.7
	7/18	627	12.8 b	13.0 a	13.0 a	83.5	88.9	92.7
			% plants tasseling and/or silking					
	7/24	696	23.0 b	39.0 a	39.5 a	107.4	108.9	111.3
	8/1	777	100.0	100.0	98.0	138.3	142.8	146.0
TPAC	5/24	174	1.7 b	-	1.9 a	0.1 b	-	0.2 a
	5/30	263	3.0 b	-	3.2 a	0.6 b	-	1.0 a
	6/4	324	3.9 b	-	4.1 a	1.7 b	-	3.1 a

[†]Cumulative GDD (^{o}C) since planting, May 7 (NEPAC), 8 (SEPAC), 9 (TPAC), and 29 (PPAC).
ST N-P-K (kg ha^{-1}) rates were 28-6-0 (SEPAC and NEPAC) and 26-10-0 (PPAC).
STH N-P-K (kg ha^{-1}) rates were 56-12-0 (SEPAC), 56-6-0 (NEPAC), 52-20-0 (PPAC), and 47-18-0 (TPAC).
Treatment means by location, sampling date, and variable followed by a different letter are different ($p \leq 0.10$).

Across all locations, average leaf collar number for both ST and STH were generally greater than the control (Table 2-4). Depending on location, differences in average leaf collar number were usually detected at the first or second sampling date. As the season progressed, the most dramatic starter effects on average leaf collar number occurred at the SEPAC and NEPAC locations. For instance, average leaf collar number for plants treated with either starter rate was roughly one greater than for control plants at around 600 to 700 GDDs after planting. At the PPAC location, at any given sampling date plants treated with starter only ranged from 0 to 0.25 additional leaf collars than the control. Across all locations, STH generally had a similar average leaf collar number as ST, except at NEPAC, where STH averaged 0.1 to 0.2 additional leaf collars than ST at the three sampling dates just prior to tassel emergence.

Across all locations, the percentage of plants with fully developed tassels or visible silks on any given sampling date was always greater for either starter treatment versus the control (Table 2-4). These results agree with other field studies that suggested starter (Gordon et al., et al., 1997; Cromley et al., 2003; Cromley et al., 2006) or popup fertilizer (Mascagni and Boquet, 1996; Kaiser et al., 2016) reduced the number of days to silking, which in our study was due to accelerated leaf collar appearance.

Total number of leaves was determined at each location except TPAC (because of earlier hail damage). Both starter treatments increased total leaf number at NEPAC and SEPAC (Table 2-5), where both treatments also accelerated leaf collar appearance (Table 1-4). At NEPAC, starter treatments, compared to the control, reduced the number of plants with 17 leaves and increased the number of plants with 18 and 19 leaves. At SEPAC, starter treatments compared to the control had more plants with 19 leaves and fewer with 18 leaves. The potential number of leaves on a maize plant is determined by the number of leaf primordia that form prior to the initiation of the

tassel primordium (Poethig, 1994), which occurs at approximately growth stage V6 (Abendroth et al., 2011).

Table 2-5. Effects of intermediate (ST) and high (STH) starter fertilizer rates applied 5 cm to one side and 5 cm below the seed on total leaf number plant^{-1} around silking at three locations in 2018.

Location	Treatment	†Avg. total leaf number plant^{-1}	Plants with 17 total leaves (%)	Plants with 18 total leaves (%)	Plants with 19 total leaves (%)	Plants with 20 total leaves (%)
NEPAC	Control	17.6 c	40	58	0	1
	ST	18.1 b	9	72	19	0
	STH	18.3 a	5	60	35	0
PPAC	Control	18.6	2	33	63	1
	ST	18.7	0	29	71	0
	STH	18.7	1	24	75	0
SEPAC	Control	18.4 b	0	56	44	0
	ST	18.8 a	2	23	71	4
	STH	18.9 a	1	14	80	5

ST N-P-K (kg ha^{-1}) rates were 28-6-0 (SEPAC and NEPAC) and 26-10-0 (PPAC).
STH N-P-K (kg ha^{-1}) rates were 56-12-0 (SEPAC), 56-6-0 (NEPAC), 52-20-0 (PPAC), and 47-18-0 (TPAC).
†Treatment means within a location followed by a different letter are different ($p \leq 0.10$).

At all three locations, the relationship between shoot dry matter and average leaf collar number was not influenced by starter fertilizer treatments (Fig. 2-3), which was consistent with results from TPAC 2016. Therefore, enhanced dry matter at a given point in time from ST, STH or PU was a function of accelerated leaf collar development as opposed to physically more robust plants of the same leaf collar number. As a result, dry matter at maturity was similar among all treatments at each location (Fig. 2-4). Bullock et al. (1993) also did not find any difference in dry matter at maturity between starter (15 kg N and 23 kg P ha^{-1}) and no-starter treatments even though development had been accelerated by the starter treatments.

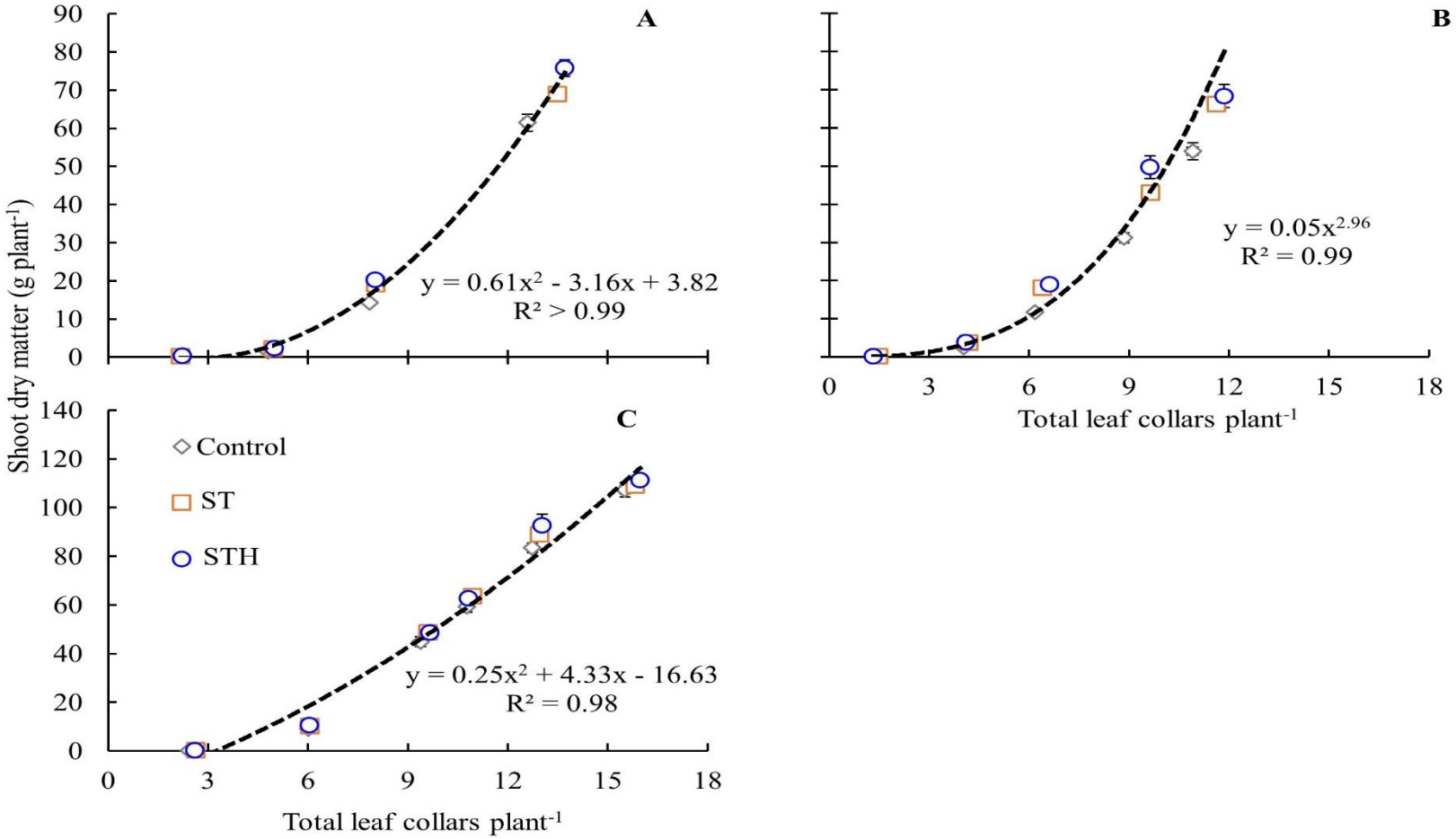

Figure 2-3. Effects of intermediate (ST) and high (STH) starter fertilizer rates applied 5 cm to one side and 5 cm below the seed on the relationship between shoot dry matter production and leaf collar development at SEPAC (A), NEPAC (B), and PPAC (C) in 2018. Starter N-P-K (kg ha^{-1}) rates were 28-6-0 (SEPAC and NEPAC) and 26-10-0 (PPAC). StarterHI N-P-K (kg ha^{-1}) rates were 56-12-0 (SEPAC), 56-6-0 (NEPAC), and 52-20-0 (PPAC). Data points are means of 4-6 replications of each treatment depending on the location. Horizontal and vertical error bars represent standard error of the mean. Dotted lines represent the power or polynomial equation averages across treatments for each location.

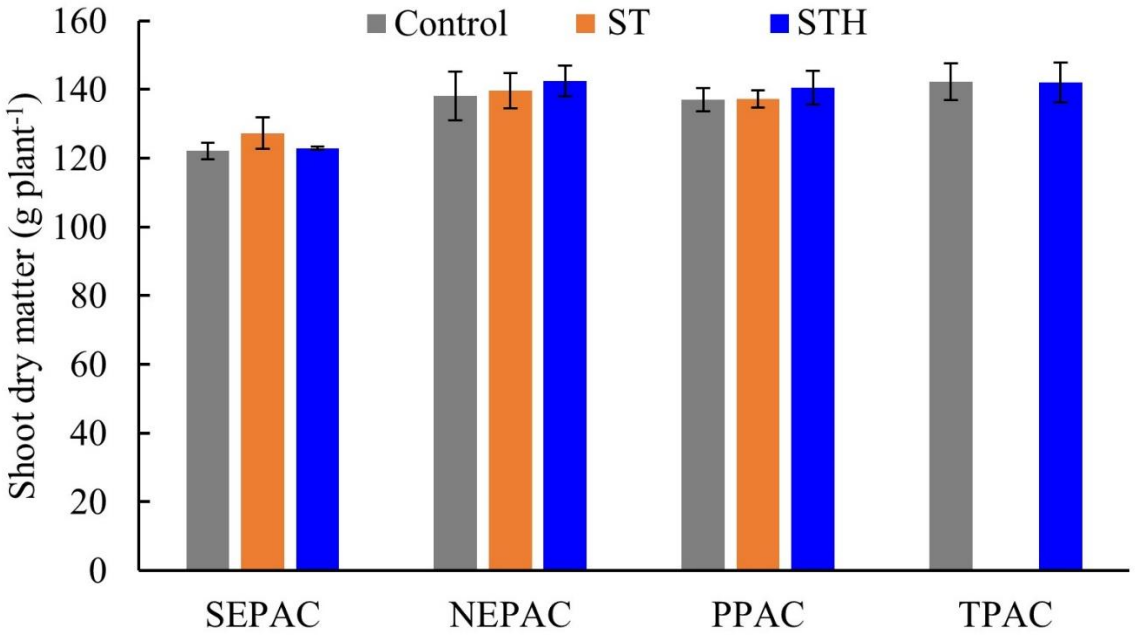

Figure 2-4. Effects of intermediate (ST) and high (STH) starter fertilizer rates applied 5 cm below and 5 cm to one side of the seed on total shoot dry matter (g plant^{-1}) at physiological maturity across four locations in 2018. Starter N-P-K (kg ha^{-1}) rates were 28-6-0 (SEPAC and NEPAC) and 26-10-0 (PPAC). StarterHI N-P-K (kg ha^{-1}) rates were 56-12-0 (SEPAC), 56-6-0 (NEPAC), 52-20-0 (PPAC), and 47-18-0 (TPAC). Error bars represent standard error of the mean. Starter fertilizer treatments had no effect on shoot dry matter (p>0.10).

2.4.4 Root Dry Matter & Shoot:Root Ratio 2016 (TPAC)

In 2016 at the TPAC location, dry matter sampling up to 199 GDD after planting, or two visible leaf collars, included the root system and were recorded on an individual plant basis to determine plant-to-plant variability. The average CV for root dry matter was 22% and was unaffected by fertilizer treatment or sampling date (treatment; date; treatment*date; p>0.10; data not shown). Average total root dry matter plant^{-1}, along with the ratio between shoot and root dry matter were not altered by ST or PU fertilizer at 120, 164, or 199 GDD after planting (treatment; treatment*date; p>0.10; data not shown). When averaged across treatments, the average total root dry matter plant^{-1} was 28, 53, and 105 mg at 120, 164, and 199 GDD after planting (date; p=<0.0001), respectively. When averaged across treatments, the ratio between shoot dry matter

and root dry matter was >1 at each sampling date (Fig. 2-5). Differences in dry matter between the shoot and root system increased at each successive sampling date (date; p=<0.0001). By 164 GDD after planting, shoot systems had twice the dry matter as roots. Root dry matter measurements were terminated after the 199 GDD sampling date because of the challenge of sampling entire root systems of larger plants.

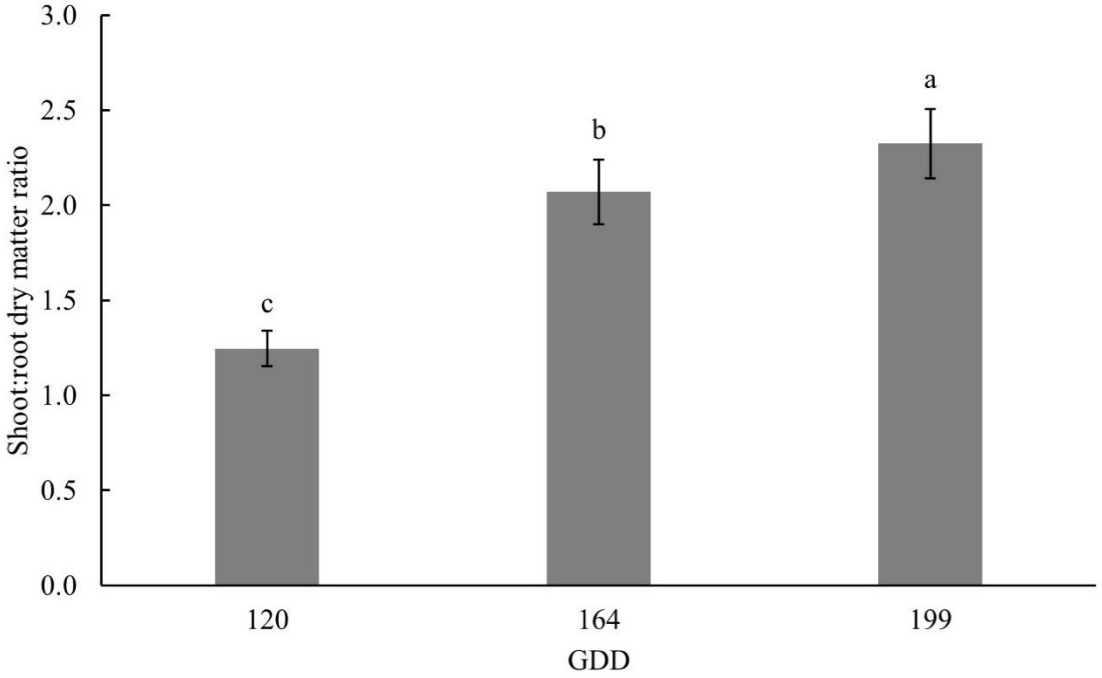

Figure 2-5. Shoot to root dry matter ratio in maize at 120, 164, and 199 GDD (°C) after planting, May 24, at TPAC in 2016. Data at each GDD are averaged across starter (ST) [(53 kg N and 21 kg P ha^{-1}) applied 5 cm to one side and 5 cm below the seed] and in-furrow popup (PU) (4 kg N and 6 kg P ha^{-1}) fertilizer treatments. Shoot to root ratio did not differ among ST, PU, or control treatments (p>0.10). Error bars represent standard error of the mean. Bars topped with different letters differ (p≤0.10).

The overall relationship between shoot and root dry matter was weak at 164 GDD and 120 GDD, irrespective of treatment. Individual plant measurements revealed a moderate, curvilinear relationship between total shoot and root dry matter at 199 GDD that varied slightly depending on fertilizer treatment (Fig. 2-6). In general, larger shoots had larger roots. For shoots weighing

between 100 and 300 mg, corresponding root systems weighed between 50 and 150 mg regardless of treatment. Plants in the ST treatment that contained shoot systems between 350 and 600 mg generally exhibited similar sized roots, whereas in the PU treatment, root size continued to increase as shoot system increased.

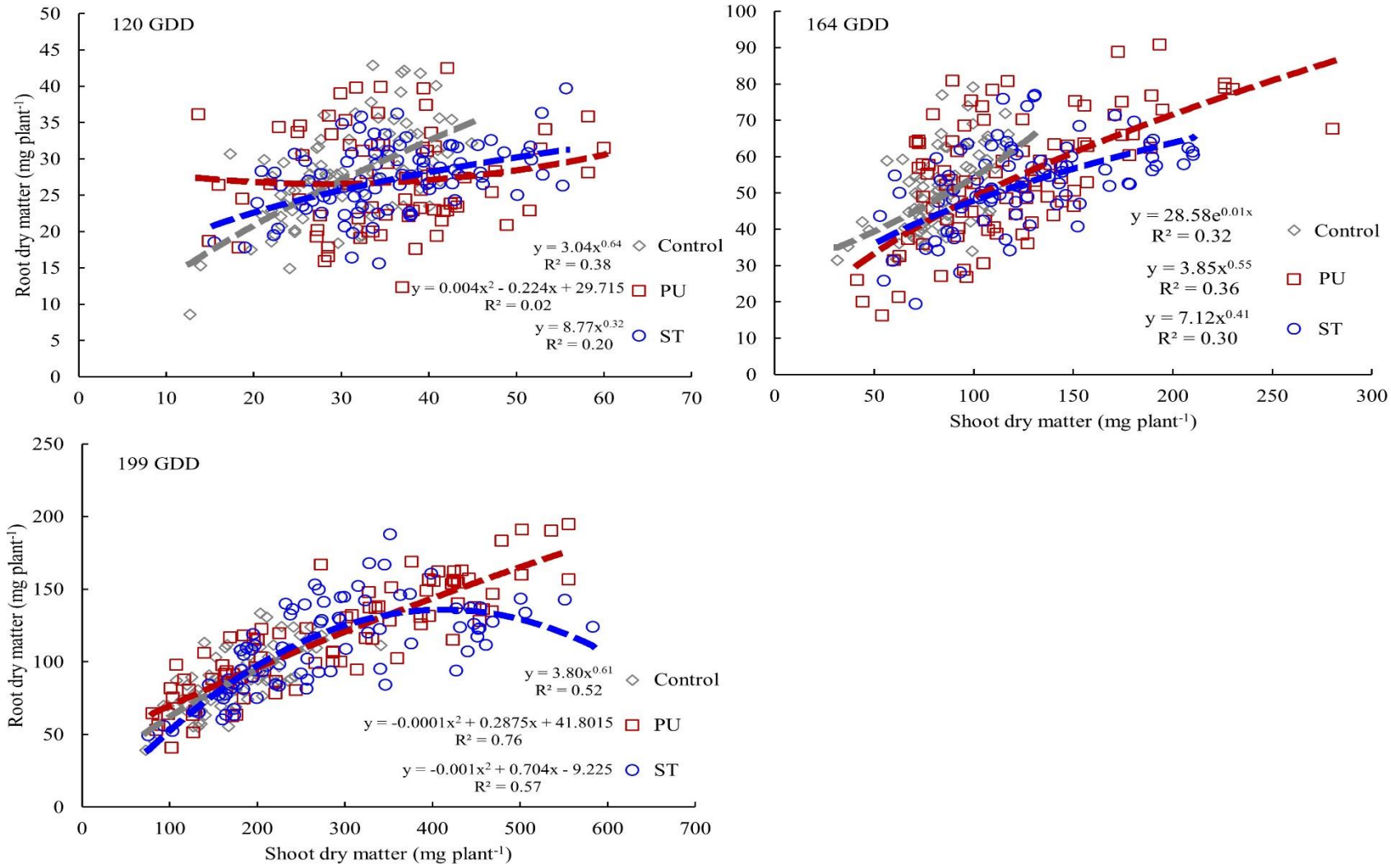

Figure 2-6. Effects of starter (ST) (53 kg N and 21 kg P ha^{-1}) applied 5 cm to one side and 5 cm below seed and in-furrow popup (PU) (4 kg N and 6 kg P ha^{-1}) fertilizer on the relationship between shoot and root dry matter (mg $plant^{-1}$) measured from individual plants at 120, 164, and 199 GDD (°C) after planting, May 24, at TPAC in 2016. At each date, dotted lines represent the regression equation for each treatment that best fit the data.

2.4.5 Nutrient Content: 2016 & 2018

In 2016, N, P and K content increased exponentially with leaf collar stage progression across all treatments (Fig. 2-7). At a given average leaf collar number, compared to the control, total N content was slightly higher in ST plants between V3 and V6, but total K content was lower. Ammonium in the UAN starter band may have hindered K^+ uptake. Although NH_4^+ concentration in the band was not directly measured in this study, results from previous research (Goos and Johnson, 1992) suggest residual NH_4^+ from UAN bands persists for several weeks after application before being completely nitrified. Previous studies across multiple plant species have demonstrated that K^+ uptake is reduced and sometimes inhibited when exposed to high concentrations of NH_4^+ (Rufty et al., 1982; Vale et al., 1987; Spalding et al., 1999; Martinéz-Cordero et al., 2005; ten Hoopen et al., 2010). Cations NH_4^+ and K^+ are nearly identical in size considering their ionic radius and hydration shell (Howitt and Udvardi, 2000). As a result, certain K^+ transporters and channels are capable of infiltrating both K^+ and NH_4^+ (Spalding et al., 1999; ten Hoopen et al., 2010), creating competition at the root surface for uptake, particularly when NH_4^+ is in excess. Enhanced N uptake accompanied by reduced K^+ uptake was not detected in the PU treatment, which provided only 4 kg N ha^{-1} compared to 53 kg N ha^{-1} with ST. Lower overall N levels in the PU treatment likely resulted in negligible competition between NH_4^+ and K^+. Compared to the control, ST and PU had little impact on total P content regardless of the average leaf collar number. Similarly, ST and PU had little effect on other cation contents, such as Ca^{2+} and Mg^{2+} (data not shown).

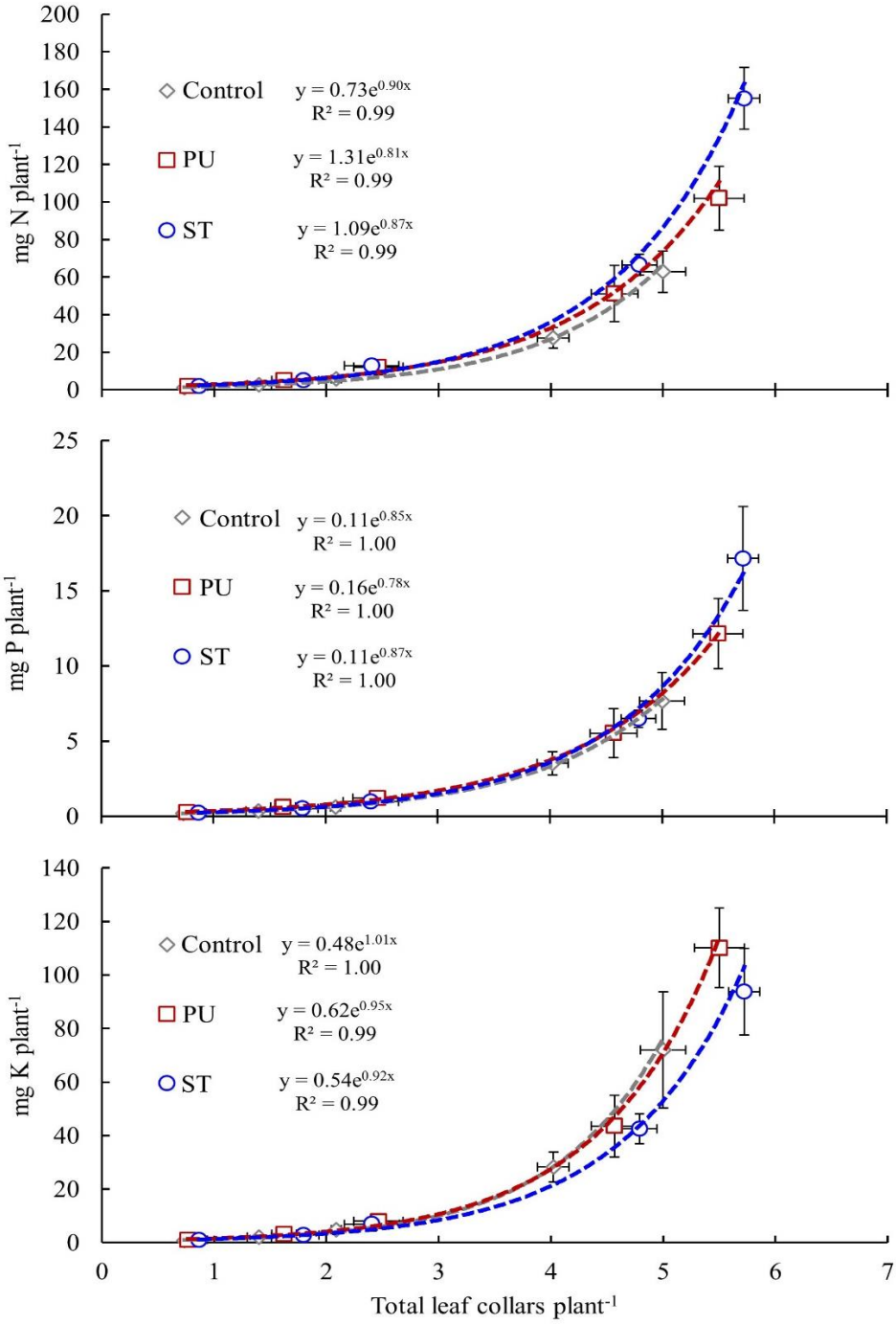

Figure 2-7. Effects of starter (ST) (53 kg N and 21 kg P ha^{-1}) applied 5 cm to one side and 5 cm below the seed and in-furrow popup (PU) (4 kg N and 6 kg P ha^{-1}) fertilizer on early season N, P, and K uptake as new leaf collars develop at TPAC in 2016. Data points are means of three replications of each treatment. Horizontal and vertical error bars represent standard error of the mean. Dotted lines represent the exponential equation for each treatment.

In 2018, plant nutrient content was measured several times throughout the vegetative growth period. As leaf collar numbers progressed, total K^+ content of either starter treatment was never lower than that of the control at all three locations, which differed from that found in 2016 study. At NEPAC (Fig. 2-8) and SEPAC (Fig. 2-9), as leaf collar numbers progressed, total N, P, or K^+ content in ST or STH treatments were sometimes greater than the control, but differences were generally small and not consistent across the two locations. At PPAC, there were no differences in N, P, or K^+ content across all average leaf collar numbers between the fertilizer treatments and control (data not shown).

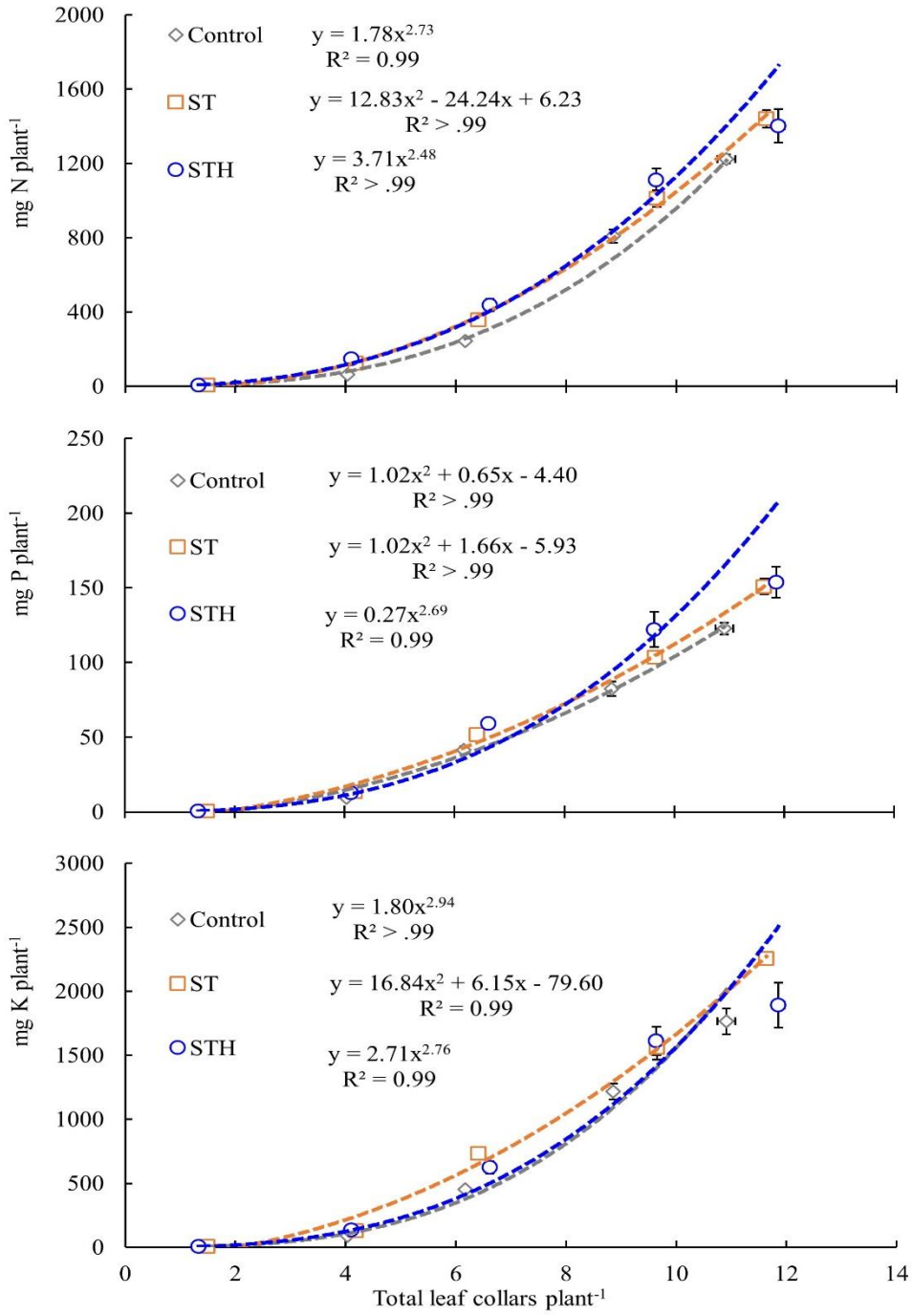

Figure 2-8. Effects of intermediate (ST) and high (STH) starter fertilizer rates applied 5 cm below and 5 cm to one side of the seed on N, P, and K uptake as new leaf collars develop at NEPAC in 2018. Fertilizer N-P-K (kg ha^{-1}) rates were 28-6-0 (ST) and 56-6-0 (STH). Data points are means of five replications of each treatment. Horizontal and vertical error bars represent standard error the mean. Dotted lines represent the polynomial or power equation for each treatment.

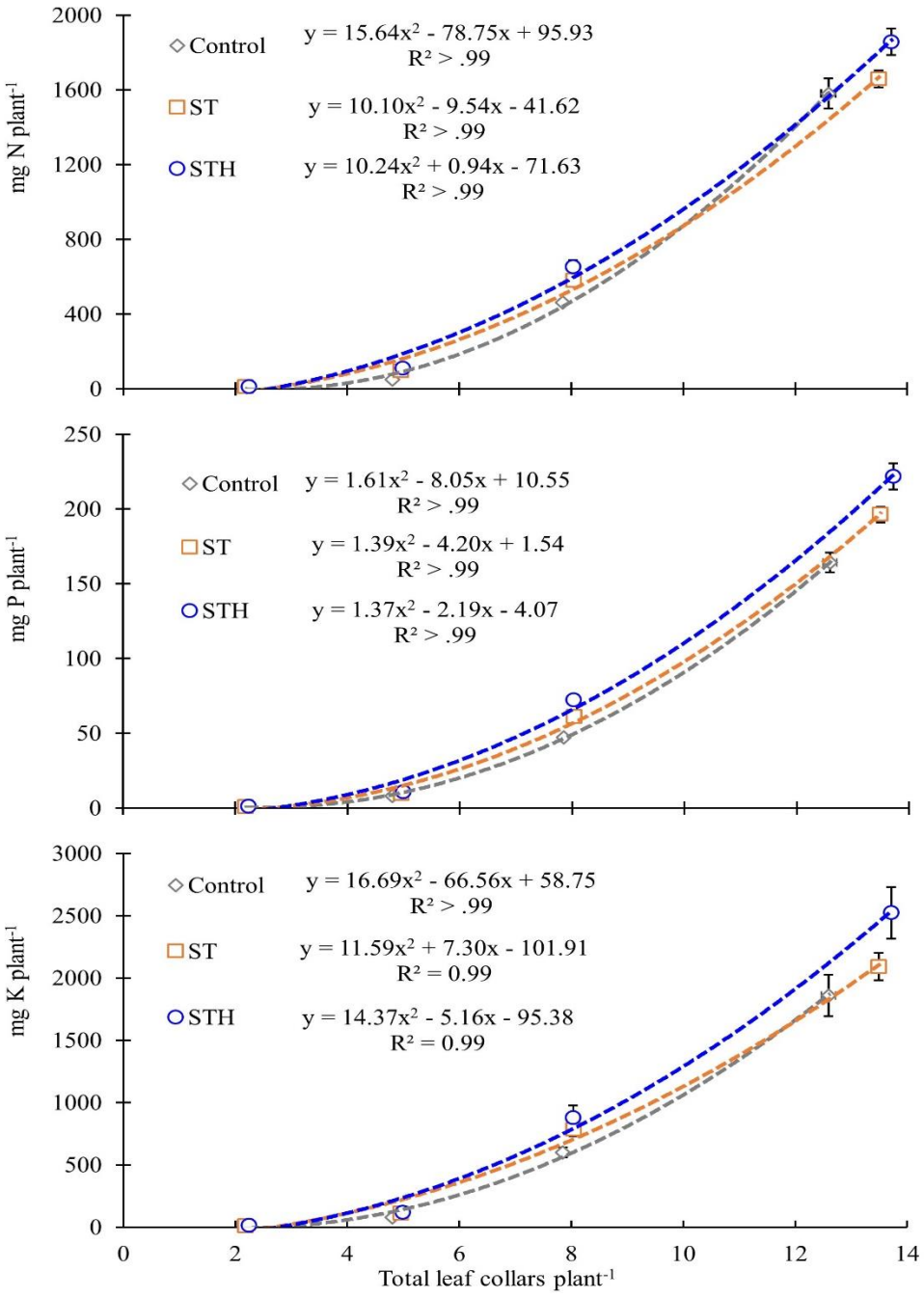

Figure 2-9. Effects of intermediate (ST) and high (STH) starter fertilizer rates applied 5 cm below and 5 cm to one side of the seed on N, P, and K uptake as new leaf collars develop at SEPAC in 2018. Fertilizer N-P-K (kg ha^{-1}) rates were 28-6-0 (ST) and 56-12-0 (STH). Data points are means of four replications of each treatment. Horizontal and vertical error bars represent standard error the mean. Dotted lines represent the polynomial equation for each treatment.

2.4.6 Grain Yield & Moisture: 2016 & 2018

Grain yield was unaffected by ST, STH, or PU treatments at all site-years (Table 2-6). Average grain yield across locations ranged from 10,844 and 12,952 kg ha^{-1}. Grain moisture, however, was reduced by ST, STH, or PU (0.1 to 0.9 g kg^{-1}), compared to the control, at 4 of 5 site-years (Table 2-7). Grain moisture was similar in PU and ST treatments at TPAC in 2016. Differences in grain moisture between ST and STH (0.1 g kg^{-1}) occurred only at NEPAC in 2018. Drier grain at harvest from ST fertilizer treatments was most likely a result of earlier grain maturation (not documented in these trials) resulting from accelerated vegetative development prior to pollination. Bullock et al. (1993) noted that plants treated with starter fertilizer reached physiological maturity two days before untreated plants, which may increase the time interval for grain drying between maturity and harvest. On average, grain drying can range from 0.8 to 0.4 percentage points day^{-1} depending on when the plant reaches maturity and subsequent environmental conditions prior to harvest (Nielsen, 2018).

Table 2-6. Effects of starter (ST or STH) applied 5 cm to one side and 5 cm below the seed and in-furrow popup (PU) (2016) fertilizer on grain yield in maize across five site-years in 2016 and 2018. Treatment means within a year and location were unaffected by treatment (p>0.10).

Year **Location**	***Treatment*** **Grain yield (kg ha^{-1})**		
2016†	*Control*	*PU*	*ST*
TPAC	10,917	10,817	10,798
2018‡	*Control*	*ST*	*STH*
SEPAC	12,768	12,617	12,749
NEPAC	12,874	13,025	12,956
PPAC	11,475	11,463	11,469
TPAC	12,699		12,573

†ST supplied 53 kg N and 21 kg P ha^{-1}, popup supplied 4 kg N and 6 kg P ha^{-1}.
‡STH N-P-K (kg ha^{-1}) rates were 28-6-0 (SEPAC and NEPAC) and 26-10-0 (PPAC). STH N-P-K (kg ha^{-1}) rates were 56-12-0 (SEPAC), 56-6-0 (NEPAC), 52-20-0 (PPAC), and 47-18-0 (TPAC)

Table 2-7. Effects of starter (ST or STH) applied 5 cm to one side and 5 cm below the seed and in-furrow popup (PU) (2016) fertilizer on grain moisture in maize across five site-years in 2016 and 2018.

Year **Location**		***Treatment*** **Moisture (g kg^{-1})**	
2016†	*Control*	*PU*	*ST*
TPAC	16.9 a	16.3 b	16.0 b
2018‡	*Control*	*ST*	*STH*
SEPAC	19.8 a	19.8 ab	19.5 b
NEPAC	18.8 a	18.3 b	18.2 c
PPAC	18.0	18.0	18.1
TPAC§	15.8 a	-	15.7 b

†ST supplied 53 kg N and 21 kg P ha^{-1}, popup supplied 4 kg N and 6 kg P ha^{-1}.
‡ST N-P-K (kg ha^{-1}) rates were 28-6-0 (SEPAC and NEPAC) and 26-10-0 (PPAC). STH N-P-K (kg ha^{-1}) rates were 56-12-0 (SEPAC), 56-6-0 (NEPAC), 52-20-0 (PPAC), and 47-18-0 (TPAC)
§ ST treatment not included in trial.
Treatment means by year and location followed by a different letter are different ($p \leq 0.10$)

2.5 Conclusion

Through examining the relationship between plant dry matter and leaf appearance rates, the goal of these trials was to gain a better understanding for why apparent early season growth enhancements from ST or PU do not always translate to increased yield. Like past studies, dry weight was generally enhanced by ST, STH, or PU compared to the control on a given day. But when compared at the same number of collared leaves, ST and PU always had similar dry matter when compared to the control. Overall, these results suggest that enhanced dry matter at a given point in time from ST, STH or PU was a function of accelerated leaf appearance as opposed to physically more robust plants of the same average leaf collar number. Consequently, whole plant dry matter at maturity was also similar among starter fertilizer and control treatments at each location.

One may argue that it is possible that the absence of dry matter differences in our study would explain why there was no increase in yield by starter fertilizer. It is important to acknowledge that since ST or PU never increased yield in this study, we are left with some uncertainty as to whether dry matter differences would have occurred if yields were significantly different. A similar follow-up study is necessary to determine if dry matter is still not increased by ST or PU even when grain yield is increased.

When compared to the control, as the average number of leaf collars progressed, it appeared that ST hindered early season K^+ uptake in the 2016 study, which we speculate was from an influx of NH_4^+ from the fertilizer band. However, when observed in the 2018 studies for a longer period up until reproduction, there was no indication that ST reduced K^+ uptake compared to the control. Furthermore, from our results ST did not appear to have a drastic or at least consistent enhancement of N, P, or K^+ uptake with leaf collar progression.

Because of accelerated leaf appearance, plants entered reproductive growth earlier with ST or STH than control plants, despite producing more total leaves. Consequently, drier grain at harvest from starter fertilizer treatments was most likely a result of earlier grain maturation (not documented in these trials) that resulted in a slightly longer drying period between physiological maturity and harvest. According to our results, early season dry matter differences between ST, PU, and control plots on a given day share no relationship with yield because ST and PU only hastened leaf appearance rates but did not actually enhance dry matter. Even though these results cannot explain why starter fertilizer applications sometimes increase yield, as shown in other studies, it does offer new direction for future studies. Rather than focusing on dry matter measurements made on a single day, future studies should focus on how accelerated leaf appearance rates affect yield in relation to starter fertilizer.

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CHAPTER 3. VISUAL ESTIMATION OF ROOTING PROFILES AND DENSITY IN THE TOP 30 CM OF SOIL USING PERFORATED CYLINDERS AND A LONG BORESCOPE EQUIPPED WITH A VIDEO RECORDING DEVICE

3.1 Abstract

Methods used to study roots in crop fields have included extracting soil cores, excavating entire root systems, using radioactive and non-radioactive chemical tracers, or using mini-rhizotrons. However, due to the intensive nature, high level of difficulty, and cost associated with these methods, their use in crop fields has been minimal. We developed an alternative method to quantify maize (*Zea mays* L.) rooting density through time. The method involved perforated cylinders installed vertically into the soil at different distances from the row, which made roots growing into the cylinder voids visible from the soil surface and possible to count at different depths. The objective of this study was to determine if the cylinder method could quantify rooting density throughout the growing season (V3, ~V7, and R2-R3) similar to the more intensive soil core method in two starter fertilizer trials [continuous maize (M/M) and maize/soybean (M/S) rotation]. Treatments included starter fertilizer (50 kg N ha^{-1} in a 5x5 cm band near the seed) and a control without starter fertilizer arranged in a randomized complete block design with 5 (M/S) or 3 (M/M) replications. Sampling occurred along either side of the maize row (disturbed and non-disturbed) within each plot. The disturbed row-side contained the coulter for starter fertilizer (coulter was left in place even in plots without starter fertilizer) and tire tracks from the planter, whereas the non-disturbed row-side had neither. Depth (0-30 cm in 5 cm increments) was also included as a treatment effect for V3 and R2-R3 measurements, and distance from the row (13 and 25 cm) was included for ~V7 measurements. Cylinders were constructed with perforated (49% voids – individual openings 0.39 x 0.42 cm) polypropylene resin to an inside diameter of 2.58 cm

and a length of 30 cm. Cylinders were painted with red and green alternating markings (5 cm) on the outside and inside walls to visually aid in identifying depth from the soil surface. After plants emerged, cylinders were inserted vertically into the soil after drilling a 3.5 cm diameter borehole. Ten perforated cylinders were installed in a parallel line 13 or 25 cm away from, and on both sides of the planted row. The total number of roots (RND) penetrating the cylinder voids at each depth marking were enumerated with the aid of a video recording device (1m-long borescope). Soil cores were also extracted at the same relative locations for conducting root extraction and subsequent calculation of root length density (RLD). Overall, when multiple depths were measured, rooting profiles at 13 or 25 cm from the row were more similar between root quantification methods at V3 than at R2-R3. The cylinder method suggested an overall deeper rooting profile than the soil core method. At V3, methods frequently resulted in the same significant ($p \leq 0.10$) or insignificant ($p > 0.10$) main and interaction effects in both fields, whereas at ~V7 and R2-R3, there were several instances where the cylinder method failed to detect the same effects as the soil core method. The cylinder method also at times detected a significant main or interaction effect as the soil core method, but the effects were opposite. For example, ~V7 (M/S), despite both methods detecting a significant distance main effect, soil cores indicated a higher rooting density at 13 than 25 cm from the row, whereas cylinders measured fewer roots at 13 than 25 cm from the row. Overall, methods were more comparable at V3 than ~V7 or R2-R3 for detecting similar treatment effects. However, since the cylinder method at times reported an opposite trend which resulted in an erroneous conclusion, further improvements to the method are necessary.

3.2 Introduction

Crop root systems in the field have been studied with several methodologies, including extraction of soil cores, excavation of entire root systems, use of radioactive and non-radioactive

chemical tracers, and the use of mini-rhizotrons. However, the intensive nature, high level of difficulty, and cost of these methods has limited their use in field experiments. Alternative methods that are easier and less expensive are needed to improve our understanding of rooting profiles in cropping systems.

The soil core method has been used in past field experiments to quantify the density of crop roots throughout the soil, as affected by various treatments (Durieux et al., 1994; Marsh and Pierzynski, 1998; Chassot et al., 2001; Qin et al., 2005; Farmaha et al., 2012). In general, hand probes or augers are used to collect intact cores from the soil, which are then later processed to separate roots from soil and non-root organic matter. Subdividing an intact core into smaller segments allows the estimation of various rooting parameters at multiple depths. Extracted soil cores typically collect volumes of soil that range in diameter from 2-5 cm and up to 10-60 cm deep. Larger square tubes (18x18x110 cm) have also been used to collect greater volumes of soil (Belford et al., 1987). The soil core method is the most common procedure used for estimating root growth due to its simplicity and perceived level of accuracy. However, the labor intensity and cost, time requirement, and level of difficulty and subjectivity when separating current-season roots from organic matter debris and soil have limited the use of this method in field crop research.

Excavation of entire root systems has been used in a few field studies (Eghball and Maranville, 1993; Pierson, 2013), but this approach is typically more suitable for plants grown in pots or boxes (Isensee et al., 1966; Passioura and Wetselaar, 1972; Drew, 1975; Zhang and Barber, 1992) where smaller volumes of soil and poorer soil structure allow easier separation of roots from soil. By using hand shovels or small trowels, excavating entire root systems in a field is effective for documenting total dry matter. However, knowing the physical location of the roots in the soil profile is immediately compromised once extracted. Preserving the entire root system in the field

without avoiding significant root loss during excavation is also difficult, especially at later growth stages. Therefore, in field studies this method is primarily restricted to young plants when root systems are small or if collecting the entire intact root system is not important.

In the late 1930s, mini-rhizotrons were introduced as an alternative root study method (Bates, 1937). Early applications consisted of inserting transparent glass tubes in the soil, allowing researchers to use angled mirrors to examine roots that contacted the glass wall. Technological advancements have enabled researchers to capture images of root growth intersecting the glass wall using small video recording devices (Upchurch and Ritchie, 1984). Higher quality cameras, in conjunction with root image analysis software, have allowed mini-rhizotrons to be used to quantify root surface area, length, diameter, and the number of root tips (Li et al., 2011). The greatest advantage of mini-rhizotrons is that they can measure root density routinely over the course of an entire growing season with minimal effort after installation (Wiesler and Walter, 1994; Liedgens et al., 2000; Nickel et al., 1995). Researchers have also used mini-rhizotrons made from cellulose acetate butyrate tubes instead of glass to reduce cost (Nickel et al., 1995).

The disruption of the natural soil environment during installation and unnatural root growth around the glass tubes has raised questions about the overall accuracy of mini-rhizotrons (Taylor et al., 1990). Several studies have found that mini-rhizotrons underestimated root density near the soil surface when compared to traditional destructive methods (Heeraman and Juma, 1993; Samson and Sinclair, 1994) and have underestimated or overestimated root density depending on plant stage (Liao et al., 2015). At reproductive stages in maize, mini-rhizotrons underestimated root density considerably in the upper 0-30 cm and overestimated root density in the deepest soil depth examined (30-90 cm) (Wiesler and Walter, 1994). In order to convert the number of roots meeting the mini-rhizotron wall to root length density (RLD), Upchurch and Ritchie (1983) used

the following formula: (root length cm) / (tube volume cm^3) in grain sorghum (*Sorghum bicolor* L.) plots where root length was the number of roots meeting the glass wall at specific depths multiplied by tube diameter. The formula correlated well with root density measured from soil cores early (May 21-28) in the season (r = 0.85 to 0.97) but was poorly correlated later (July 10-29) in the season (r = 0.30 to 0.26). Exclusion of measurements made near the soil surface (0-20 cm) improved the correlation later in the growing season (r = 0.46 to 0.66).

In a preliminary field experiment, we developed an alternative to the mini-rhizotron or soil core method for measuring the effect of starter fertilizer on root development in maize. The method consisted of installing perforated cylinders at various distances from the row that allowed roots to grow through the cylinder voids at their normal trajectory. The roots were then visible from the soil surface by looking down into the cylinders. By monitoring the cylinders every other day, we were able to monitor root development at 13 and 25 cm from the row over time based on the change in percentage of cylinders with new visible roots.

Researchers in Florida recently developed a similar method as our perforated cylinder method to study root-rhizome biomass accumulation in perennial forage fields over time (Cooley et al., 2019). Their method consisted of inserting 7.5 cm diameter wire cages wrapped in 4 mm mesh screen down to 28 cm deep in plots of different rhizoma peanut (*Arachis glabrata* Benth.) cultivars. The cylinders were filled with soil and buried just beneath the surface for ~100 days to allow roots to penetrate the mesh screen and accumulate inside the wire cages. Afterwards, roots inside the cages were removed, cleaned of soil, and then oven dried and weighed in order to estimate total root biomass. Overall, the method provided a successful means for measuring root biomass accumulation over time and was capable of discerning differences between cultivars. This

method appeared to be a viable option to measure rooting density over time but not rooting patterns or distribution throughout the upper soil surface.

The new method developed by our lab was an effective way to quantify the rate of root movement over time during a short period with minimal effort and low cost. In retrospect, counting the number of roots at different depths on each day would have provided a means for estimating root density and distribution throughout the top 30 cm of soil. Currently, in order to measure root density and distribution over time, researchers are primarily limited to collecting soil cores on multiple dates or monitoring them with mini-rhizotrons. The perforated cylinder method developed by our lab could be a viable alternative that is less labor intensive than collecting soil cores and much cheaper than mini-rhizotrons. Moving forward, our objective was to determine if the perforated cylinder method could be used to accurately measure rooting density compared to the traditional soil core and extraction method.

3.3 Materials & Methods

3.3.1 Field Description & Trial Design

In 2017, two field experiments were established near West Lafayette, IN (40.485288 lat., -87.005739 long.). The two trials were approximately 100 m apart. One field was continuous maize (M/M) for 11 years and the other was a maize/soybean [*Glycine max* (L.) Merr.] rotation (M/S) for several decades. Both fields were chisel plowed in the fall and prepared for planting in the spring using a field cultivator equipped with a rolling harrow. The predominant soil types and characteristics for each field are described in Table 3-1.

Table 3-1. Predominant soil types and properties of the two experimental fields, determined by Bray for available P, ammonium acetate extraction for K, and loss-on-ignition for organic matter (O.M.).

Field	Series	Texture	Classification	Bray-P1 (mg kg^{-1})	K (mg kg^{-1})	O.M. (g kg^{-1})	pH
M/S	Chalmers-87%†	Silty clay loam	fine-silty, mixed, superactive, mesic Typic Endoaquolls	29	122	39	6.3
M/M	Raub-Brenton-54%	Silt loam	fine-silty, mixed, superactive, mesic Aquic Argiudolls	19	159	35	6.2
	Chalmers-46%	Silty clay loam	fine-silty, mixed, superactive, mesic Typic Endoaquolls				

† Percentage of field area mapped to this soil series.

Average monthly temperatures from April to October were near the 30-year average (Fig. 3-1), except for May and August, which were slightly cooler than normal. Total precipitation from April to August was above the 30-year average, especially in May and July, but then precipitation was below average in September and October (Fig. 3-1).

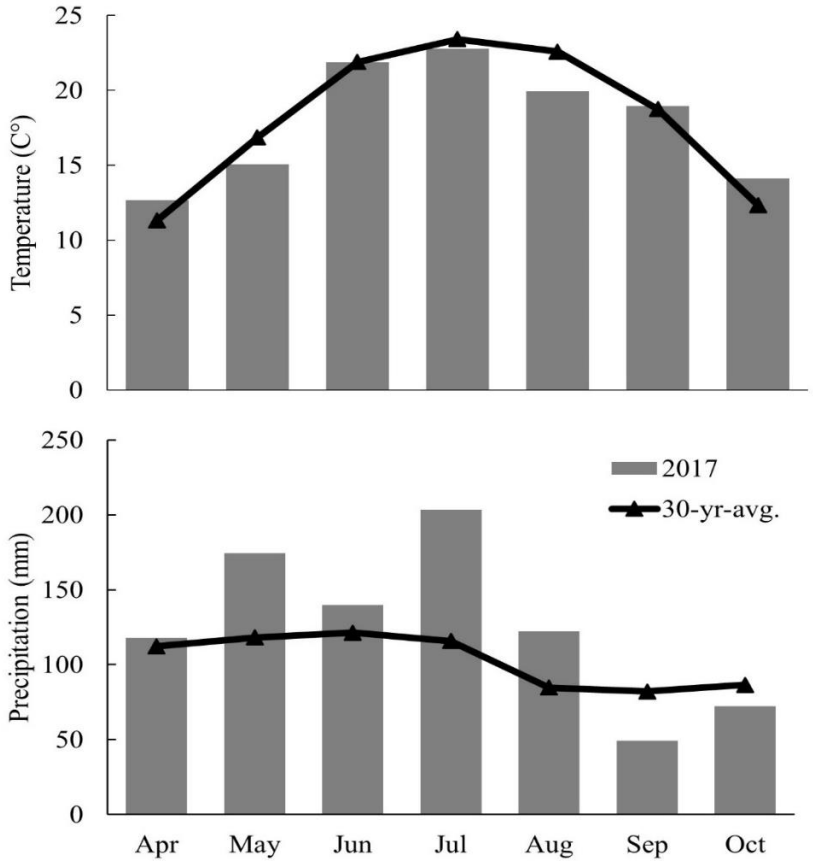

Figure 3-1. Average daily temperature and total precipitation near West Lafayette, IN. Thirty-year average (1988-2017) represents the #4-climate division for Indiana. All data were acquired through NWS Cooperative Observer Program (US-COOP) obtained from the Midwestern Regional Climate Center, cli-MATE (MRCC Application Tools Environment).

Both fields were planted 18 May with the same maize hybrid (RM 108) at 73,100 seeds ha^{-1} using a 6-row commercial planter. Each row unit on the planter was equipped with separate

coulters capable of positioning liquid starter fertilizer 5 cm below and 5 cm to one side of the seed. Two treatments, starter fertilizer (SF) and no-starter fertilizer (control), were arranged in a randomized complete block design with five (M/S) or three (M/M) replications. Starter fertilizer was urea-ammonium-nitrate (UAN; 28-0-0) at a rate of 50 kg N ha^{-1}. Even though no fertilizer was applied in the control treatment, coulters were left in place to disturb the soil similarly in both treatments.

On 20 June, both fields were sidedressed at approximately the V6 stage of development (Abendroth et al., 2011) by dribbling 28% UAN directly on the soil surface with a high clearance sprayer equipped with drop nozzles. Nitrogen was surface dribbled rather than coulter injected to avoid hitting the cylinders. Control treatments received an additional 50 kg N ha^{-1} at sidedress in order to equalize total N across all treatments. Total N applied including starter was 196 kg ha^{-1} (M/S) and 241 kg ha^{-1} (M/M). To ensure that only maize roots were growing in the field, two post-emergence herbicide applications were administered on 24 May and 20 June achieving complete weed control.

3.3.2 Field Measurements

Root distribution was measured with both soil core and cylinder methods three times during the growing season. Estimates of root development were made at vegetative stages V3 and ~V7, and at reproductive stage R2-R3 (Abendroth et al., 2011).

For the cylinder method, we utilized commercially available perforated cylinders (49% voids – individual openings 0.39 x 0.42 cm) constructed with polypropylene resin to an inside diameter of 2.58 cm and a length of 30 cm (Industrial Netting, Minneapolis, MN). Before field installation, cylinders were painted with red and green alternating markings (5 cm) on the outside

and inside walls (Fig. 3-2). The markings were used to visually determine depth from the soil surface in 5-cm increments.

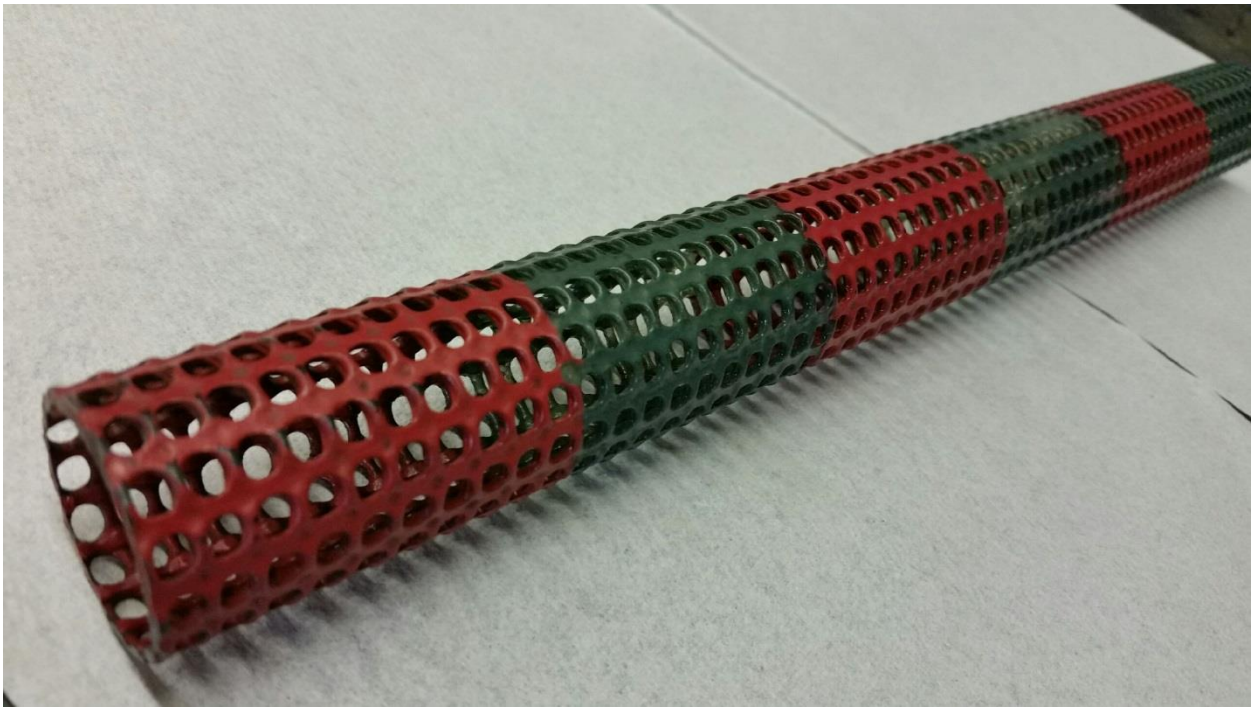

Figure 3-2. Perforated polypropylene resin cylinders (2.58 cm diameter by 30 cm long with 49% voids, Industrial Netting, Minneapolis, MN). Alternating painted markings in 5-cm long increments.

Shortly after plants emerged, cylinders were inserted vertically into the soil after drilling a borehole with an auger wood drilling bit (diam. 3.5 cm). Ten perforated cylinders were installed in a parallel line 13 or 25 cm away from, and on both sides of, the planted row (Fig. 3-3). Each group of 10 cylinders was spaced directly across from every other plant, approximately 36 cm apart. In order to prevent rain and light from entering, cylinders were capped with florescent orange disks measuring 108 mm in diameter (White Flyer Div., Reagent Chemical & Research Inc., Ringoes, NJ). Cylinders were installed on both sides of the row due to the anticipated difference in root growth where only one side contained a starter fertilizer band (SFB) and wheel traffic from

the planter and the other side did not contain wheel traffic or SFB. The intent was to determine if the cylinder method was sensitive enough to detect anticipated differences in root growth as affected by wheel traffic and SFB compared to neither factor. Row-side positions are illustrated in Fig. 3-4, in which positions #1 and #2 located on the disturbed [with or without starter fertilizer band (SFB) depending on treatment and planter tire tracks] row-side and positions #3 and #4 located on the non-disturbed row-side.

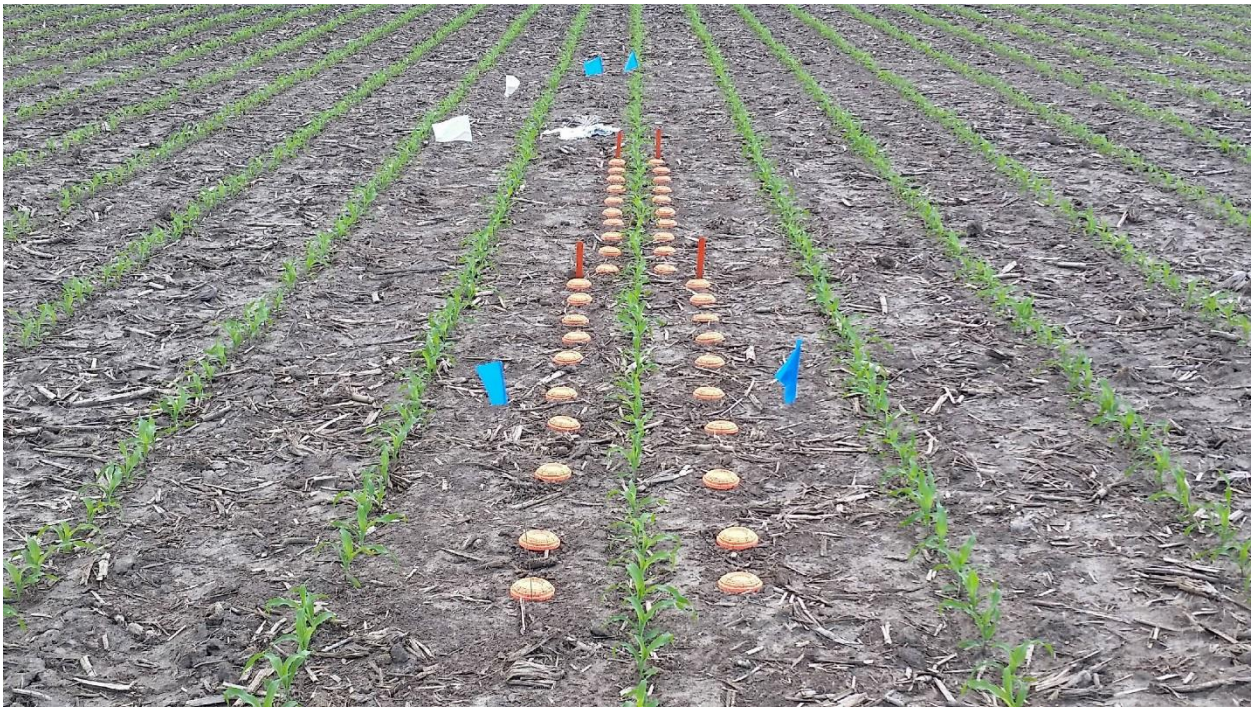

Figure 3-3. Plot location of perforated cylinders at 13 and 25 cm on both sides of the row capped with 108 mm fluorescent orange disks.

Cylinders were first inspected at V3 using a 1m-long industrial borescope equipped with a 640x480 pixel video camera probe with an adjustable LED light source at the tip (NTS200 Industrial Endoscope, Teslong, Shenzhen, China). A video was recorded while slowly lowering and raising the borescope inside each cylinder. All the videos were stored on a 64 GB microSDXC card (SanDisk Corp., Milpitas, CA). On the same day, ten 30-cm long intact soil cores were

extracted from the same row-sides and distances from the row as the cylinders. A soil recovery probe (AMS, Inc., American Falls, ID) was used to keep each individual core intact inside of a plastic-tubular liner (diam. 2.54 cm).

At ~V7, cylinders were inspected again using the video borescope and soil cores were extracted from the same row-side positions (Fig. 3-4) as the cylinders. Instead of using the soil recovery probe, due to the limited availability of plastic liners from the first sampling date, 30-cm long soil cores were extracted with a normal hand soil probe (diam. 1.905 cm) and then stored in individual plastic zip lock bags. Cylinders were inspected for the final time around R2-R3. Again, due to the limited availability of plastic liners for the soil recovery probe, only five intact soil cores were extracted from the first and fourth sampling positions (Fig. 3-4). Consequently, only the same number of cylinders were inspected. Soil cores from all three observation dates were immediately stored at -20°C following field sampling until further processing.

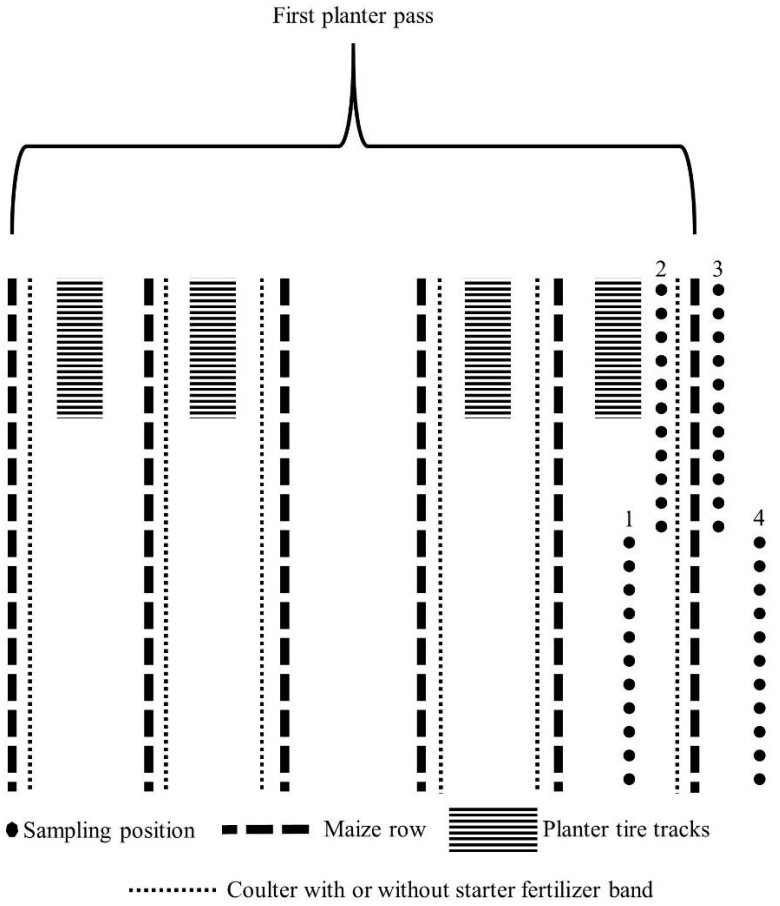

Figure 3-4. One of two planter passes for each 12-row plot, along with cylinder and soil sampling positions. Coulter row-side applied with 50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed (starter treatment) or without (control). Sampling position 1) 25 cm from row on starter fertilizer row-side 2) 13 cm from row on starter fertilizer row-side 3) 13 cm from row not on starter fertilizer row-side 4) 25 cm from row not on starter fertilizer row-side.

3.3.3 Soil Core Processing

After thawing to room temperature, intact soil cores were subdivided into six 5-cm long segments that represented different soil depths (0-5, 5-10, 10-15, 15-20, 20-25, and 25-30 cm), which were the same as the painted markings on the inside of the cylinder walls. Each individual segment was then soaked in warm soapy water to initiate soil dispersion before being poured over a mesh screen (1.5 x 1.5 mm openings). Soil cores collected with the normal hand probe could not be subdivided; therefore, the entire 30 cm sample was soaked in warm soapy water before being poured over the mesh screen. While continuously flushing with water, the mesh screen was carefully examined for any roots until all the soil had been flushed through the screen. A bucket placed beneath the screen that captured the water and soil was then poured back over the screen for a second observation of any missed roots. All the root pieces collected from the first and second observations were stored in a plastic vial with clean water at -20°C until further analyzing.

3.3.4 Root Scanning & Video Imaging

Individual vials were thawed completely to room temperature prior to scanning. Root pieces from each vial were poured into transparent trays and then filled with water. Root-containing trays were scanned with an EPSON Perfection V800 flatbed scanner (Seiko EPSON Corporation, Shinjuku, Tokyo, Japan) and total root length determined from the scanned images using WinRHIZO image analysis software (Regent Instruments Inc., Quebec City, Canada). The total root length (cm) for each sample was then divided by the total volume of soil for each subdivided segment for soil cores that were collected intact in order to calculate root length density (RLD). For samples collected with the normal hand probe, total root length for each sample was divided by the entire core sample volume.

Video images from inspection of root cylinders were uploaded to a computer and then viewed with Windows Media Player (Microsoft Corp., Redmond, WA). Video settings such as brightness and play speed were adjusted to accentuate the ability to detect roots (Fig. 3-5). In each cylinder, the total number of roots penetrating the cylinder void were counted separately for each depth based on the color-coding of the sidewall. Root measurements for the cylinder method were therefore defined as the total number of roots per 5 cm of cylinder (root number density - RND). We hypothesized that RND and RLD would be highly correlated, and thus, would detect similar root growth affects.

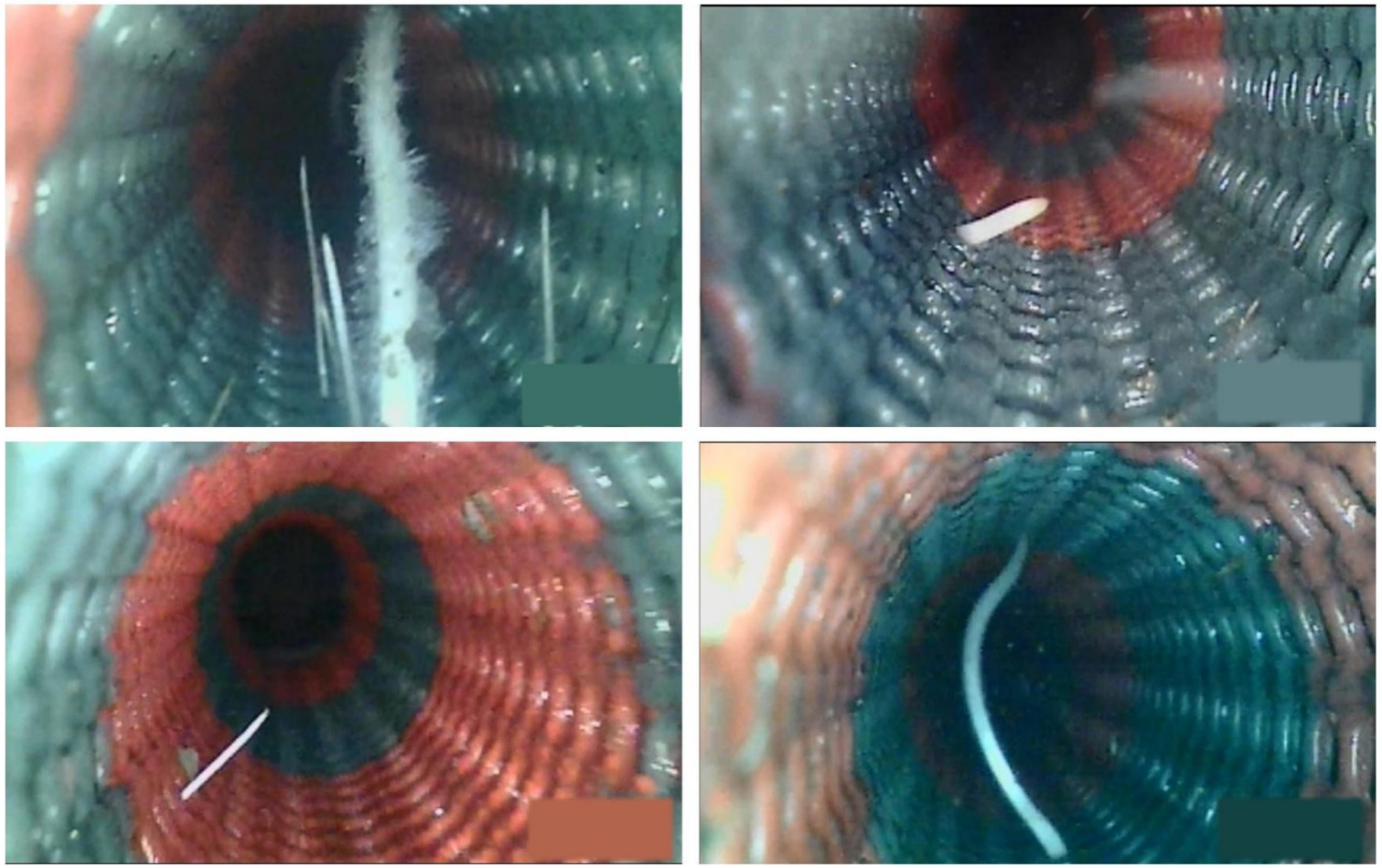

Figure 3-5. Borescope images showing maize roots penetrating the cylinder voids at growth stage V3 at different depths as indicted by the different color markings.

3.3.5 Statistical Procedures

Root length density (soil core method) and RND (cylinder method) were analyzed individually by field and sampling date. All measurements were analyzed using a generalized linear mixed model (GLMM) in which fertilizer treatment, row-side, distance from the row, and depths were considered fixed effects and replication was considered a random effect. Where root growth measured from either the soil core or root cylinder method was insufficient, particularly at the first sampling date in the top and bottom depths, those depths were omitted from the analysis. The GLMM for each variable was executed using the MIXED procedure in SAS version 9.4 (SAS Institute Inc., Cary, North Carolina). Fixed effects were specified in the MODEL statement, whereas random effects were specified in the RANDOM statement. Prior to the analysis, an extension of Levene's test was performed on the absolute value of the residuals for each variable to test for homogeneity of variance among the different main effects and interactions of main effects. When appropriate, a REPEATED statement and GROUP option was used to allow for unequal variance. If a main effect was significant ($p \leq 0.10$), least square means were computed using the LSMEANS statement. If an interaction of main effects was significant ($p \leq 0.10$), least square means were computed using the LSMEANS statement and SLICE option.

The cylinder and soil core methods were evaluated for their relative ability to detect differences in root growth due to fertilizer treatment, row-side, and/or depth. The soil core method is generally considered to provide the most accurate assessment of root growth and is commonly used in root studies. For soil core and cylinder measurements at V3, root growth effects from fertilizer treatment, row-side, and depth were analyzed by distance from the row due to unequal variance in root measurements between 13 and 25 cm from the row. This resulted in 14 possible outcomes of different fertilizer treatment, row-side, and depth main and interaction effects to

compare between methods. Meanwhile, measurements at R2-R3 only included seven possible outcomes including main and interaction effects between fertilizer treatment, row-side, and depth as RND was only determined in cylinders 25-cm from the row. Measurements at ~V7 were different than those at V3 and R2-R3, in that root measures ~V7 were not determined in the entire 0-30 cm depth, not in 5-cm increments. Therefore, without depth this resulted in seven possible outcomes of main and interaction effects between fertilizer treatment, row-side, and distance from the row comparisons between methods.

3.4 Results

At V3, 23 and 40% of cylinders in the M/S and M/M field, respectively, were void of any roots. In the remaining cylinders, few roots (avg. 2-3 through the entire 30-cm depth) penetrated each cylinder, and thus, were easy to count. At ~V7, every cylinder in both fields contained an average of 17 total roots throughout the 30-cm depth, which although was not excessive, made counting difficult and somewhat subjective as many exhibited a proliferation of lateral branching, grew in a spiral pattern around the inside of the cylinder walls, or grew straight down. Consequently, it was difficult to determine if a root was branched from an existing root within the cylinder or penetrated the cylinder from the soil. Approximately 20 and 15% of cylinders at ~V7 in the M/S and M/M field, respectively, contained a level of lateral root proliferation that made counting difficult. Even in cylinders without lateral root proliferation, counting was often difficult due to roots obstructing the camera lens. At R2-R3, total roots per cylinder was less [avg. 15 (M/M) or 11 (M/S)] than ~V7, but were again difficult to count for the same reasons at ~V7 and additionally because roots were brown and often covered in soil that had seeped through the cylinder walls. Furthermore, organic debris that had fallen into the cylinders was also difficult to distinguish from roots.

3.4.1 Method Comparisons at V3

Overall, at V3, the two root quantification methods frequently resulted in the same significant ($p \leq 0.10$) or insignificant ($p > 0.10$) main and interaction effects in both fields (Table 3-2). Methods agreed in 9 of 14 comparisons in M/S and 14 of 14 in M/M with the most agreements where there was no treatment effect. It is important to note that in the M/S field there were also two instances where both methods detected the same significant main and interaction effect but resulted in opposite conclusions.

Table 3-2. Total occurrences where the soil core [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 5 cm of cylinder] methods measured identical significant ($\alpha = 0.10$) main and interaction rooting effects at V3 due to fertilizer treatment (starter† and no-starter fertilizer), row-side‡ (disturbed and non-disturbed), and depth§ measured at 13 and 25 cm from the row in the maize after soybean (M/S) and continuous maize (M/M) fields. Main and interaction effects included fertilizer treatment (FT), row-side, FT*row-side, depth, FT*depth, row-side*depth, FT*row-side*depth at 13 and 25 cm from the row, which totals 14 possible comparisons between methods. Significant treatment effects on maize root growth were analyzed with generalized linear mixed models where fixed effects were FT, row-side, and depth, and replication was a random effect.

	M/S field	**M/M field**
Soil core method and cylinder method $p \leq 0.10$, same conclusion	2	2
Soil core method and cylinder method $p > 0.10$	7	12
Soil core method and cylinder method $p \leq 0.10$, opposite conclusion	2	0
Soil core method $p \leq 0.10$ and cylinder method $p > 0.10$	1	0
Soil core method $p > 0.10$ and cylinder method $p \leq 0.10$	2	0

†Starter applied 50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed.
‡The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither.
§Six 5-cm depth increments between 0 and 30 cm below the soil surface.

The coefficient of variation, C.V., for both root methods at V3 was large in both fields. In the M/S field, the C.V. was around 50 and 80% at 13 and 25 cm from the row, regardless of method. In the M/M field, C.V. values were around 90% at 13 cm from the row, regardless of method, and 130 (cylinders) and 160% (soil cores) at 25 cm from the row. These ranges of C.V. values are

similar to those reported in other published root research (Anderson et al., 1987; Chassot et al., 2001) and reflect the inherent variability in root growth and the difficulty in measuring roots.

Both methods consistently detected differences in root growth with depth at both distances from the row, averaged over fertilizer treatments and row-sides (Table 3-3). In fact, depth was the only effect detected by either method in the M/M field. In the M/S field, some treatment effects were identified by one method, but not the other. For example, root growth differences from fertilizer treatment at 13 cm from the row, averaged over row-side and depth, were only detected by the soil core method, in which the starter treatment averaged lower RLD (0.08 cm cm^{-3}) than the control (0.11 cm cm^{-3}) in the M/S field. In the M/S field, even though both methods discerned a significant row-side main effect and row-side by depth interaction, the methods differed in terms of which row-side contained a higher rooting density. In the M/S field, when averaged across fertilizer treatments, estimates of RND at 13-cm from the row were generally greater for the disturbed row-side than for the non-disturbed row-side, while estimates of RLD generally showed the opposite (Fig. 3-6). At 25 cm from the row, when averaged across fertilizer treatments, estimates of RND were greater on the non-disturbed than disturbed row-side at certain depths, whereas estimates of RLD discerned no differences between row-sides.

Table 3-3. Level of significance (*Pr > F*) for maize root growth differences using the soil core method [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 5 cm of cylinder] method at V3 in the maize after soybean (M/S) and continuous maize (M/M) fields due to fertilizer treatment (starter† and no-starter fertilizer), row-side‡ (disturbed and non-disturbed), and depth§ measured at 13 and 25 cm from the planted row.

	13 cm		25 cm	
	Soil Cores	**Cylinders**	**Soil Cores**	**Cylinders**
	----------*M/S field*----------			
Fertilizer treatment (FT)	**0.06**	0.67	0.33	0.51
Row-side	**0.01**	**0.003**	0.12	**0.06**
FT*Row-side	0.12	0.75	0.81	0.46
Depth	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
FT*Depth	0.19	0.95	0.13	0.89
Row-side*Depth	**0.03**	**0.01**	0.50	**0.05**
FT*Row-side*Depth	0.45	0.77	0.27	0.14
C.V. (%)	50	52	84	75
	----------*M/M field*----------			
FT	0.16	0.79	0.75	0.44
Row-side	0.86	0.28	0.67	0.31
FT*Row-side	0.77	0.69	0.64	0.31
Depth	**<0.0001**	**0.001**	**0.06**	**0.01**
FT*Depth	0.41	0.83	0.52	0.66
Row-side*Depth	0.20	0.11	0.71	0.55
FT*Row-side*Depth	0.32	0.72	0.33	0.34
C.V. (%)	95	88	158	130

† Starter applied 50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed.

‡ The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither.

§ Six 5 cm depth increments between 0 and 30 cm below soil surface.

Significant effects were analyzed with a generalized linear mixed model where fixed effects were treatment, row-side, and depth, and random effect (not shown) was replication. Experimental design was a randomized complete block with 5 (M/S) or 3 (M/M) replications where treatment was the main plot, row-side as the sub plot, and depth as the sub-sub plot.

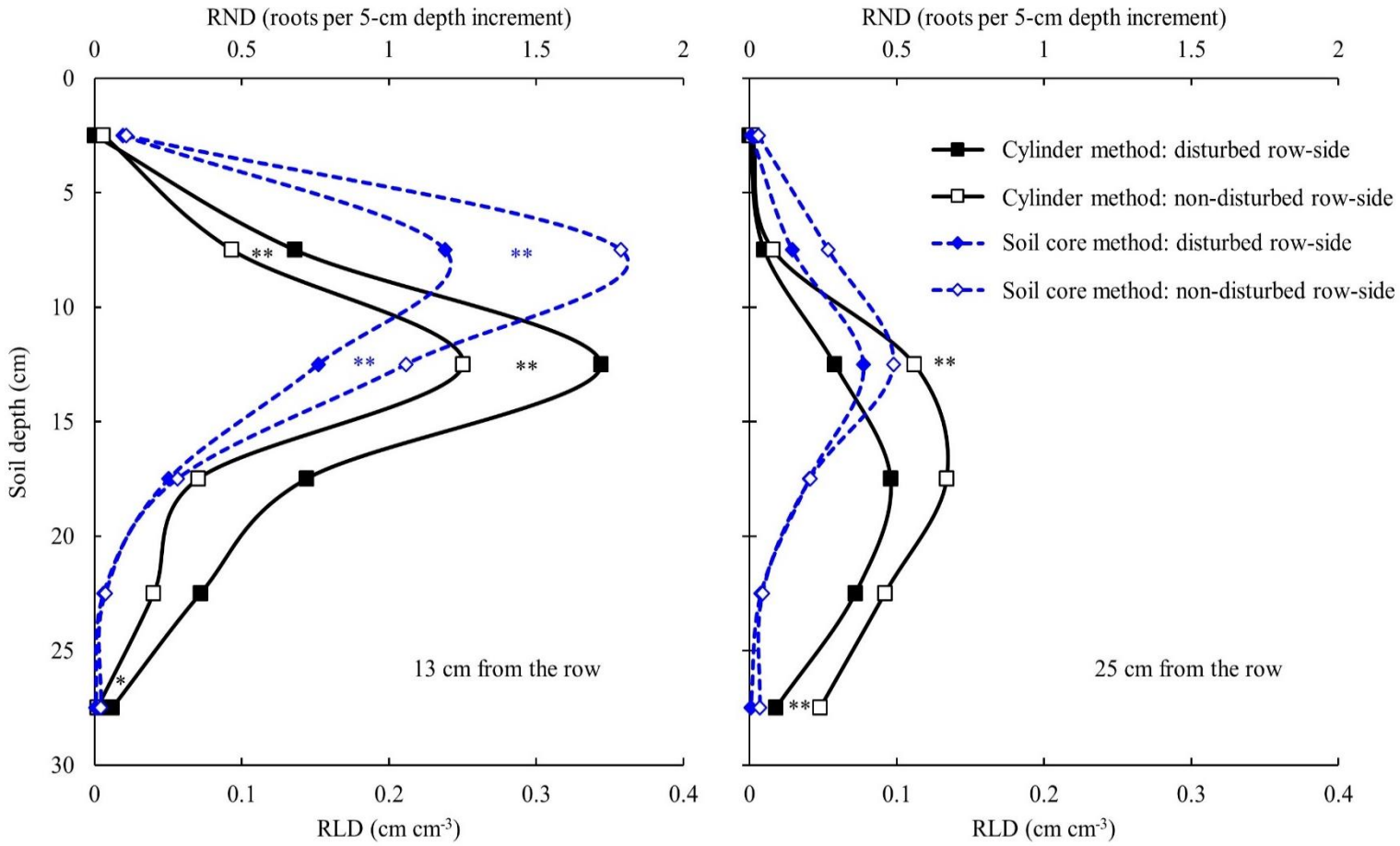

Figure 3-6. Maize rooting patterns at V3 in the maize after soybean (M/S) field with respect to distance from the row, row-side, and depth measured by the soil core method [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 5 cm of cylinder] method. The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither. A row-side*depth interaction ($p \leq 0.10$) was noted for the cylinder and soil core method at 13 cm, but only for the cylinder method at 25 cm. Data points are averaged across two fertilizer treatments that had no interaction with row-side and depth ($p > 0.10$) for either method. Within each method, statistical differences between row-sides at each depth are signified by **, * at $p \leq 0.05$, $p \leq 0.10$.

Estimates of rooting profiles in the top 30-cm of soil at V3 indicated a similar pattern between the two methods but distributed differently among depths. Part of the dissimilarity consisted of maximum RND estimates using the cylinder method occurring at deeper depths than the RLD estimates using the soil core method at either distance from the row in the M/S field (Fig. 3-6) and at 13-cm from the row in the M/M field (Fig. 3-7). In the M/M field, maximum RLD occurred between 5 and 10 cm deep at 13-cm from the row and between 10 and 15 cm deep at 25 cm from the row. With cylinders, maximum RND occurred between 10 and 15 cm deep at both 13 and 25 cm from the row.

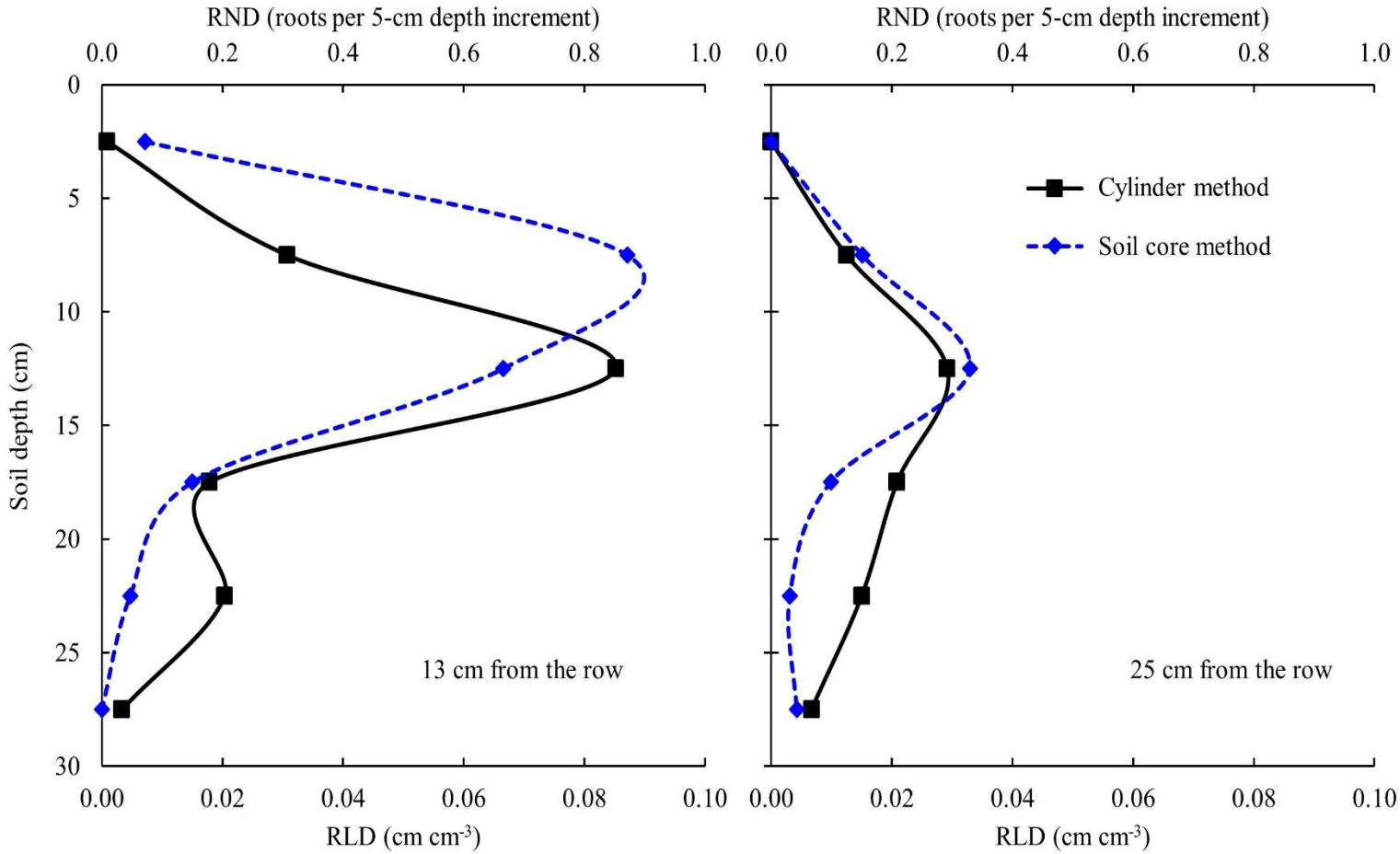

Figure 3-7. Maize rooting patterns at V3 in the continuous maize (M/M) field with respect to distance from the row and depth measured by the soil core method [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 5 cm of cylinder] method. Rooting density differed by depth ($p \leq 0.10$) for either method. Data points are averaged across both row sides and two fertilizer treatments that had no effect on rooting density for either method ($p > 0.10$).

3.4.2 Method Comparisons at V7

Overall, at ~V7 the two root quantification methods often did not result in the same significant or insignificant main and interaction effects in either field (Table 3-4). Methods agreed in 3 of 7 comparisons in M/S and 4 of 7 in M/M field. There were multiple instances in both fields where an effect of the treatments was only detected by one method and not the other. It is noteworthy that on one occasion in the M/S field both methods detected the same significant main effect but resulted in opposite conclusions.

Table 3-4. Total occurrences where the soil core [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 30 cm cylinder] methods measured identical significant (α=0.10) main and interaction rooting effects at ~V7 due to fertilizer treatment (starter† and no-starter fertilizer), row-side‡ (disturbed and non-disturbed), and distance from the row (13 and 25 cm) in the maize after soybean (M/S) and continuous maize (M/M) fields. Main and interaction effects included fertilizer treatment (FT), row-side, FT*row-side, distance, FT*distance, row-side*distance, and FT*row-side*distance, which totals seven possible comparisons between methods. Significant root growth effects were analyzed with generalized linear mixed models where fixed effects were fertilizer treatment, row-side, and distance from the row (13 and 25 cm), and random effect (not shown) was replication.

	M/S field	**M/M field**
Soil core method and cylinder method $p \leq 0.10$, same conclusion	1	0
Soil core method and cylinder method $p > 0.10$	2	4
Soil core method and cylinder method $p \leq 0.10$, opposite conclusion	1	0
Soil core method $p \leq 0.10$ and cylinder method $p > 0.10$	1	1
Soil core method $p > 0.10$ and cylinder method $p \leq 0.10$	2	2

†Starter applied 50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed.
‡The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither.

At ~V7, RND estimates using the cylinder method had a lower overall C.V. value in both fields than the RLD estimates using the soil core method. In the M/S field, C.V. values were 32 and 10% for the soil core and cylinder method, respectively. In the M/M field, C.V. values were 30 and 15% for the soil core and cylinder method, respectively.

At ~V7, only the soil core method detected a significant fertilizer treatment main effect in the M/S field (Table 3-5). There was also one instance at ~V7 in the M/S field where both methods detected a significant distance main effect but resulted in opposite conclusions. For example, estimates of RLD were higher at 13 compared to 25 cm from the row, whereas estimates of RND were lower at 13 compared to 25 cm from the row (data not shown). Soil cores also indicated there was no fertilizer treatment*row-side interaction, whereas cylinders detected a significant fertilizer treatment*row-side interaction in both fields. Estimates of RND in the starter treatment were significantly less than that in the control treatment for the non-disturbed row-side in both fields, whereas RLD (while numerically lower for the starter treatment) was not significantly different among the two treatments on either row-side (Fig. 3-8). It is important to note that in the M/M field, it does appear that RLD estimates are in a relative agreement with RND estimates in that both show the control exhibiting a lower rooting density than the starter treatment on the non-disturbed row-side. The soil core method likely failed to detect the fertilizer treatment*row-side interaction due to the higher level of variability as noted by the higher C.V. value. The methods did however show some notable similarity at ~V7 in that both detected a fertilizer treatment*distance interaction in the M/S field (Table 3-5). Regardless of method, root growth estimated by either method was lower for the starter treatment than the control, but only at 25 cm from the row (Fig. 3-9).

Table 3-5. Level of significance (*Pr > F*) for maize root growth differences using the soil core method [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 30 cm cylinder] method at ~V7 between 0 and 30 cm below the soil surface in the maize after soybean (M/S) and continuous maize (M/M) fields due to fertilizer treatment (starter† and no-starter fertilizer), row-side‡ (disturbed and non-disturbed), and distance (13 and 25 cm) from the row using the cylinder or soil core method.

	M/S field		**M/M field**	
	Soil cores	**Cylinders**	**Soil cores**	**Cylinders**
Fertilizer treatment (FT)	**0.10**	0.39	0.30	0.46
Row-side (RS)	0.25	**0.002**	0.78	**0.02**
FT*RS	0.73	**0.02**	0.17	**0.06**
Distance (D)	**0.01**	**0.01**	**0.001**	0.16
FT*D	**0.003**	**0.01**	0.76	0.62
RS* D	0.41	0.17	0.25	0.46
FT*RS* D	0.32	0.16	0.49	0.13
C.V. (%)	32	10	30	15

† Starter applied 50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed.

‡ The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither.

§Measurements were only done at 25 cm from the row.

Significant effects were analyzed with a generalized linear mixed model where fixed effects were treatment, row-side, and distance, and random effect (not shown) was replication. Experimental design was a randomized complete block with 5 (M/S) or 3 (M/M) replications where treatment was the main plot, row-side as the sub plot, and distance as the sub-sub plot.

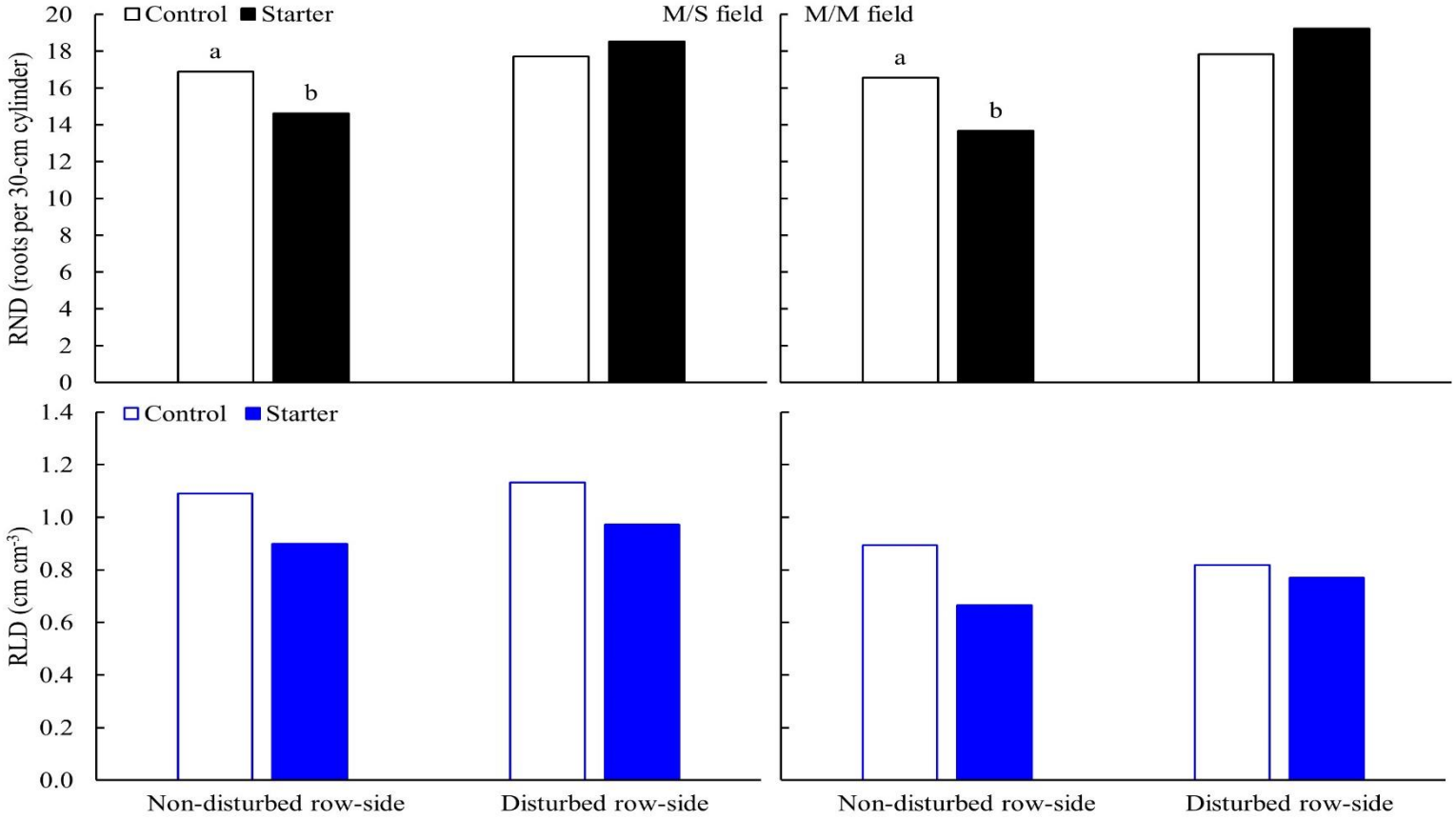

Figure 3-8. Maize rooting density at ~V7 between 0 and 30 cm below the soil surface in the maize after soybean (M/S) and continuous maize (M/M) fields in relation to fertilizer treatments and row-side measured by the soil core method [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 30 cm of cylinder] method, estimates averaged across 13 and 25 cm distances from the row. A significant ($p \leq 0.10$) treatment*row-side effect was only detected for the cylinder method. Means from the treatment*row-side interaction for the soil core method are still shown for comparison sake between methods even though the interaction was not significant ($p > 0.10$). Bars topped with different letters are statistically different ($p \leq 0.10$) by method and row-side. Fertilizer treatments were starter (50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed) and no-starter control. The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither.

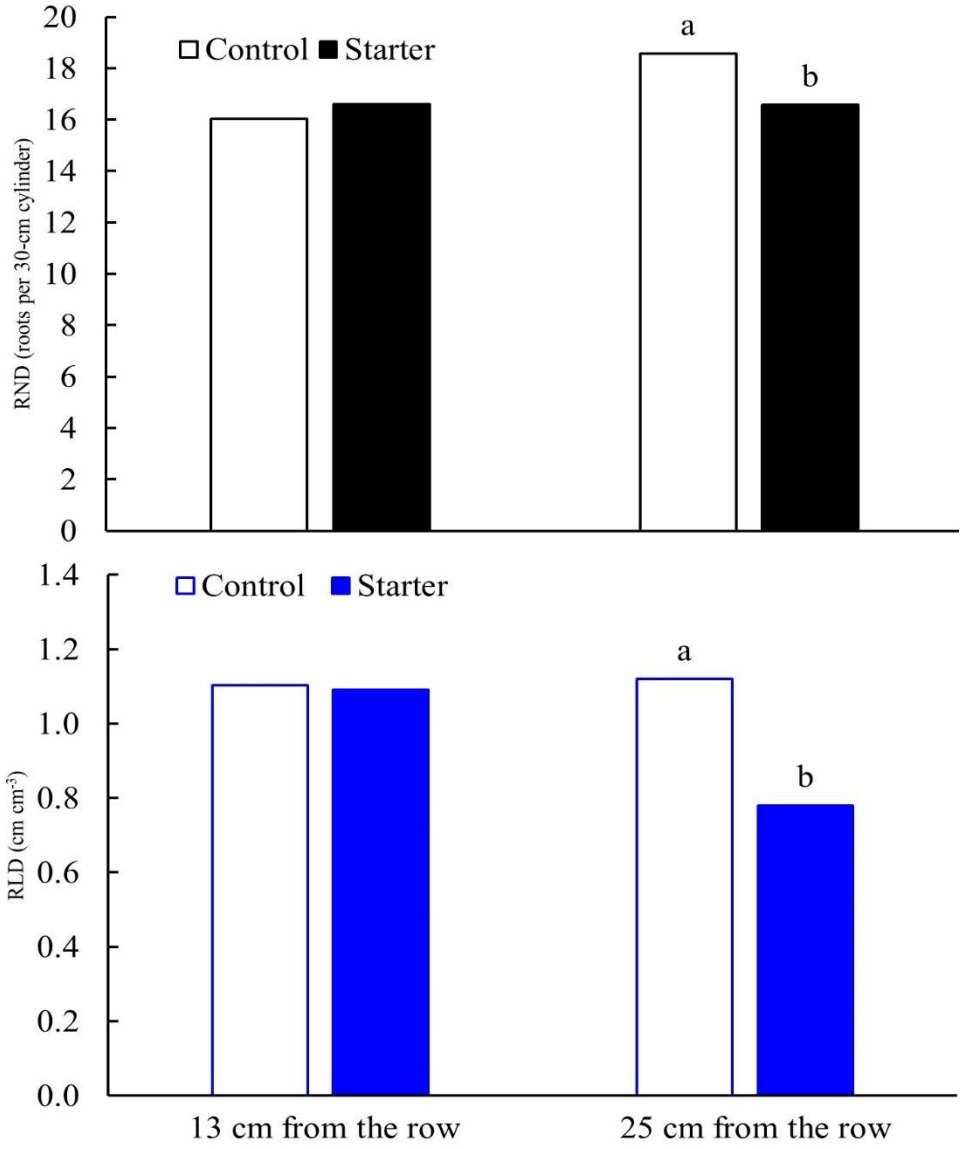

Figure 3-9. Maize rooting density at ~V7 between 0 and 30 cm below the soil surface in the maize after soybean (M/S) field in relation to fertilizer treatment and distance from the row measured by the soil core method [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 30 cm of cylinder] method. A significant ($p \leq 0.10$) treatment*distance effect was detected for both methods. Bars topped with different letters are statistically different ($p \leq 0.10$) by method and distance from the row. Fertilizer treatments were starter (50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed) and no-starter control.

3.4.3 Method Comparisons at R2-R3

At R2-R3, the two methods often did not detect the same significant or insignificant main and interaction effects in the M/S field (Table 3-6). In fact, five of the seven possible comparisons in M/S were only detected with the soil core method and not by the cylinder method. The methods were more consistent in the M/M field considering they frequently resulted in the same significant or insignificant main and interaction effects. Methods agreed in six of seven comparisons in which three of the same treatment effects were found to be significant with either method and three of the same treatment effects were insignificant based on either method.

Table 3-6. Total occurrences where the soil core [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 5 cm of cylinder] methods measured identical significant (α=0.10) main and interaction rooting effects at R2-R3 due to fertilizer treatment (starter† and no-starter fertilizer), row-side‡ (disturbed and non-disturbed), and depth§ measured 25 cm from the row in the maize after soybean (M/S) and continuous maize (M/M) fields. Main and interaction effects included fertilizer treatment (FT), row-side, FT*row-side, depth, FT*depth, row-side*depth, and FT*row-side*depth, which totals seven possible comparisons between methods. Significant root growth effects were analyzed with generalized linear mixed models where fixed effects were FT, row-side, and depth, and random effect (not shown) was replication.

	M/S field	**M/M field**
Soil core method and cylinder method $p \leq 0.10$, same conclusion	1	3
Soil core method and cylinder method $p > 0.10$	1	3
Soil core method and cylinder method $p \leq 0.10$, opposite conclusion	0	0
Soil core method $p \leq 0.10$ and cylinder method $p > 0.10$	5	1
Soil core method $p > 0.10$ and cylinder method $p \leq 0.10$	0	0

†Starter applied 50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed.
‡The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither.
§Six 5 cm depth increments between 0 and 30 cm below soil surface.

At R2-R3, the C.V. values were similar between methods in both the M/S (40%) and M/M (35%) fields (Table 3-7). In the M/S field, the only commonality between methods was detecting differences in root growth by depth. The soil core method discerned multiple effects not detected

by the cylinder method including a three-way interaction between fertilizer treatment, row-side, and depth. In the M/M field, both methods detected differences due to depth, row-side, and a row-side*depth interaction. The only inconsistency in the M/M field was that only the soil core method detected a fertilizer main effect, in which starter (1.31 cm cm^{-3}) exhibited fewer roots than the control (1.75 cm cm^{-3}), when averaged across row-side and depth.

Table 3-7. Level of significance (*Pr > F*) for maize root growth differences using the soil core method [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 5 cm of cylinder] method at R2-R3 in the maize after soybean (M/S) and continuous maize (M/M) fields due to fertilizer treatment (starter† and no-starter fertilizer), row-side‡ (disturbed and non-disturbed), and depth§ measured at 25 cm from the row using the cylinder or soil core method.

	M/S field		**M/M field**	
	Soil cores	**Cylinders**	**Soil cores**	**Cylinders**
Fertilizer treatment (FT)	**0.08**	0.13	**0.10**	0.24
Row-side	**0.01**	0.74	**0.01**	**0.02**
FT*Row-side	0.86	0.31	0.11	0.33
Depth	**<0.0001**	**<0.0001**	**<0.0001**	**0.001**
FT*Depth	**0.02**	0.84	0.39	0.67
Row-side*Depth	**0.001**	0.37	**0.003**	**0.10**
FT*Row-side*Depth	**0.09**	0.40	0.13	0.86
C.V. (%)	40	41	34	35

† Starter applied 50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed.
‡ The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither.
§Six 5 cm depth increments between 0 and 30 cm below soil surface.
Significant effects were analyzed with a generalized linear mixed model where fixed effects were treatment, row-side, and depth, and random effect (not shown) was replication. Experimental design was a randomized complete block with 5 (M/S) or 3 (M/M) replications where treatment was the main plot, row-side as the sub plot, and depth as the sub-sub plot.

At R2-R3 in either field (Fig. 3-10 and 3-11), the methods portrayed rooting profiles that were less alike than they were at V3. The highest density of roots measured with soil cores was in the upper 10 cm, whereas the highest number of roots per cylinder was between 5 and 10 cm deep. In the M/S field, RLD was greater on the non-disturbed row-side from 0 to 10 cm deep, regardless

of fertilizer treatment. The effect of row side appeared to be greater in the control than starter treatment near the soil surface. Although there was no three-way interaction for RND, similar row-side effects were visible for RND values at the 5-10 cm depth. The rooting profiles were slightly more similar in the M/M field, although RND estimates did not gradually decrease with depth like RLD estimates (Fig. 3-11). Both methods measured a lower rooting density on the disturbed than non-disturbed row-side in the upper soil depths (0-15 cm).

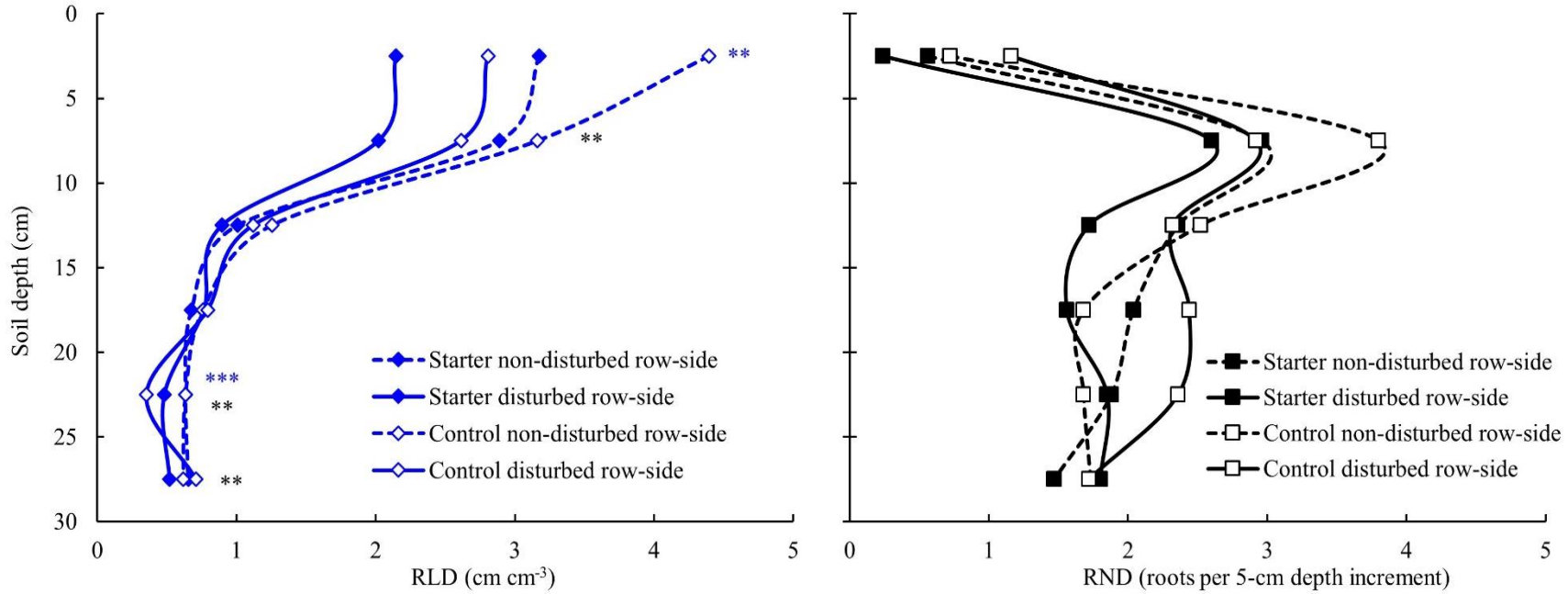

Figure 3-10. Maize rooting profiles at R2-R3 in the maize after soybean (M/S) field with respect to fertilizer treatment, row-side, and depth measured by the soil core method [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 5 cm of cylinder] method. The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither. Fertilizer treatments consisted of starter (50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed) and no-starter. A treatment*row-side*depth interaction ($p \leq 0.10$) was only detected for the soil core method. Means from the treatment*row-side*depth interaction for the cylinder method are still shown for comparison sake between methods even though the interaction was not significant ($p > 0.10$). Within the soil core method, statistical differences between row-sides at each depth are signified by blue ** ($p \leq 0.05$) or *** ($p \leq 0.001$) within the control treatment and black ** ($p \leq 0.05$) within the starter treatment.

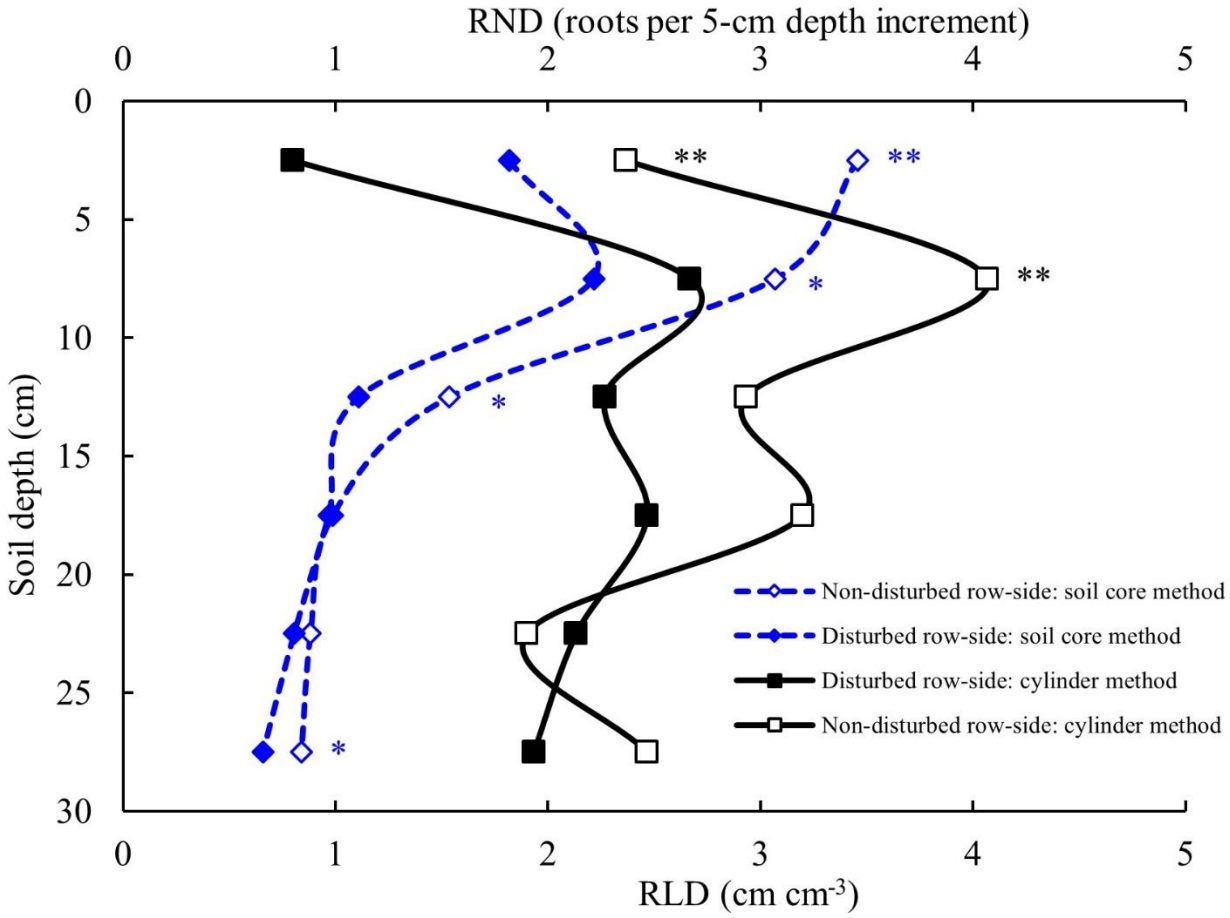

Figure 3-11. Maize rooting profiles at R2-R3 in the continuous maize (M/M) field with respect to row-side and depth measured by the soil core method [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 5 cm of cylinder] method. The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither. A row-side*depth interaction ($p \leq 0.10$) was noted for the soil core and cylinder method. Data points are averaged across two fertilizer treatments that did not differ by depth for either method ($p > 0.10$). Within each method, statistical differences between row-sides at each depth are signified by **, * at $p \leq 0.05$, $p \leq 0.10$.

3.4.4 Methods Standardized across V3, V7, and R2-R3 Comparisons

Most of the variation in V3 and R2-R3 measurements was from analyzing fertilizer treatment and row-side effects by depth. Since C.V. values were often excessively high, a second approach was then used to compare methods by summing root measures over the entire 30 cm core or cylinder and determine whether the methods were more or less comparable in their ability to discern fertilizer treatment and row-side effects on RLD and RND.

Overall, excluding depth from the model lowered C.V. values for both methods at each observation, but did not greatly improve the consistency between methods in relation to identifying similar main and interaction effects (Table 3-8). At V3 and R2-R3, there were still several instances in both fields where only the soil core method detected a certain main or interaction effect that was not detected by the cylinder method. In contrast, the cylinder method detected more significant effects than the soil core method at V7 in both fields. In the M/S field, methods agreed in four of seven, three of seven, and one of three comparisons at V3, ~V7, and R2-R3, respectively, in which most of the agreement was when there was no treatment effect. In the M/M field, methods agreed in six of seven, four of seven, and one of three comparisons at V3, ~V7, and R2-R3, respectively, in which most of the agreement was when there was no treatment effect.

Table 3-8. Total occurrences where the soil core [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 30 cm cylinder] methods measured identical significant (α=0.10) main and interaction rooting effects at V3, ~V7, and R2-R3 due to fertilizer treatment (starter† and no-starter fertilizer), row-side‡ (disturbed and non-disturbed), and distance (only at V3 and ~V7) from the row (13 and 25 cm) in the maize after soybean (M/S) and continuous maize (M/M) fields. At V3 and ~V7, main and interaction effects included fertilizer treatment (FT), row-side, FT*row-side, distance, FT*distance, row-side*distance, and FT*row-side*distance, which totals seven possible comparisons between methods. At R2-R3, main and interaction effects included FT, row-side, and FT*row-side, which totals three possible comparisons between methods. Significant root growth effects were analyzed with generalized linear mixed models where fixed effects were fertilizer treatment, row-side, and distance (V3 and ~V7) from the row (13 and 25 cm), and random effect (not shown) was replication.

	M/S field	**M/M field**
----------*V3*----------		
Soil core method and cylinder method $p \leq 0.10$, same conclusion	1	1
Soil core method and cylinder method $p > 0.10$	3	5
Soil core method and cylinder method $p \leq 0.10$, opposite conclusion	1	0
Soil core method $p \leq 0.10$ and cylinder method $p > 0.10$	2	1
Soil core method $p > 0.10$ and cylinder method $p \leq 0.10$	0	0
----------*V7*----------		
Soil core method and cylinder method $p \leq 0.10$, same conclusion	1	0
Soil core method and cylinder method $p > 0.10$	2	4
Soil core method and cylinder method $p \leq 0.10$, opposite conclusion	1	0
Soil core method $p \leq 0.10$ and cylinder method $p > 0.10$	1	1
Soil core method $p > 0.10$ and cylinder method $p \leq 0.10$	2	2
----------*R2-R3*§----------		
Soil core method and cylinder method $p \leq 0.10$, same conclusion	0	1
Soil core method and cylinder method $p > 0.10$	1	0
Soil core method and cylinder method $p \leq 0.10$, opposite conclusion	0	0
Soil core method $p \leq 0.10$ and cylinder method $p > 0.10$	2	2
Soil core method $p > 0.10$ and cylinder method $p \leq 0.10$	0	0

†Starter applied 50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed.
‡The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither.
§Measurements were only done at 25 cm from the row.

Excluding depth from the model and standardizing across all growth stages also allowed us to compare which method identified similar root growth effects throughout the season (Table 3-9). Overall, it appeared the soil core method detected the same significant main or interaction effects at each growth stage more so than the cylinder method but there were certainly some

inconsistencies noted with either method. For example, in the M/S field, soil cores detected a main fertilizer effect at each growth stage, whereas cylinders never detected a difference. The soil core method was also inconsistent at times because in the M/S field, soil cores measured differences in rooting due to row-side at V3 and R2-R3 but detected no differences due to row-side at ~V7. On the other hand, the cylinder method detected several row-side and fertilizer main and interaction effects ~V7 but little and zero at V3 and at R2-R3, respectively, in the M/S field. In the M/M field, soil cores somewhat consistently found little difference in rooting due to fertilizer or row-side until R2-R3 and always found a difference due to distance when measured. On the other hand, cylinders in the M/M field only measured differences due to distance at V3 and not ~V7, and only once at ~V7 detected a single fertilizer effect. In fact, the only consistency shown by the cylinder method in the M/M field was measuring a difference due to row-side both ~V7 and R2-R3.

Table 3-9. Level of significance (*Pr > F*) for maize root growth differences using the soil core method [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 30 cm cylinder] method at V3, ~V7, and R2-R3 between 0 and 30 cm below the soil surface in the maize after soybean (M/S) and continuous maize (M/M) fields due to fertilizer treatment (starter† and no-starter fertilizer), row-side‡ (disturbed and non-disturbed), and distance (13 and 25 cm) from the row using the cylinder or soil core method.

	M/S field		**M/M field**	
	Soil cores	**Cylinders**	**Soil cores**	**Cylinders**
	----------*V3*----------			
Fertilizer treatment (FT)	**0.07**	0.54	0.32	0.48
Row-side (RS)	**0.01**	0.42	0.76	0.79
FT*RS	0.29	0.78	0.79	0.37
Distance (D)	**<0.0001**	**<0.0001**	**0.003**	**0.02**
FT*D	0.14	0.84	**0.10**	0.77
RS* D	**0.09**	**0.0001**	0.72	0.12
FT*RS* D	0.16	0.36	0.64	0.69
C.V. (%)	35	34	63	60
	----------*V7*----------			
FT	**0.10**	0.39	0.30	0.46
RS	0.25	**0.002**	0.78	**0.02**
FT*RS	0.73	**0.02**	0.17	**0.06**
D	**0.01**	**0.01**	**0.001**	0.16
FT*D	**0.003**	**0.01**	0.76	0.62
RS* D	0.41	0.17	0.25	0.46
FT*RS* D	0.32	0.16	0.49	0.13
C.V. (%)	32	10	30	15
	----------*R2-R3*§----------			
FT	**0.10**	0.14	**0.10**	0.23
RS	**0.02**	0.75	**0.01**	**0.01**
FT*RS	0.89	0.32	**0.10**	0.19
C.V. (%)	20	22	19	14

† Starter applied 50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed.

‡ The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither.

§Measurements were only done at 25 cm from the row.

Significant effects were analyzed with a generalized linear mixed model where fixed effects were treatment, row-side, and distance, and random effect (not shown) was replication. Experimental design was a randomized complete block with 5 (M/S) or 3 (M/M) replications where treatment was the main plot, row-side as the sub plot, and distance as the sub-sub plot.

3.4.5 Correlations

Pearson correlation analyses between methods were conducted to determine if RND measurements from cylinders could accurately describe RLD measurements from soil cores. Because of the known discrepancies by depth between methods, as indicated by the deeper rooting profiles from cylinders at V3 and R2-R3, correlations were performed by the total roots cylinder^{-1} and average RLD summed over the 0-30 cm depth. At V3, measurements between methods were moderately correlated in the M/S field (r=0.52) but poorly correlated in the M/M field (r=0.31; Fig. 3-12). At ~V7, measurements between methods were moderately correlated in the M/M field (r=0.42), but poorly correlated in the M/S field (r=0.31; Fig. 3-13). At R2-R3, measurements between methods were highly correlated in the M/M field (r=0.85), but poorly correlated in the M/S field (r=0.24; Fig. 3-14).

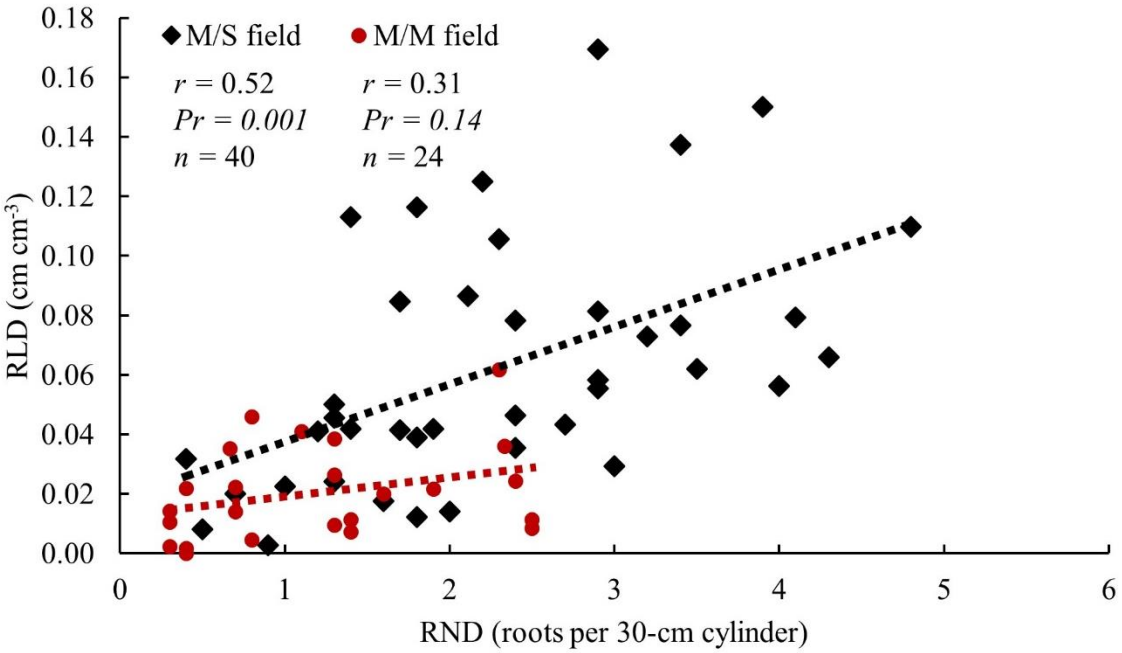

Figure 3-12. Pearson correlation between soil core [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 30 cm cylinder] measurements at V3 in the maize after soybean (M/S) and continuous maize (M/M) fields. Individual values represent the average of 10 cylinders or soil core samples from each side of the maize row and both fertilizer treatments collected at 13 and 25 cm from the row across 10 (M/S) or 6 (M/M) plots. Individual values from both RLD and RND measurements represent the average across 0 to 30 cm of soil below the surface.

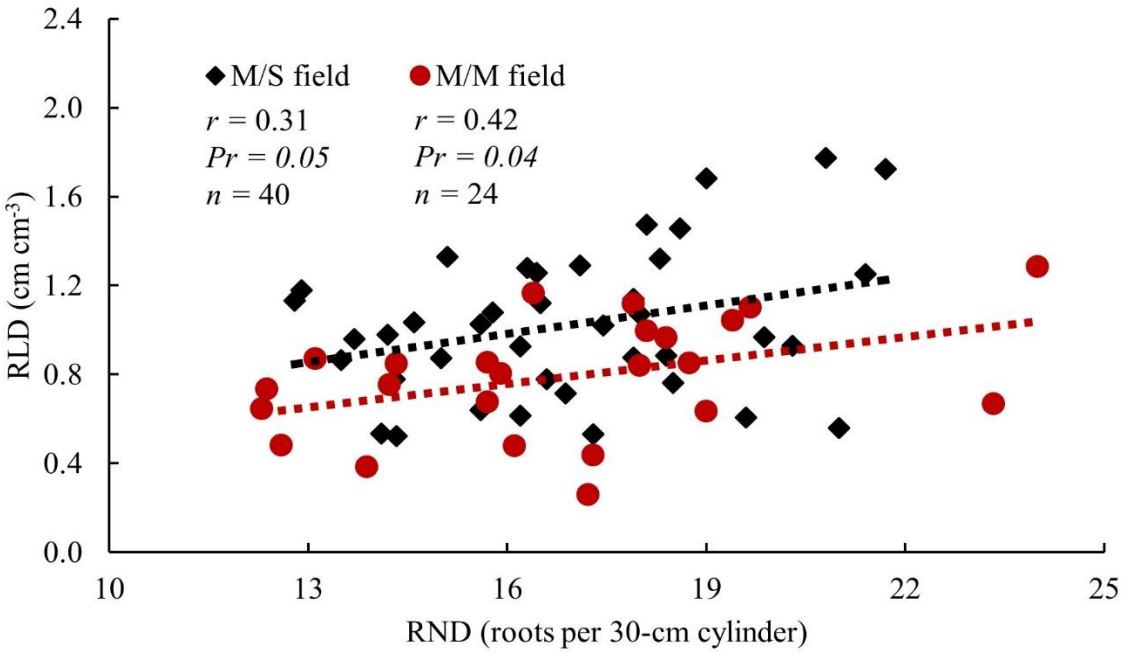

Figure 3-13. Pearson correlation between soil core [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 30 cm cylinder] measurements at ~V7 in the maize after soybean (M/S) and continuous maize (M/M) fields. Individual values represent the average of 10 cylinders or soil core samples from each side of the maize row and both fertilizer treatments collected at 13 and 25 cm from the row across 10 (M/S) or 6 (M/M) plots. Individual values from both RLD and RND measurements represent the average across 0 to 30 cm of soil below the surface.

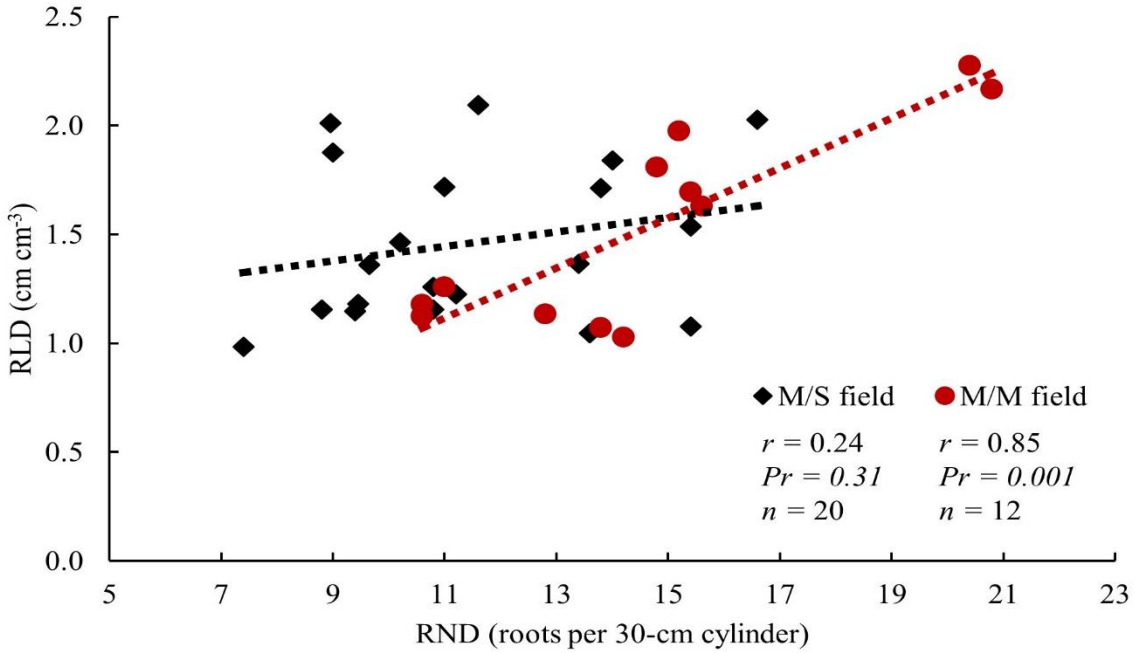

Figure 3-14. Pearson correlation between soil core [(RLD) root length density cm cm^{-3}] and cylinder [(RND) total number of roots per 30 cm cylinder] measurements at R2-R3 in the maize after soybean (M/S) and continuous maize (M/M) fields. Individual values represent the average of 10 cylinders or soil core samples from each side of the maize row and both fertilizer treatments collected at 13 and 25 cm from the row across 10 (M/S) or 6 (M/M) plots. Individual values from both RLD and RND measurements represent the average across 0 to 30 cm of soil below the surface.

3.4.6 Cost Estimates & Labor Requirements

The original objective was to evaluate the two root estimation methods at three separate growth stages. However, since we could not process samples from the intact cores fast enough, only a limited number of plastic liners were available after the first sampling date. Therefore, samples at ~V7 were not collected intact and samples at R2-R3 were only collected at 25 cm from the row. To simplify, the cost evaluation discussed here is an estimation of cost for either method to complete the original objective. The initial primary expense for the cylinder method was the perforated cylinders, whereas the soil core method required two soil recovery probes, plastic liners and caps, and the root image analysis software (Table 3-10). For either method, slightly more than 50% of the cost was labor. Approximately 48 hr of student labor was required to install the

perforated cylinders in both fields. The approximate time to record videos for 400 cylinders (i.e. one field), was approximately 12 hr with one person and one camera. The amount of time to record videos in one field (i.e. 400 cylinders) was equivalent to the time required for two people to collect the same number of intact soil cores.

Table 3-10. Cost estimate to use the cylinder or soil core method to measure root distribution in the M/S and M/M field from two fertilizer treatments (starter† and no-starter fertilizer), both sides of the maize-row‡ (disturbed and non-disturbed), two distances from the row (13 and 25 cm), three times during the growing season (V3, ~V7 and R2-R3).

Description	Information	Total
----------Cylinder method----------		
Perforated cylinders	*800 @ $1.37 each + $150 (setup)*	$1,246
Video recorder	*With borescope*	$ 130
MicroSD card	*2 @ $22 each*	$ 44
Electric drill	*Corded drill with 7.8 amp motor*	$ 150
Generator	*2500 watt generator rental - $140 per week*	$ 140
Drill bit	*Auger bit (diam. 3.5 cm) + (46 cm) extension rod*	$ 35
Estimated labor§	*Cylinder installation – 48 hr student labor ($528)*	$1,980
	Record videos – 72 hr student labor ($792)	
	Analyze videos – 60 hr student labor ($660)	
	Grand total	$3,725
----------Soil core method----------		
Soil recovery probe	*2 @ $211.10 each*	$ 422
Plastic liners	*2,400 @ $1.80 each*	$4,320
Plastic end caps	*4,800 @ $0.40 each*	$1,920
Root scanner	*Scanner (EPSON Perfection V800) plus software (WinRHIZO)*	$3,840
Root washing supplies	*Soap, mesh screens, tweezers*	$ 30
Estimated labor§	*Field sampling – 144 hr student labor ($1,584)*	$14,784
	Soil processing – 1,200 hr student labor ($13,200)	
	Grand total	$25,316

† Starter applied 50 kg N ha^{-1} 5 cm below and 5 cm to one side of the seed.
‡ The disturbed row-side contained coulter for starter fertilizer and tire tracks from the planter, whereas the non-disturbed row-side had neither.
Experimental design was a randomized split plot with 5 replications per field.
§Assumed labor cost \$11 hr^{-1} rate.

The estimated cost of the cylinder method was $3,725, substantially less than the estimated $25,316 for the soil core method. The major difference was cost of the root scanner and plastic

liners and the estimated time in labor to process root cores after field sampling. Separating root material from the soil by hand washing required approximately 1,200 hr to process all the intact soil cores by depth (i.e. 6 subdivisions for each core) for both fields and three sampling dates. The estimated time to count the number of roots within the same number of cylinders at each depth increment and across three sampling dates and both fields was 60 hr.

3.4.8 Discussion

Overall, with respect to soil depth, cylinders often tended to portray a deeper rooting profile than soil cores and consistently underestimated growth near the soil surface. Discrepancies in rooting depth might arise by a combination of multiple sources of error. First, soil core samples collected in the plastic liners could have compressed slightly as the probe was driven into the ground. When measuring from the surface of the soil this would inadvertently cause rooting depths to be shifted upwards. Second, during installation, many of the cylinders were forced into the ground until flush with the soil mounds that were created at the soil surface after drilling with the auger bit. Once the mound of soil around the top of the cylinders had settled, some of the cylinders were a few cm above the soil surface (Fig. 3-15). Roots growing at 0-5 cm soil depths, for example, would have then penetrated the cylinder void at the 5-10 cm painted markings if the cylinders were not flush with the ground surface. Third, underestimation of rooting density near the surface was likely due to, in part, by an actual lower incidence of root growth, but also perhaps because installation of the cylinders may have inhibited root growth by altering temperature and moisture conditions in the immediate area near the surface. Although we did not measure temperature and moisture around the cylinders, one could speculate that the open cylinder enhanced soil drying around, and then consequently altered soil temperature.

Figure 3-15. Representation of perforated cylinders from the soil surface where one image (left) shows a properly installed cylinder that is even with the soil surface and the second image (right) shows a poorly installed cylinder. Cylinders installed a few cm above the soil surface (right) were forced into the ground during installation until even with the soil mounds that were created at the soil surface after drilling with the augur bit. Once the mound of soil around the top of the cylinders had settled, it was revealed that the cylinders were actually a few cm above the soil surface. Roots growing at 0-5 cm deep, for example, would have then penetrated the cylinder void at the 5-10 cm painted markings.

Methods were comparable in discerning similar root growth effects due to fertilizer treatment, row-side, and/or depth depending on observation time and field, but in many situations, were not that comparable. It is important to acknowledge that an exact agreement between different root study methods is unlikely given the inherent challenges with current methodologies. One of the biggest challenges with either method used in this study is the difficulty in collecting large enough sample sizes to not only account for sampling and processing errors, but also the natural spatial variation in root growth across fields. This was especially evident based on the high C.V. values associated with either method, particularly early season. Chassot et al. (2001) reported C.V.s between 105 and 177%, depending on site-year and distance from the row, when rooting density was measured with soil cores at V6. Kuchenbuch and Barber (1987) reported a wide range in C.V. values (28 and 56%) depending on year of a multi-year study where soil cores were used to measure rooting density 1-2 weeks after R1 (silking) at multiple soil depths down to 75 cm deep. In our study, totaling RND in cylinders across all depths, and calculating RLD by the entire core length rather than depth, reduced C.V. values considerably in both fields and across all observation times. However, excluding depth did not improve comparisons between methods. Cylinder RND estimates ~V7 showed a significantly lower C.V. in both fields than RLD estimates from soil cores. However, roots counted per cylinder ranged by two-fold in either field, whereas RLD values ranged by nearly 3.5- (M/S) or 5-fold (M/M) at ~V7. The greater range in values associated with RLD would result in a higher level of variability across plots, and thus, a higher C.V. The smaller range in root counts in cylinders may have been in part due to the inability to account for every root due to the visual obstruction from other roots.

Even though the cylinder method at times was capable of discerning similar root growth effects as the soil core method, there were a few instances that may seriously question its overall

accuracy. There were three main instances that warrant further discussion. First, when using the soil core method at V3, the peak RLD 13 cm from the row occurred 5-10 cm deep, whereas the peak RLD 25 cm from the row occurred 10-15 cm deep. This outcome was perhaps expected based on the trajectory of roots from maize plants noted in previous research (Millar, 1930; Pagès et al., 1989; York et al., 2015). However, when using the cylinder method in the M/M field, the peak RND at 13 or 25 cm from the row occurred at the same depth, or at least no apparent difference in rooting depth between the two distances. In other words, the results using the cylinder method did not suggest that roots grew in a downward angle from the plant.

Second, the soil core method generally measured a lower RLD on the disturbed than non-disturbed row-side at either distance from the row and in both fields. The cylinder method did not show consistent row-side differences between fields or at different observation times. With the cylinder method in the M/S field (V3), a higher RND was measured on the disturbed than non-disturbed row-side at 13 cm from the row, but then at 25 cm from the row, the disturbed row-side had a lower RND. In the M/M field, the soil core method only detected a significant row-side difference at R2-R3, in which the disturbed row-side exhibited a lower RLD than the non-disturbed row-side. In contrast, the cylinder method resulted in no row-side effects around V3, a higher RND on the disturbed row-side ~V7, and then a lower RND on the disturbed row-side at R2-R3.

Third, the cylinder method also resulted in questionable differences in root growth with respect to distance from the row. In either field, the soil core method always measured a higher RLD at 13 than 25 cm from the row. Past studies that measured RLD from soil samples collected at V6 also found a higher concentration of roots closer to the plant (Marsh and Pierzynski, 1998; Chassot et al., 2001). With the cylinder method, RND in the M/S field was higher at 13 than 25 cm from the row at V3, but then at ~V7, RND was higher at 25 cm from the row. In the M/M field,

RND was higher at 13 than 25 cm from the row at V3, but then at ~V7, no differences between distances were detected. We suspect that cylinders installed at 13 cm were too close to the row and affected root growth from disruption of soil structure after installation or some alteration in moisture or temperature conditions.

The correlation between RND and RLD between 0 and 30 cm was better than expected in most situations considering the variation in root growth and difficulty of counting roots ~V7 and at R2-R3. On average, methods were slightly or moderately correlated at V3 and ~V7 depending on field. Methods were highly correlated in the M/M field at R2-R3 but were greatly influenced by the clustered data points at different positions along the vertical axis coupled with a low sample size. In general, only moderate correlations were obtained because regardless of how many lateral branches one root contained, it was still considered a single root when counted inside the cylinders. In other words, one small root shared the same value as a larger root with several lateral branches. On the contrary, RLD measured the total length in cm of each root, including lateral branches.

The installation of cylinders also required a change from the usual method of sidedress N application. In order to avoid damaging the cylinders, sidedress N was surface applied in a concentrated band rather than coulter injected which is the norm in Indiana. Measuring root growth in a non-typical growing environment from having to modify a fertilizer application for the sake of conducting the measurement may be an undesirable approach in some studies. It is possible that surface N applications may result in roots concentrated closer to the soil surface than subsurface coulter injected N. Furthermore, the inherent risk of greater N loss via volatilization with surface applied N could also reduce the overall N availability to the root system versus a subsurface application. One benefit of the cylinder method, though, was that it required substantially less processing time and was significantly less expensive than the soil core method. Even though both

methods required similar field time, counting roots in the videos was a more cost-effective approach than extracting roots from soil via handwashing. Recording videos in the field was far less strenuous than collecting intact soil cores, and additional cameras could also help reduce field time. Automated hydropneumatic elutriation systems (Gillison's Variety Fabrication, Benzonia, MI) to separate roots from soil are available and may reduce labor time for the soil core method.

3.5 Conclusion

Despite the cylinder method showing maximum rooting density at deeper depths, the overall pattern with respect to depth was like that of the soil core method at V3. The deeper rooting issue though could be solved by ensuring that cylinders are installed level with the actual soil surface. The major differences though in rooting profiles R2-R3 suggest that the cylinder method is insufficient for late season measurements. Unfortunately, one of our hypothesized benefits was the ability to efficiently measure root growth overtime and throughout the season. It is also important to recognize that too often the cylinder method failed to identify treatment effects similar to the soil core method at each observation, and at times indicated opposite conclusions. This discrepancy or inconsistency makes us cautious in recommending the cylinder method until further improvements are made. Certain improvements could be made to possibly improve the accuracy of the method. First, installing cylinders earlier in the season prior to planting would allow time for soil to settle around the cylinders. Second, use a higher resolution camera and root counting software to make counting less subjective. Another possible improvement could be to install a transparent tube inside the perforated cylinders. This would allow a clear path for the borescope and avoid obstruction of view from roots. Further testing is certainly needed to evaluate these potential improvements.

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CHAPTER 4. CORN RESPONSE TO STARTER FERTILIZER & IN-FURROW BIOLOGICAL & PLANT GROWTH REGULATORS

4.1 Abstract

In-furrow biological (BIO) and plant growth regulator (PGR) products, otherwise known as biostimulants, are becoming increasingly available in the commercial corn market. These products are commonly marketed as stimulating plant growth and increasing nutrient uptake and grain yield. Farmers may consider these products as an additional component to their starter fertilizer. The focus of this study was to compare the effects of several commercially available in-furrow biostimulant products on corn growth and development, nutrient uptake, and grain yield to starter fertilizer in large-plot field trials. The study was conducted across five locations in 2016, and three locations each in 2017 and 2018 at Purdue University research farms. At each location, treatments consisted of four different BIO or PGR products plus starter fertilizer, starter fertilizer only, and an untreated control. Biological and PGR products were selected based on their perceived prevalence in the marketplace. The same products were evaluated across all locations within each year of the study; however, from year-to-year the exact products tested varied slightly. Treatments were evaluated by measuring plant dry weight (DW), leaves with visible leaf collars, and nutrient concentrations ~V6 at most site-years. Grain yield was measured each site-year. Compared to the control, starter-only increased early season DW 18 to 104%, exhibited slightly more leaf collars at a given date, and enhanced N and P content at most site-years. Adding any of the BIO or PGR products did not consistently further enhance DW, visible leaf collars, or nutrient uptake across site-years, when compared to starter-only. Compared to the control, starter-only increased grain yield at 7 of 8 site-years in 2016 and 2018 ranging from 2 to 12 bu ac^{-1}, depending on location, but no increase was found at any of the 3 locations in 2017. Grain yield was increased

(3 of 11 site-years) or decreased (2 of 11 site-years) by some of the BIO or PGR products, but in 6 of 11 site-years none of the products affected yield compared to starter-only. None of the products individually showed a consistent response across multiple site-years.

4.2 Introduction

In corn production, starter fertilizers are often applied with or near the seed at planting to stimulate early season growth and potentially increase grain yield. Results from past studies have shown that starter fertilizer reduced the number of days to reach tasseling (Bullock et al., 1993) and silking (Mascagni and Boquet, 1996; Gordon et al., 1997; Cromley et al., 2003, 2006) and increased early season dry matter production (Niehues et al., 2004; Kim et al., 2013). Despite enhanced plant growth, grain yield response to starter does not always ensue (Bundy and Andraski, 1999). In recent years, other early season amendments, such as in-furrow biologicals (BIO) or plant growth regulators (PGR), have begun to appear in the commercial corn market for applications with starter fertilizer. Biological and PGR products are collectively referred to as biostimulants. These products are commonly marketed as amendments that will stimulate plant growth, enhance nutrient uptake, and increase grain yield.

Biological products contain various microorganisms that reportedly provide numerous benefits to plants, including disease suppression (Elad et al., 1980; Kinkel et al., 2012; Harman et al., 2004), increased solubility and uptake of essential nutrients (Qin et al., 2011; Chen et al., 2014), or enhanced plant development through the microbial production of plant growth hormones (Tien et al., 1979; Idris et al., 2007; Cassán et al., 2009). Plant growth regulators contain different forms of plant hormones, such as auxins, gibberellic acids, and/or cytokinins that are directly involved with physiological processes in the plant (Rademacher, 2015). These processes can be related to

cell elongation or division, development of reproductive organs, and/or the initiation of shoots and roots.

A limited number of independent field trials have evaluated the responses of corn to biostimulants. A recent study conducted in 11 Wisconsin corn fields reported that a PGR product containing cytokinin, gibberellic acid, and indole butyric acid marketed for corn did not increase yield relative to untreated checks (Lauer, 2017). Nevertheless, the marketing claims behind biostimulants can appeal to farmers, especially considering their low-cost relative to other inputs. The goal of this study was to evaluate the effects of several commercially available in-furrow biostimulant products on corn growth and development, nutrient uptake, and grain yield under multiple growing environments in field scale trials.

4.3 Materials & Methods

4.3.1 Field Descriptions & Trial Designs

Field scale trials were conducted in 2016, 2017, and 2018 across multiple locations that provided different growing conditions. Depending on year, trials were established at five different Purdue University research farms located in southeast (SEPAC; 39.035778, -85.529082), northeast (NEPAC; 41.104553, -85.398854), east central (DPAC; 40.253837, -85.150098), northwest (PPAC; 41.443402, -86.930340), and west central (TPAC; 40.296413, -86.902976) Indiana (Fig. 4-1).

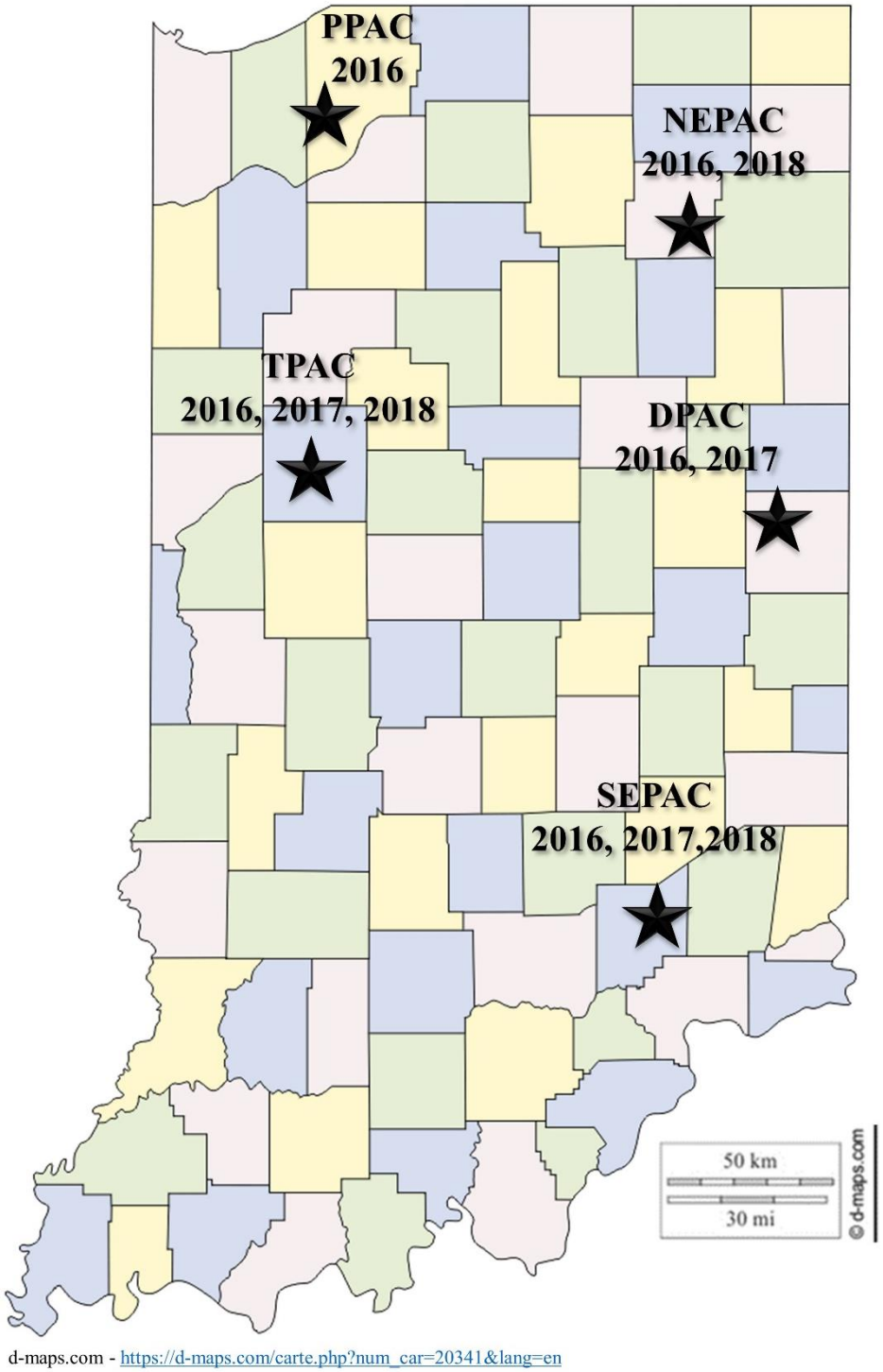

Figure 4-1. Site-years for in-furrow biological and plant growth regulator field trials conducted at multiple Purdue Agricultural Centers.

Trials ranged in size from 10 to 40 acres and were all in a corn and soybean rotation for at least 10 years. Trials in 2016 and 2018 at the SEPAC and TPAC farms were conducted on the same fields, whereas a new field was selected at the remaining farms each year of the study. Soil types, fertility levels, and management practices varied by field (Table 4-1).

Table 4-1. In-furrow biological and plant growth regulator field trial descriptions across multiple Purdue Ag. Centers and years.

Farm	**SEPAC**			**NEPAC**		**DPAC**		**PPAC**	**TPAC**		
Year	**2016**	**2017**	**2018**	**2016**	**2018**	**2016**	**2017**	**2016**	**2016**	**2017**	**2018**
†Soil texture	SiL			L, SL	L, CL	SiL, SiCL		L	SiL, SiCL		
Predominant soil types	Nabb, Ryker-Muscatatuck			Haskins, Rawson	Glynwood, Haskins, Mermill, Morley	Glynwood, Pewamo	Blount, Pewamo	Sebewa	Toronto-Millbrook complex, Lauramie, Drummer	Throck-morton, Toronto-Millbrook complex, Drummer	Toronto-Millbrook complex, Lauramie, Drummer
Tillage	No-till			No-till		Fall strip-till		Conv.	Conv.		
Planting date	5/26	4/26	5/2	5/23	5/8	5/24	5/16	5/20	5/31	6/6	5/11
Harvest date	10/6	9/27	9/18	10/24	10/17	10/31	10/26	10/19	11/1	11/28	11/12
‡SF (lbs. ac^{-1})	40-20-0			30-27-0-3S	25-12-0-5S	32-28-0	40-36-0	24-21-0	21-19-0	42-37-0	
¶N (lbs. ac^{-1})	196	175	177	220	225	225	215	193	191	175	177
#STP (ppm)	17	13	23	20	28	29	34	26	-	29	33
††STK (ppm)	100	105	156	111	111	135	122	121	-	137	144
pH	5.9	6.3	6.1	6.3	6.1	7.2	6.8	6.4	-	6.0	6.4
O.M. (%)	2.3	2.5	2.4	2.4	1.9	2.9	3.2	3.2	-	2.6	2.3

Conventional tillage practices consisted of fall (chisel plow) and spring (field cultivator) applications.

† >50% of total plot area; SiL (silt loam); L (loam); SL (sandy loam); CL (clay loam); SiCL (silty clay loam).

‡ Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O) applied 5 cm below and 5 cm to one side of the seed.

¶ Total N was amount of N received via SF plus sidedress. Total N was balanced across all treatments at sidedress.

Bray-1 P, †† ammonium acetate extractable.

Biological and PGR products were selected based on their perceived prevalence in the marketplace (Table 4-2). At each location, treatments consisted of four different in-furrow BIO or PGR products combined with traditional 2x2 row starter fertilizer, row starter fertilizer only, and an untreated control. The same products were evaluated across all locations within each year of the study; however, from year-to-year the exact product list varied slightly. Treatments were arranged in a randomized complete block design replicated three to six times per trial, depending on field size. Individual plots were 30 ft wide (12-rows) and varied in length from 400 to 1,800 ft.

Table 4-2. Active ingredients and application rates of in-furrow biological and plant growth regulator products tested across multiple Purdue Ag. Centers and years.

Treatment	Active Ingredients	CFU/ml†	Application Rate	Year evaluated
BIO-1	*Bacillus licheniformis* *Bacillus megaterium* *Bacillus pumilus*	$1x10^3$ $1x10^3$ $1x10^3$	48 fl. oz. ac^{-1}	2016, 2017
PGR	0.03 oz. cytokinin $qt.^{-1}$ 0.015 oz. indole-3-butyric acid $qt.^{-1}$ 0.01 oz. gibberellic acid $qt.^{-1}$		6 fl. oz. ac^{-1}	2016, 2017, 2018
BIO-2	*Rhodopseudomonas palustris* *Bacillus brevis* *Bacillus licheniformis* *Streptomyces griseus* *Bacillus megaterium* *Rhodococcus rhodochrous* *Lactobacillus plantarum*	$2.26x10^3$ $2.26x10^3$ $2.26x10^3$ $2.26x10^3$ $2.26x10^3$ $2.26x10^3$ $2.26x10^3$	32 fl. oz. ac^{-1}	2016, 2017, 2018
BIO-3A‡	Combination of *Trichoderma* strains	$4.5x10^9$	0.16 fl. oz. ac^{-1}	2016
BIO-3B‡	*Trichoderma harzianum* *Trichoderma atroviride*	$4x10^7$ $4x10^7$	1 fl. oz. ac^{-1}	2018
BIO-4	*Bacillus amyloliquefaciens* *Trichoderma virens*	$2.1x10^8$ cfu/g $5.0x10^7$ cfu/g	16 g 80,000 $seeds^{-1}$	2017, 2018

† Colony-forming unit (CFU) per ml for liquid products and per g for dry product (BIO-4) as shown on the product label.
‡ Same product name but different formulation and rate between 2016 and 2018.

All the BIO or PGR products were liquid formulations except BIO-4, which was a dry powder. The liquid products were applied in-furrow directly on the seed at planting at their respective recommended labeled rates (Table 4-2). Water was added as a carrier to bring the total volume of solution to a 3 gallon per acre rate for each treatment application. The BIO-4 product was mixed directly with the seed just prior to planting per manufacturer instructions. Starter fertilizer was applied in a 2x2 inch band below and to the side of the seed at planting. Starter nutrient rates varied by location (Table 4-1). At all locations except NEPAC, the starter fertilizer contained a combination of liquid urea-ammonium-nitrate (UAN, 28-0-0) and ammonium polyphosate (APP, 10-34-0). At NEPAC, ammonium thiosulfate (ATS, 12-0-0-26S) was included in the row starter mix to provide all treatments, including the control, with an additional 3 or 5 lbs S ac^{-1} in 2016 and 2018, respectively, due to known S deficiencies at that location.

The same hybrid (112 CRM, 2860 GDDs planting to black layer) was used for all trials in 2016 and 2017 at 32,000 and 30,000 seeds ac^{-1}, respectively. In 2018, a different hybrid (111 CRM, 2730 GDDs planting to maturity) was selected for all locations and was planted at 30,000 seeds ac^{-1}. The two hybrids used in this study contained both herbicide tolerance and insect resistance transgenic traits, except at SEPAC 2018, which used the non-transgenic version of the same hybrid used at other locations. At all locations except NEPAC, plots were sidedressed with UAN at targeted rates selected to equalize the total applied N rate across all treatments (Table 4-1). At NEPAC, ATS was added to the sidedress fertilizer mix to provide all treatments with an additional 17-20 or 15 lbs S ac^{-1} in 2016 and 2018, respectively, due to known S deficiencies at that location. Even though total S applied varied slightly (3 lbs ac^{-1}) by treatment at NEPAC 2016, S concentrations in earleaf samples at ~R1 were 0.19% across all treatments, which is above the minimum (0.16%) sufficiency level for this growth stage (Vitosh et al., 1995). Although the rate

of P applied via starter fertilizer was not equalized between control and treatments that received starter, all soil test P levels exceeded the critical level of 15 ppm (Vitosh et al., 1995), except SEPAC 2017, which was slightly below the optimum.

4.3.2 Plant Growth & Nutrient Uptake

Whole plant dry weight (DW), number of leaves with visible leaf collars, and plant tissue nutrient concentrations were measured once at most site-years sometime between V4 and V8 (Abendroth et al., 2011). Total plant dry weight was determined from 20 to 48 whole plants per plot, depending on field size, cut at the soil surface and dried in a forced air oven at 140°F for several days until sample weights stabilized. Samples were ground to a fine powder to pass through a 2 mm mesh screen and a subsample was analyzed for N, P, and K concentration by A&L Great Lakes Laboratories (Fort Wayne, IN). Nutrient concentrations (%) reported from the lab were converted to total nutrient content per acre, based on the total weight per plant, multiplied by the number of plants per acre (assumed to be 95% of the target seeding rate). The number of visible leaf collars plant^{-1} was determined for 40 to 60 plants per plot, depending on field size, once at most site-years. In 2016 and 2017, the stalk node number of the primary ear and the total final number of leaves plant^{-1} were also recorded at about the time silks began to appear on the ears.

4.3.3 Grain Yield

Grain yield was measured by harvesting the middle six rows of each 12-row plot with commercial combines equipped with GPS-enabled yield monitors. Yield monitors were calibrated the day of harvest based on guidelines provided by the manufactures. Data processing and cleaning procedures with GIS software included removing data points 1) within 50 to 75 ft from end-rows, 2) from anomalous poor areas of the field (i.e. gullies, wet spots, extreme slopes, etc., and 3) greater

than ± two standard deviations from each treatment mean. All remaining data points within each plot were extracted using GIS software and averaged to obtain a single yield and moisture value for the individual plot.

4.3.4 Statistical Procedures

Within each location, variables were analyzed with *a priori* contrast statements to compare the starter-only treatment to the untreated check and each BIO or PGR treatment (each included starter fertilizer). The analyses were performed using the GLM procedure in SAS version 9.4 (SAS Inst., Cary, NC). Treatment means were considered significantly different at $\alpha \leq 0.10$.

The percentage of plants exhibiting a specific total leaf number per location was compared between the starter-only and control treatments using a two-sided Z-test and were considered significantly different at $\alpha \leq 0.10$. Within a given total leaf number per location, the percentage of plants that contained a given position of the primary ear was compared between starter and no-starter (control) based on a two-sided Z-test and were considered significantly different at $\alpha \leq 0.10$.

4.4 Results & Discussion

4.4.1 Growth & Development & Nutrient Uptake

Compared to the control, the starter fertilizer treatment increased early season DW from 18 to 104% over the 11 site-years (Table 4-3). However, in most of the trials, none of the in-furrow BIO or PGR products further increased early season DW above and beyond that caused by the row starter alone. The only additional increase in DW occurred at SEPAC 2016, where BIO-2 increased DW ~20% above the row starter-only treatment. In four trials, early season DW decreased in response to the in-furrow application of certain individual BIO or PGR treatments in addition to the row starter fertilizer.

Table 4-3. Effect of in-furrow biological or plant growth regulator products on whole plant dry weight (lbs ac^{-1}) relative to the starter-only treatment. Sample growth stage was ~V6. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	**NEPAC**	**DPAC**	**PPAC**	**TPAC**
2016	----------	----------	*lbs ac^{-1}*	----------	----------
BIO-1	18	34	-44	-20	-40
PGR	-‡	34	52	**-38***	-43
BIO-2	**56***	-9	1	4	-50
BIO-3A	-	14	43	**-34***	-45
Control	**-52***	**-118***	**-192***	**-89***	**-103***
Starter-only mean	270	294	376	360	540
Trial mean	275	287	353	331	494
C.V.	8.4	15.1	19.4	6.3	10.9
2017	----------	----------	*lbs ac^{-1}*	----------	----------
BIO-1	-2	-	144	-	-21
PGR	**-27***	-	16	-	-24
BIO-2	-19	-	-22	-	-12
BIO-4	-2	-	-36	-	-33
Control	**-69***	-	**-494***	-	**-58***
Starter-only mean	175	-	1177	-	377
Trial mean	155	-	1112	-	352
C.V.	15.2	-	13.3	-	17.0
2018	----------	----------	*lbs ac^{-1}*	----------	----------
PGR	-9	19	-	-	**-36***
BIO-2	-4	47	-	-	-4
BIO-4	**-36***	6	-	-	-2
BIO-3B	-7	-3	-	-	-4
Control	**-87***	**-186***	-	-	**-85***
Starter-only mean	388	503	-	-	320
Trial mean	364	484	-	-	298
C.V.	7.9	8.9	-	-	8.1

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17,18); 31-27-0-3S (NEPAC 16); 25-12-0-5S (NEPAC 18); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 21-19-0 (TPAC 16); 42-37-0 (TPAC 17, 18).
‡Products not evaluated in a trial represented by " - ".

Crop growth stage (number of leaf collars) at the early season sampling date was slightly more advanced for the starter-only treatment compared to the control at 10 of the 11 site-years, but by less than 1 full leaf collar difference (Table 4-4). In most of the trials, none of the BIO or PGR

products greatly affected the number of total visible leaf collars compared to the starter-only treatment.

Table 4-4. Effect of in-furrow biological or plant growth regulator products on the total number of visible leaf collars plant^{-1} relative to the starter-only treatment around V6. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	**NEPAC**	**DPAC**	**PPAC**	**TPAC**
2016	----------*total leaf collars plant^{-1}*----------				
BIO-1	0	0	0	0	0
PGR	-‡	0.1	0.2	0	**-0.1***
BIO-2	**0.1***	-0.1	0.1	0	0
BIO-3A	-	0	0.2	0	-0.1
Control	**-0.2***	**-0.2***	**-0.4***	**-0.1***	**-0.3***
Starter-only mean	5.7	6.1	4.4	6.0	6.6
Trial mean	5.7	6.0	4.4	6.0	6.6
C.V.	1.6	1.8	5.0	0.5	2.0
2017	----------*total leaf collars plant^{-1}*----------				
BIO-1	0	-	-	-	0
PGR	-0.1	-	-	-	**-0.1***
BIO-2	0	-	-	-	**-0.1***
BIO-4	0	-	-	-	-0.1
Control	**-0.3***	-	-	-	0
Starter-only mean	5.1	-	-	-	6.1
Trial mean	5.0	-	-	-	6.0
C.V.	1.4	-	-	-	1.7
2018	----------*total leaf collars plant^{-1}*----------				
PGR	-0.1	0.1	-	-	0
BIO-2	0	0	-	-	0
BIO-4	-0.1	0.1	-	-	-0.1
BIO-3B	**-0.1***	**0.1***	-	-	0
Control	**-0.4***	**-0.4***	-	-	**-0.4***
Starter-only mean	6.7	6.6	-	-	5.8
Trial mean	6.6	6.6	-	-	5.7
C.V.	1.4	1.2	-	-	2.1

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17,18); 31-27-0-3S (NEPAC 16); 25-12-0-5S (NEPAC 18); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 21-19-0 (TPAC 16); 42-37-0 (TPAC 17, 18).

‡Products not evaluated in a trial represented by " - ".

The starter-only treatment enhanced N and P content over the control at each site-year, and K content at most site-years (Table 4-5, 4-6, and 4-7), but this was primarily a function of increased DW. One of the purported benefits of biostimulants is their ability to increase nutrient uptake. Across site-years, total N, P, or K content at ~V6 was generally similar between the biostimulants and starter-only treatments (Table 4-5, 4-6, and 4-7). In a few instances, total nutrient content was increased or decreased by one or more of the BIO or PGR products, but no product consistently increased nutrient content across multiple site-years.

Table 4-5. Effect of in-furrow biological or plant growth regulator products on N content (lbs ac^{-1}) relative to the starter-only treatment around V6. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	**NEPAC**	**DPAC**	**PPAC**	**TPAC**
2016	----------	----------	*lbs ac^{-1}*	----------	----------
BIO-1	0.4	1.1	-1.0	-0.5	-2.1
PGR	-‡	1.0	1.2	**-1.8***	-1.6
BIO-2	**2.1***	-0.7	0.7	0.5	-1.5
BIO-3A	-	0.9	1.4	**-1.3***	-1.4
Control	**-4.3***	**-5.3***	**-6.5***	**-4.9***	**-6.8***
Starter-only mean	10.5	10.9	11.5	15.6	21.8
Trial mean	10.1	10.4	10.8	14.3	19.6
C.V.	9.7	19.5	19.1	6.5	11.0
2017	----------	----------	*lbs ac^{-1}*	----------	----------
BIO-1	-0.4	-	3.6	-	-0.9
PGR	-0.8	-	0.5	-	-1.1
BIO-2	-0.7	-	-0.3	-	-0.5
BIO-4	0.1	-	-1.9	-	-1.1
Control	**-3.4***	-	**-16.6***	-	**-3.6***
Starter-only mean	6.9	-	39.2	-	15.5
Trial mean	6.0	-	36.8	-	14.3
C.V.	14.1	-	14.2	-	18.9
2018	----------	----------	*lbs ac^{-1}*	----------	----------
PGR	-0.6	0.1	-	-	**-1.2***
BIO-2	-0.2	0.2	-	-	0.4
BIO-4	-1.6	-1.2	-	-	0.3
BIO-3B	-0.4	-1.3	-	-	0.3
Control	**-4.8***	**-8.9***	-	-	**-5.5***
Starter-only mean	18.0	18.5	-	-	14.1
Trial mean	16.7	16.7	-	-	13.2
C.V.	8.8	12.9	-	-	8.4

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17); 31-27-0-3S (NEPAC 16); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 21-19-0 (TPAC 16); 42-37-0 (TPAC 17).
‡Products not evaluated in a trial represented by " - ".

Table 4-6. Effect of in-furrow biological or plant growth regulator products on P content (lbs ac^{-1}) relative to the starter-only treatment around V6. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	**NEPAC**	**DPAC**	**PPAC**	**TPAC**
2016	----------	----------	*lbs ac^{-1}*	----------	----------
BIO-1	0.1	0.1	-0.2	-0.1	-0.1
PGR	-‡	0	0.1	**-0.2***	0
BIO-2	**0.3***	-0.1	0	0.1	-0.1
BIO-3A	-	0	0.2	**-0.2***	-0.1
Control	**-0.3***	**-0.5***	**-0.7***	**-0.5***	**-0.5***
Starter-only mean	1.1	1.0	1.8	1.9	2.3
Trial mean	1.1	0.9	1.7	1.8	2.1
C.V.	10.8	24.6	20.7	6.9	12.9
2017	----------	----------	*lbs ac^{-1}*	----------	----------
BIO-1	0.1	-	0.2	-	0
PGR	-0.1	-	-0.1	-	-0.1
BIO-2	0	-	-0.1	-	0
BIO-4	0	-	0	-	-0.1
Control	**-0.2***	-	**-2.1***	-	**-0.3***
Starter-only mean	0.6	-	4.2	-	1.4
Trial mean	0.6	-	3.9	-	1.3
C.V.	25.9	-	15.1	-	15.4
2018	----------	----------	*lbs ac^{-1}*	----------	----------
PGR	0	0.1	-	-	-0.1
BIO-2	0.1	0.1	-	-	0
BIO-4	-0.1	0.1	-	-	0.1
BIO-3B	0	0	-	-	0.1
Control	**-0.4***	**-0.7***	-	-	**-0.4***
Starter-only mean	1.9	2.1	-	-	1.4
Trial mean	1.8	2.0	-	-	1.4
C.V.	10.2	14.8	-	-	9.5

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17); 31-27-0-3S (NEPAC 16); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 21-19-0 (TPAC 16); 42-37-0 (TPAC 17).
‡Products not evaluated in a trial represented by " - ".

Table 4-7. Effect of in-furrow biological or plant growth regulator products on K content (lbs ac^{-1}) relative to the starter-only treatment around V6. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	NEPAC	DPAC	PPAC	TPAC
2016	----------	----------	*lbs ac^{-1}*	----------	----------
BIO-1	0.6	1.6	-2.0	-0.2	-1.9
PGR	-‡	1.4	3.4	-0.7	**-4.0***
BIO-2	**2.0***	-1.2	3.0	0.9	**-4.4***
BIO-3A	-	1.0	1.2	-1.0	-2.8
Control	-1.5	**-5.2***	**-5.4***	-1.9	**-5.0***
Starter-only mean	10.4	11.7	12.6	13.7	28.6
Trial mean	10.7	11.3	12.7	13.2	25.6
C.V.	11.4	25.4	22.0	12.0	12.3
2017	----------	----------	*lbs ac^{-1}*	----------	----------
BIO-1	-0.3	-	1.4	-	-1.1
PGR	**-1.5***	-	-1.8	-	**-2.0**
BIO-2	**-2.1***	-	-1.3	-	-0.5
BIO-4	0.1	-	4.3	-	-1.6
Control	**-2.6***	-	**-12.7***	-	**-3.5***
Starter-only mean	6.8	-	30.0	-	16.1
Trial mean	5.7	-	28.3	-	14.6
C.V.	23.7	-	19.7	-	27.4
2018	----------	----------	*lbs ac^{-1}*	----------	----------
PGR	-0.1	3.3	-	-	**-1.8***
BIO-2	-0.9	0.2	-	-	0
BIO-4	-2.9	-2.7	-	-	0.2
BIO-3B	-0.4	-3.6	-	-	0.1
Control	**-5.2***	**-9.1***	-	-	**-4.1***
Starter-only mean	21.4	21.9	-	-	15.3
Trial mean	19.9	19.9	-	-	14.3
C.V.	16.4	12.6	-	-	11.7

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17); 31-27-0-3S (NEPAC 16); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 21-19-0 (TPAC 16); 42-37-0 (TPAC 17).
‡Products not evaluated in a trial represented by " - ".

Average final leaf number was slightly greater for the starter-only treatment compared to the control at SEPAC (both years) and PPAC, but not at the other 3 site-years. At SEPAC, a greater percentage of plants in the starter-only plots had 19 total leaves, whereas the control plots had a greater percentage of plants with 18 or 17 total leaves (Table 4-8). At PPAC 2016, the starter-only

plots had a greater percentage of plants with 20 total leaves than the control plots, which possessed more plants with 19 total leaves. None of the BIO or PGR treatments consistently affected final leaf number (data not shown).

Table 4-8. Effect of starter fertilizer across multiple site-years† on the average total number of leaves plant^{-1} and the percentage of plants within each treatment that exhibited a certain total leaf number. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. Treatment means followed by a different letter are statistically different ($p \leq 0.10$) within each location. Treatment means represented by " * " and bolded are statistically different ($p \leq 0.10$) within each location based on a two-sided Z-test.

Loc.	Trt.	Avg. total leaves plant^{-1}	Plants with 16 total leaves	Plants with 17 total leaves	Plants with 18 total leaves	Plants with 19 total leaves	Plants with 20 total leaves	Plants with 21 total leaves
2016			----------%----------					
DPAC	Control	18.7	0	3	**36***	**55***	**6***	0
	Starter	18.9	0	1	**23***	**62***	**14***	0
NEPAC	Control	18.5	0	3	**44***	**53***	1	0
	Starter	18.5	0	0	**53***	**44***	3	0
PPAC	Control	19.3 b	0	0	3	**66***	**31***	1
	Starter	19.5 a	0	0	0	**46***	**53***	1
SEPAC	Control	18.0 b	1	**21***	**55***	**22***	0	0
	Starter	18.5 a	0	**5***	**46***	**46***	3	0
2017			----------%----------					
SEPAC	Control	18.4 b	0	4	**54***	**42***	1	0
	Starter	18.6 a	1	0	**39***	**58***	2	0
TPAC	Control	19.0	0	0	14	**70***	**17***	0
	Starter	19.1	0	0	11	**67***	**22***	0

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17); 31-27-0-3S (NEPAC 16); 32-28-0 (DPAC 16); 24-21-0 (PPAC 16); 21-19-0 (TPAC 16); 42-37-0 (TPAC 17).

In general, among plants that exhibited the same final leaf number, the primary ear position (stalk node number) of the starter-only treatment tended to be lower than that of the control plants (Fig. 4-2). For example, a higher percentage of plants with 18 total leaves in the control plots had a primary ear positioned at the 13th stalk node, whereas starter-only plants with 18 total leaves had

a higher percentage of the primary ears positioned at the 12th stalk node. Overall, these results suggest that although starter fertilizer tended to increase the total number of leaves on plants, the position of the primary ear occurred at lower stalk nodes of the plants.

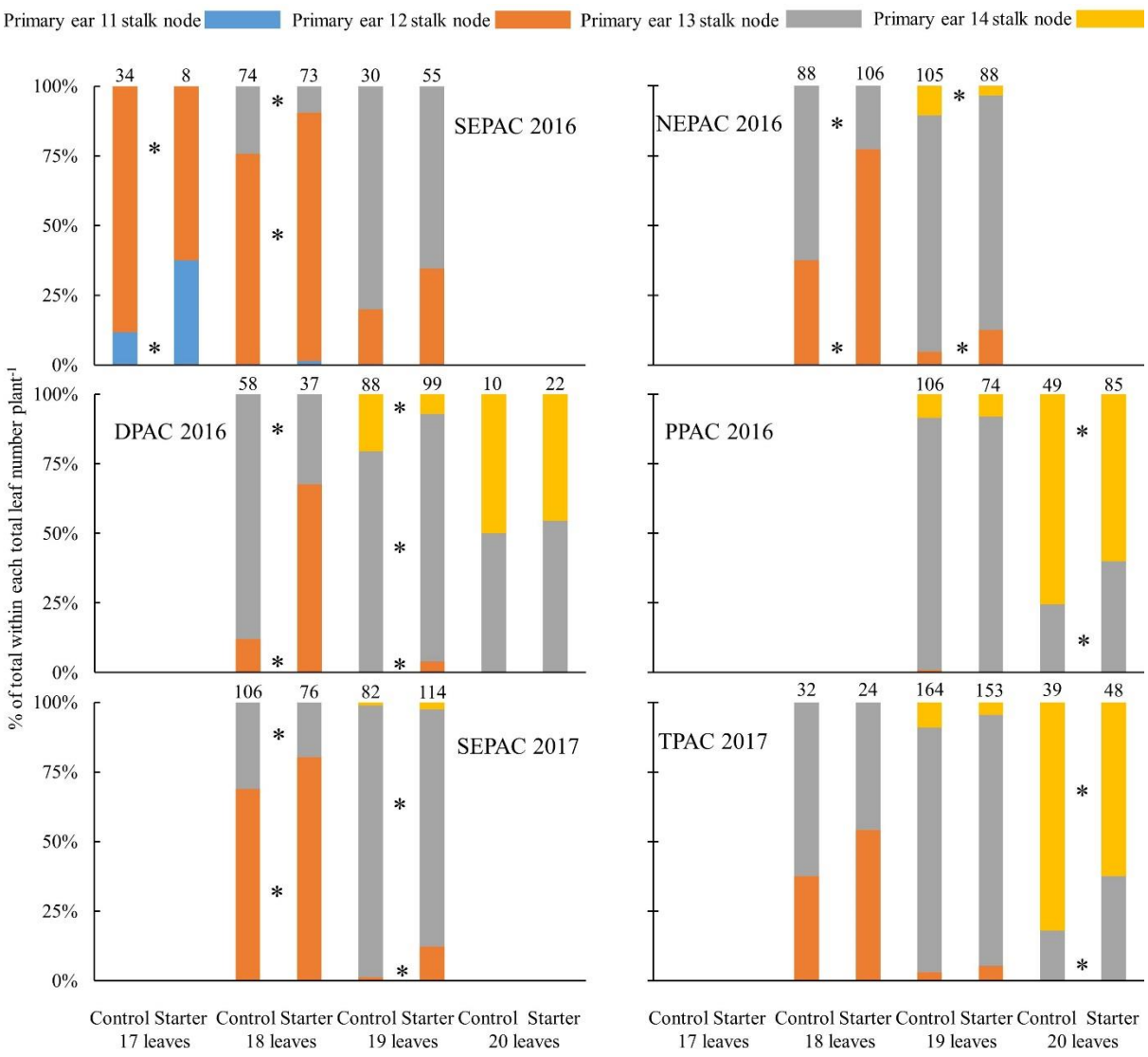

Figure 4-2. Effect of starter fertilizer on the position of the primary ear at a specific stalk node in relation total to leaf number across multiple site-years. Columns that do not contain data had <3% of the total plants sampled that fit the criteria within a site-year. Bars separated by " * " within a given total leaf number per location represent a statistically different ($p \leq 0.10$) percentage of plants that contained a given position of the primary ear between starter and no-starter (control) based on a two-sided Z-test. Starter fertilizer applied 2 in. below and 2 in. to the side of the seed. Values at the top of each bar represent the total number of plants observed. Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17); 31-27-0-3 (NEPAC 16); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 42-37-0 (TPAC 17).

4.4.2 Grain Yield

In 2016 and 2018, the starter-only treatment increased yield over the control at every location except NEPAC in 2016 (Table 4-9). Yield increases ranged from 2 to 12 bu ac^{-1}, varying by location. However, in 2017, the starter-only treatment did not affect yield at any of the three locations. At all locations in 2017 and at NEPAC in 2016, dry matter was increased at ~V6 by starter-only, irrespective of whether or not a significant yield increase occurred.

Table 4-9. Effect of in-furrow biological or plant growth regulator products on grain yield (bu/ac) relative to the starter-only treatment. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	**NEPAC**	**DPAC**	**PPAC**	**TPAC**
2016	----------	----------	*(bu ac^{-1})*	----------	----------
BIO-1	1	4	3	-1	-2
PGR	-‡	2	4	-1	-1
BIO-2	1	-4	**5***	1	**-3***
BIO-3A	-	-1	-2	-2	-1
Control	**-11***	-5	**-12***	**-5***	**-4***
Starter-only	212	158	210	225	190
Mean trial yield	210	158	210	224	188
C.V.	1.1	3.9	1.7	1.3	1.7
2017	----------	----------	*(bu ac^{-1})*	----------	----------
BIO-1	0	-	-5	-	**6***
PGR	**6***	-	-3	-	**8***
BIO-2	-2	-	0	-	2
BIO-4	**7***	-	-5	-	**8***
Control	1	-	-4	-	-3
Starter-only	201	-	218	-	216
Mean trial yield	203	-	215	-	219
C.V.	1.6	-	2.5	-	1.3
2018	----------	----------	*(bu ac^{-1})*	----------	----------
PGR	-2	0	-	-	0
BIO-2	-2	3	-	-	0
BIO-4	-2	0	-	-	**-5***
BIO-3B	-3	0	-	-	-2
Control	**-8***	**-5***	-	-	**-2***
Starter-only	257	261	-	-	226
Mean trial yield	254	260	-	-	225
C.V.	1.7	1.0	-	-	1.2

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17,18); 31-27-0-3S (NEPAC 16); 25-12-0-5S (NEPAC 18); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 21-19-0 (TPAC 16); 42-37-0 (TPAC 17, 18).

‡Products not evaluated in a trial represented by " - ".

Grain yield response to the biostimulants was inconsistent over the 11 trials. In 2016, no yield responses occurred at 3 of the 5 locations (Table 4-9). At DPAC, compared to starter-only, BIO-2 increased yield 5 bu ac^{-1}, but at TPAC BIO-2 decreased yield 3 bu ac^{-1}. In 2017, the PGR and BIO-4 treatments increased yields at DPAC and TPAC, while the BIO-1 treatment increased yields at TPAC. None of the other biostimulants affected yield in 2017. In 2018, none of the BIO or PGR products increased yield over the starter-only treatment, but BIO-4 decreased yield 5 bu ac^{-1} at TPAC.

The inconsistent effects of the BIO products on yield were not surprising for a multitude of reasons. For example, previous research has documented that microbial inoculants interact differently with certain corn hybrids and may increase, decrease, or have no effect on yield depending on hybrid (Harman, 2006; de Salomone and Döbereiner, 1996). The interaction between the microbial inoculant and the native microbial community is difficult to predict. Not only can microbial inoculants alter the natural microbial community, but their ability to colonize on host plants is highly contingent on the native microbial community as well (Vázquez et al., 2000), which ultimately may reduce or enhance their efficacy under certain situations.

The PGR product evaluated in our study increased yield at 2 of the 10 site-years where it was evaluated and had no effect at the other 8 site-years. A recent field study evaluated the same PGR product across 11 different locations in Wisconsin using separate in-furrow, foliar, and in-furrow + foliar treatment applications (Lauer, 2017). Regardless of application method, no significant yield increases over the untreated control were observed at any of the 11 locations. At one location, the PGR applied in-furrow or in-furrow + foliar reduced yield by 11 to 14 bu ac^{-1}.

4.5 Conclusion

Traditional row starter fertilizer (2 in. x 2 in.) applications consistently increased yield across most locations in 2 of the 3 years in this study. Enhanced DW at ~V6 from starter fertilizer did not always subsequently result increased yield. In 4 site-years of previous research (Lee, 2019), row starter fertilizer accelerated the appearance of leaf collars but did not affect DW of plants when compared at common growth stages. Similarly, observed differences in DW between the starter-only and control treatments in this study, measured at a single point in time, were likely a result of the starter-only plants being further along in development (more leaf collars).

None of the biostimulants used in this study consistently affected plant development, DW, N, P, and K uptake, or yield versus starter-only. For the BIO products, a yield decrease was nearly as likely as observing a yield increase. If any of the biostimulants did improve nutrient availability, their effects may have been negated by the N and P supplied through the starter fertilizer. However, the goal of this project was to determine how the biostimulants affected plant growth and yield in addition to the effects of starter fertilizer given that many growers commonly use starter fertilizer.

In summary, we strongly encourage corn growers who are considering biostimulants to first conduct replicated strip trials across multiple fields and years before investing in biostimulants across large acres.

4.6 References

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APPENDIX

Root length density measurement directly in starter fertilizer band

In 2017, two starter fertilizer trials were established near West Lafayette, IN (40.485288 lat., -87.005739 long.). Fields were approximately 100 m apart and one was continuous maize (M/M) for 12 yr and the other was a maize/soybean [*Glycine max* (L.) Merr.] rotation (M/S) for at least 12 yr. Both fields were chisel plowed in the fall and smoothed in the spring with a field cultivator equipped with a rolling harrow in preparation for planting.

On May18th, both fields were planted with the same hybrid (RM 108) at 73,086 seeds ha^{-1} using a 6-row commercial planter. Each row unit on the planter was equipped with separate coulters capable of positioning liquid fertilizer 5 cm below and 5 cm to one side of the seed (starter). Two treatments arranged in a randomized complete block design with five (M/S) or three (M/M) replications were established at planting, starter fertilizer (SF) and no-starter fertilizer (control). Starter fertilizer contained urea-ammonium-nitrate (UAN; 28-0-0) at a rate of 50 kg N ha^{-1}. Even though no fertilizer was applied, coulters were left in place for the control treatment in order to maintain a similar level of soil disturbance.

One component of the study was to measure root growth directly in the starter fertilizer band (SFB). In both the M/S and M/M fields, 10 30-cm long intact soil cores were extracted in a parallel line 5 cm from both sides of the planted row, or in other words, directly over top of the SFB. Soil samples were only collected within the starter fertilizer treatments and are discussed as either coming from the SFB or non-SFB row-side. Immediately after sampling, soil cores were then stored at -20°C until further processing.

After thawing at room temperature, intact soil cores were subdivided into six 5-cm long segments that represented different soil depths (0-5, 5-10, 10-15, 15-20, 20-25, and 25-30 cm).

Root-like material was then separated out from the soil through fragmenting with hand tools. The fragmented soil was carefully examined and then all visible root pieces were placed in a labelled vial with clean water. The vials were labelled with the appropriate plot number, distance from the row, row-side, and depth information before being stored at -20°C until further analyzing. Leftover soil was composited by depth and then placed in a forced air dryer at 38°C for several days. Inorganic N was extracted from a 10 g subsample for each plot, row-side and depth combination using 1 *M* KCl and then analyzed colorimetrically following EPA methods for NH_4^- (AGR-210-A Rev 1) and NO_3^- (AGR-231-A Rev 0) using an AQ2 Discrete Seal Analyzer (Seal Analytical, Southampton, Hampshire, United Kingdom). Soil pH was also measured in a separate 10 g subsample with deionized water in a 1:1 soil slurry.

In both fields, NH_4-N (Fig. A-1 B and F) and NO_3-N (Fig. A-1 C and G) levels were increased on the SFB row-side primarily around 2.5 and 7.5 cm deep (row-side*depth; $p \leq 0.05$), which is the approximate area SF was applied. At depths away from the SFB, NH_4-N and NO_3-N levels were generally similar on either side of the row. Soil pH was also reduced in the upper soil depths where SF was applied (row-side*depth; $p \leq 0.05$; fig. 2-6D and H); however, it is important to acknowledge that the apparent acidity measured near the SFB may have been overestimated due to the effects of the fertilizer on the osmotic potential of the soil.

In the M/S field, RLD was increased nearly two-fold on the SFB row-side around 7.5 cm deep compared to the same depth on the non-SFB row-side (row-side*depth; $p=0.05$; fig. 2-6A). At all other depths away from the SFB, RLD was similar on both sides of the row. In the M/M field, RLD was similar regardless of row-side at each depth (row-side*depth; $p=0.84$; fig. 2-6E), even around 7.5 cm deep where the SFB was applied. It is not clear why root proliferation in the SFB occurred in the M/S field but not in the M/M field.

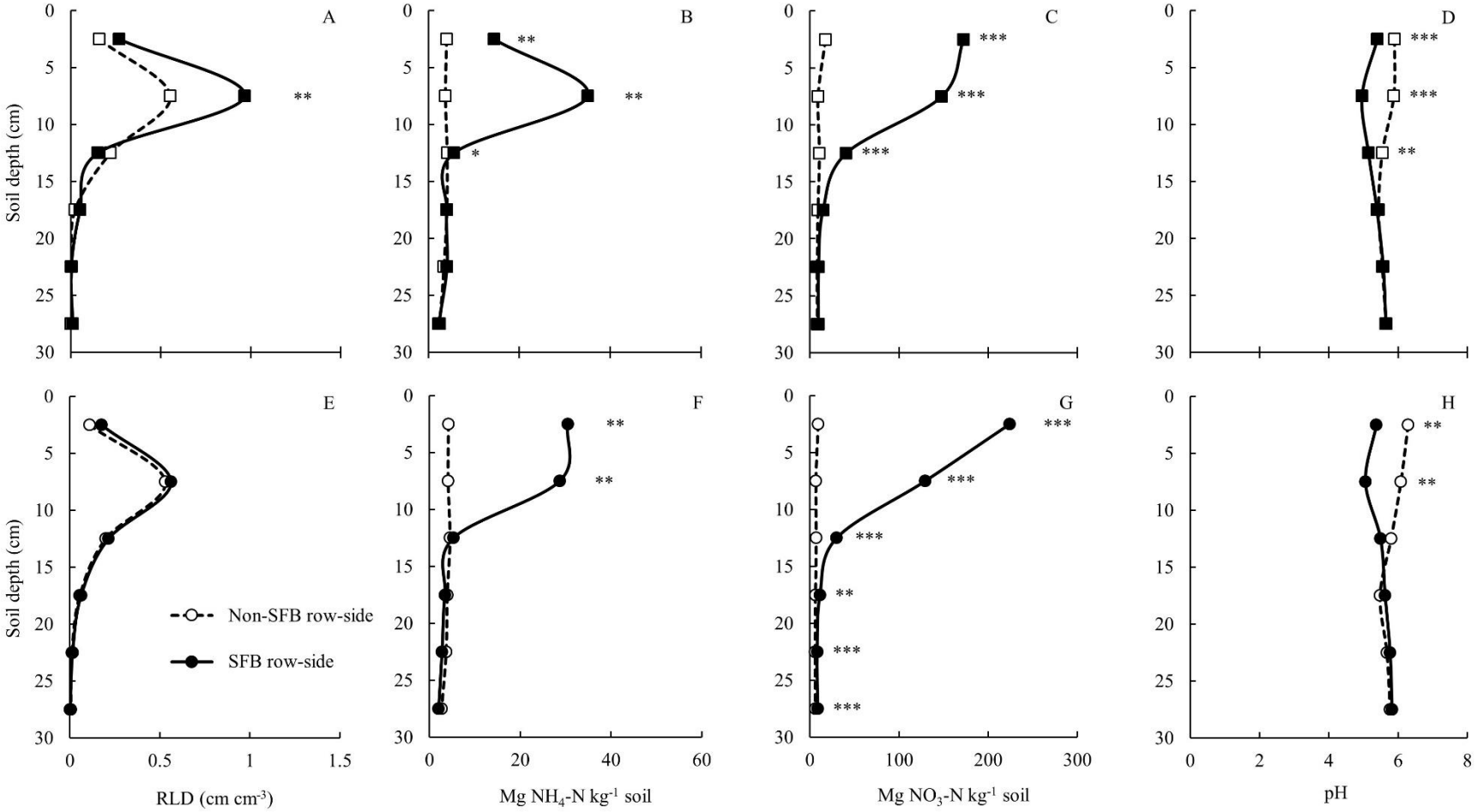

Figure A-1. Root length density (RLD) determined by the soil core method in the region of the fertilizer band, inorganic nitrogen content, and pH measured on either side of the maize-row with and without a starter fertilizer band applied 5 cm below and 5 cm to one side of the seed at 50 kg N ha^{-1} as 28% urea ammonium nitrate. Top row (A-D; squares) was maize following soybean rotation, whereas the bottom row (E-H; circles) was maize following maize rotation. Statistical differences between row-side at each depth are signified by ***, **, and * at $p \leq 0.0001$, $p \leq 0.05$, and $p \leq 0.10$, respectively.

Supplementary Tables for Chapter 4

Table A-2. Effect of in-furrow biological or plant growth regulator products on the total number of visible leaf collars plant^{-1} relative to the starter-only treatment between V10 and V12. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	**NEPAC**	**DPAC**	**PPAC**	**TPAC**
2016	----------*total leaf collars plant^{-1}*----------				
BIO-1	0	0.1	0	0	0
PGR	-‡	0.2	0.3	-0.1	**-0.2***
BIO-2	**0.2***	-0.2	0.2	0	0
BIO-3A	-	0.1	0.1	-0.2	**-0.1***
Control	**-0.6***	**-0.8***	**-1.1***	**-0.5***	**-0.4***
Starter-only mean	10.5	10.8	10.2	12.3	11.5
Trial mean	10.4	10.7	10.1	12.1	11.3
C.V.	1.3	2.7	2.9	1.2	1.3
2017	----------*total leaf collars plant^{-1}*----------				
BIO-1	0	-	-	-	-0.1
PGR	-0.3	-	-	-	**-0.3***
BIO-2	-0.2	-	-	-	-0.2
BIO-4	0	-	-	-	-0.1
Control	**-1.2***	-	-	-	**-0.3***
Starter-only mean	12.6	-	-	-	11.5
Trial mean	12.4	-	-	-	11.3
C.V.	2.3	-	-	-	2.7

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17); 31-27-0-3S (NEPAC 16); 32-28-0 (DPAC 16); 24-21-0 (PPAC 16); 21-19-0 (TPAC 16); 42-37-0 (TPAC 17).
‡Products not evaluated in a trial represented by " - ".

Table A-3. Effect of in-furrow biological or plant growth regulator products on total leaf number plant^{-1} relative to the starter-only treatment. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	**NEPAC**	**DPAC**	**PPAC**	**TPAC**
2016	----------*total leaf number plant^{-1}*----------				
BIO-1	-0.1	0.1	0.1	-0.1	-
PGR	-‡	0.1	**0.3***	0	-
BIO-2	0	**0.2***	**0.2***	0	-
BIO-3A	-	0.1	0.2	-0.1	-
Control	-**0.5***	0	-0.2	-**0.3***	-
Starter-only mean	18.5	18.5	18.9	19.5	-
Trial mean	18.3	18.6	19.0	19.5	-
C.V.	1.0	0.8	1.0	0.5	-
2017	----------*total leaf number plant^{-1}*----------				
BIO-1	-0.1	-	-	-	**0.2***
PGR	-0.2	-	-	-	0.1
BIO-2	-**0.3***	-	-	-	0.1
BIO-4	-0.1	-	-	-	**0.2***
Control	-**0.2***	-	-	-	-0.1
Starter-only mean	18.6	-	-	-	19.1
Trial mean	18.5	-	-	-	19.2
C.V.	1.0	-	-	-	1.0

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17); 31-27-0-3S (NEPAC 16); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 42-37-0 (TPAC 17).
‡Products not evaluated in a trial represented by " - ".

Table A-4. Effect of in-furrow biological or plant growth regulator products on N concentration (%) in the earleaf around silking relative to the starter-only treatment. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	**NEPAC**	**DPAC**	**PPAC**	**TPAC**
2016	----------%----------				
BIO-1	0	-0.1	0	0	-
PGR	-‡	0	0	0.1	-
BIO-2	**-0.1**	0	0	0	-
BIO-3A	-	0.1	0	0	-
Control	0.1	**0.1***	**0.1***	0	-
Starter-only mean	3.0	2.6	2.8	2.5	-
Trial mean	3.0	2.7	2.8	2.5	-
C.V	1.9	3.2	2.1	4.0	-
2017	----------%----------				
BIO-1	-0.1	-	-0.1	-	0
PGR	0	-	0	-	0
BIO-2	0	-	-0.1	-	0
BIO-4	0.1	-	0	-	0.1
Control	**0.2***	-	0	-	**0.1***
Starter-only mean	3.1	-	3.0	-	3.1
Trial mean	3.1	-	2.9	-	3.1
C.V.	3.2	-	3.2	-	3.2

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17); 31-27-0-3S (NEPAC 16); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 42-37-0 (TPAC 17).
‡Products not evaluated in a trial represented by " - ".

Table A-5. Effect of in-furrow biological or plant growth regulator products on P concentration (%) in the earleaf around silking relative to the starter-only treatment. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	**NEPAC**	**DPAC**	**PPAC**	**TPAC**
2016	----------%----------				
BIO-1	0.01	-0.01	**-0.02***	**-0.02***	-
PGR	-‡	0	0.01	-0.02	-
BIO-2	-0.02	-0.01	0.01	-0.01	-
BIO-3A	-	0.01	0	0	-
Control	0	0	-0.01	-0.01	-
Starter-only mean	0.43	0.26	0.38	0.31	-
Trial mean	0.42	0.26	0.37	0.30	-
C.V.	3.6	5.8	4.5	4.6	-
2017	----------%----------				
BIO-1	0	-	-0.01	-	0.01
PGR	0	-	-0.01	-	0
BIO-2	-0.02	-	-0.01	-	0
BIO-4	0	-	0.01	-	0.01
Control	0.01	-	0.01	-	-0.01
Starter-only mean	0.37	-	0.44	-	0.38
Trial mean	0.37	-	0.44	-	0.38
C.V.	7.1	-	4.8	-	5.3

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17); 31-27-0-3S (NEPAC 16); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 21-19-0; 42-37-0 (TPAC 17).
‡Products not evaluated in a trial represented by " - ".

Table A-6. Effect of in-furrow biological or plant growth regulator products on K concentration (%) in the earleaf around silking relative to the starter-only treatment. Starter fertilizer applied 2 in. below and 2 in. to one side of the seed. All products were applied with the same rate and formulation of starter as the starter-only treatment, but rates and formulations varied by location and year†. Values represent [Product] or [Control] minus [Starter-only]. Significant differences between product or control and starter-only represented by "*" ($p \leq 0.10$) and bolded.

	SEPAC	**NEPAC**	**DPAC**	**PPAC**	**TPAC**
2016	----------%----------				
BIO-1	-0.04	0.02	-0.13	-0.05	-
PGR	-‡	0.08	0.10	-0.04	-
BIO-2	-0.09	0.05	0.04	-0.02	-
BIO-3A	-	0.06	0.04	-0.05	-
Control	0.11	-0.09	0.03	0.04	-
Starter-only mean	2.57	2.08	2.07	1.99	-
Trial mean	2.56	2.10	2.08	1.97	-
C.V.	6.1	4.8	6.0	2.2	-
2017	----------%----------				
BIO-1	**-0.22***	-	-0.04	-	-0.07
PGR	**-0.22***	-	-0.17	-	0
BIO-2	**-0.32***	-	0	-	0.06
BIO-4	**-0.34***	-	0.02	-	0.01
Control	-0.06	-	0.11	-	-0.05
Starter-only mean	2.74	-	2.51	-	2.57
Trial mean	2.55	-	2.50	-	2.56
C.V.	6.7	-	6.7	-	8.2

†Starter fertilizer (lbs ac^{-1} N-P_2O_5-K_2O-S) 40-20-0 (SEPAC 16,17); 31-27-0-3S (NEPAC 16); 32-28-0 (DPAC 16); 40-36-0 (DPAC 17); 24-21-0 (PPAC 16); 42-37-0 (TPAC 17).
‡Products not evaluated in a trial represented by " - ".