Corn and Soybean Hormone and Antioxidant Metabolism Responses to Biosolids under Two Cropping Systems

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ABSTRACT

Biosolids applied at agronomic rates have been shown to improve hormone metabolism and drought tolerance in greenhouse trials, but no research has demonstrated such effects in the field. This study was designed to investigate effects of lime-stabilized (LS) and anaerobically digested (AD) biosolids on hormone and antioxidant metabolism and grain yields in a corn (*Zea mays* L.)–soybean (*Glycine max* L.) rotation under both conventional tillage (CT) and no tillage (NT) practices during 2009 to 2011. Application of both biosolids increased leaf photochemical efficiency (PE), hormones (indole-3-acetic acid (IAA) and *trans*-zeatin riboside (ZR)), proline, and superoxide dismutase (SOD) activity in corn at all sampling dates. The soybean grown in the plots previously amended with the biosolids exhibited greater PE, IAA, ZR, and SOD activity when compared with the control. The LS and AD biosolids increased grain yield by 87% and 77%, respectively, in corn and 15% and 18%, respectively, in soybean compared with the control. No difference in PE, hormone, proline levels, and SOD activity was found between CT and NT practices. The research demonstrated that biosolids application improves leaf antisenescence hormones, osmoprotectant, and antioxidant metabolism to increase grain yields, especially under drought stress.

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Abbreviations: ABA, abscisic acid; AD, anaerobically digested; ANOVA, analysis of variance; CCE, calcium carbonate equivalent; CT, conventional tillage; IAA, indole-3-acetic acid; LS, lime stabilized; NT, no tillage; PAW, plant available water; PE, photochemical efficiency; ROS, reactive oxygen species; SOD, superoxide dismutase; TKN, total Kjeldahl N; WP, water potential; ZR, zeatin riboside.

Drought stress reduces crop yield because adequate moisture is essential for successful growth and development of summer crops during sensitive reproductive stages. Drought stress causes a series of physiological changes, leading to plant senescence and metabolic dysfunction (Hirt, 2009). Hormones play important roles in plant adaptation to drought stress (Davies, 2010). Cytokinins have antisenescence effects (Strivastava, 2002). Man et al. (2011) noted that a tall fescue (*Festuca arundinacea* Schreb.) cultivar with greater cytokinin content had better drought tolerance than one with less cytokinin. Exogenous cytokinins have been shown to improve drought tolerance in creeping bentgrass (*Agrostis stolonifera* L.) (Zhang and Ervin, 2004).

Auxin is associated with root development and has been shown to have antisenescence properties (Davies, 2010). Abscisic acid (ABA) plays an important role in plant responses to drought stress. Abscisic acid serves as a signal molecule in inducing stomatal closure and conserving water under soil moisture deficit. Abscisic acid, along with hydrogen peroxide (H_2O_2), can

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stimulate the antioxidant defense system against drought stress (Hirt, 2009).

Drought stress damages cells through an over production of toxic reactive oxygen species (ROS) such as superoxide radicals ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($HO^{-\cdot}$). Plants have developed efficient antioxidant defense systems to scavenge ROS and protect cells from stress injury. Superoxide dismutases (SOD) are metallo-enzymes which convert $O_2^{-\cdot}$ to H_2O_2, and are considered as the "primary defense" against ROS (Perl-Treves and Perl, 2002). Plants with higher SOD activity may effectively remove superoxide radicals and improve plant survival under stress.

Biosolids are valued as sources of plant nutrients, soil organic matter, and, in the case of alkaline-stabilized materials, liming agents (USEPA, 2007; Zhang et al., 2009). Addition of organic materials enhances the biological, physical, and chemical properties of the soil, with yield improvements usually being associated with increased nutrient availability (Akrivos et al., 2000; Bugbee, 2002; Gilmour et al., 2003) and, sometimes, to improved soil physical properties (Klock-Moore, 2000). It has been reported that biosolids may contain biologically active substances such as humic acids and phytohormones that enable crops to withstand environmental stresses (e.g., drought, salinity, pathogens) and/or positively affect crop growth and quality (Subler et al., 1998; Zhang et al., 2005, 2007, 2009).

Application of biosolids and other organic amendments has been shown to improve crop yield, especially under drought stress conditions. Application of organic amendments increased water stress resistance of sweet corn leaves (HuiLan et al., 1998). Sahs and Lesoing (1985) observed higher sweet corn yields in plots amended with beef feedlot manure than those that were inorganically fertilized during drought years. Improved drought tolerance of crops grown in organically amended soils has been linked to increases in antisenescence hormone content and photosynthetic function (Zhang et al., 2009).

It has been documented that biosolids may provide hormones directly to soils and also serve as substrates from which soil microorganisms can produce hormones for subsequent plant uptake (Arshad and Frankenberger, 1993). Zhang et al. (2009) found that soil auxin and cytokinin contents were significantly greater in biosolids-amended soil than the control. They noted that biosolids application increased plant leaf IAA and cytokinin contents which were associated with higher PE and less leaf wilting under drought stress. Bowden et al. (2010) found that organic amendments increased PE, chlorophyll content, and corn grain yield in field conditions. Few studies have reported on the influence of land application of biosolids on crop hormone metabolism under field conditions.

Wang (2010) noted that application of cytokinin may enhance nitrate reductase activity and improve nitrogen use efficiency of creeping bentgrass. Exogenous auxin has been shown to increase creeping bentgrass root growth. Application of humic acid and cytokinin-containing seaweed extract increased creeping bentgrass antioxidant superoxide dismutase activity and improved drought tolerance under greenhouse conditions (Zhang and Ervin, 2004).

Biosolids may contain amino acids (Zhang et al., 2005). Amino acids are readily absorbed and translocated by plant tissues. Once absorbed, they have the capacity to function as compatible osmolytes, regulate ion transport, serve as signaling molecules, modulate stomatal opening, and detoxify heavy metals (Kauffman et al., 2007). Exogenous application of amino acids can increase endogenous amino acids in plant leaves (Vidmar et al., 2000). Amino acids play important roles in plant metabolism and stress tolerance. Two primary examples are tryptophan and proline. Tryptophan is a precursor for auxin biosynthesis (Davies, 2010), while proline is an important osmoprotectant (Zhang et al., 2009).

It has been shown that application of biosolids enhanced proline content and SOD activity in tall fescue under greenhouse conditions (Zhang et al., 2009). Bowden et al. (2010) noted organic amendments did not improve antioxidant enzyme activity in corn under field conditions. Li et al. (2013) noted that LS and AD biosolids increased spring soil nitrate N, plant tissue N at silking, post-season corn stalk nitrate N, and grain yield. Effects of biosolids on nitrogen and antioxidant metabolism in common crops like corn and soybean have not been well documented.

Objectives of this study were to investigate effects of soil application of biosolids on hormones and antioxidants of corn and soybean under conventional tillage and no tillage systems in a sandy soil in the Coastal Plain of Virginia and determine if these changes in hormones and antioxidants may be associated with improvement in crop drought tolerance and grain yields.

MATERIALS AND METHODS

Study Site

This study was conducted on a commercial farm in Sussex County, VA, USA on an Orangeburg loamy sand (*Fine-loamy, kaolinitic, thermic Typic Kandiudults*) whose mean productivity for corn is 7.54 Mg ha^{-1} according to the Virginia Agronomic Land Use Evaluation System (VALUES) (Simpson et al., 1993). Identical treatments, varying only in crop rotation, were laid out on each of two adjacent parcels of land. The dimensions of both study sites were 22.86 m × 87.78 m (2007 m^2).

Experimental Design

A split plot design was used with four replications. Tillage type was the main plot and fertility treatments were the subplots. The tillage treatments included CT and NT. Conventional tillage consisted of disking and NT consisted of seed drilling directly into the undisturbed soil through the stubble remaining from the previously harvested crop. The six fertility treatments included two biosolids sources, Blue Plains lime-stabilized (LS; Wastewater Treatment Plant, Washington, DC) and Alexandria anaerobically digested (AD; Alexandria Sanitation Authority, VA), applied at agronomic nitrogen rates for corn in the corn–soybean rotation and four fertilizer N rates (0x, 0.5x, 1.0x, and 1.5x agronomic (Ag) N rate). Each experimental plot had an area of 9.14 m × 3.66 m (33.45 m^2). Agronomic N rates for the natural Orangeburg soil were based on yield estimates using the Virginia Land Use Evaluation System (VALUES; Simpson et al., 1993). The 1x agronomic N rate for corn grain of 156 kg ha^{-1} was reduced by either 51 kg N ha^{-1} (following peanut) or 23 kg N ha^{-1} (following soybean) each year based on estimated residual N availability of the previous legume crop (Virginia Department of Conservation and Recreation, 2005).

Biosolids Analysis, Application, and Crop Planting

In March 2009, subsamples from the two biosolids were collected and moisture, organic matter, and nutrients were determined by A & L Eastern Laboratories, Inc. (Richmond, VA) using the procedures described by Zhang et al. (2009). Biosolids application rates were calculated from previous biosolids analyses. The actual nutrient rates applied were calculated each year from analyses of the biosolids applied. Ten subsamples were randomly collected from the biosolids stockpiled at the site, placed on ice, and sent to a commercial laboratory (A&L Eastern Laboratories, Inc.) for content analysis. Analyses performed included total solids (SM-2540G), total Kjeldahl N (SM-4500-TKN), ammonium N (SM-4500-NH3) (American Public Health Association, 1992), phosphorus (SW-846–6010C), potassium (SW-846–6010C) (USEPA, 1986) and other macro and micro nutrients.

In early April 2009, fresh biosolids were weighed in the field, surface-applied to each plot, and uniformly raked. Commercial urea fertilizers [$CO(NH_2)_2$] were applied to plots by hand before planting the corn. The plots were then subjected to either CT or NT. All experimental plots also received supplemental basal inorganic P ($NH_4H_2PO_4$) and K (KCl) fertilizers based on soil testing recommendations (Donohue and Heckendorn, 1994).

Corn (Pioneer 31G20; Pioneer Hi-Bred International, Inc., Johnston, IA) was planted at a row spacing of 91 cm and seeding rate of 69,300 seeds ha^{-1} (resulting in a plant population of 63,000 plant ha^{-1}) in mid-April 2009 and thinned to approximately 52,000 plants ha^{-1} 3 wk after emergence. Corn was harvested in September 2009.

In April 2010, corn was planted in new field plots adjacent to the 2009 plots. *Bradyrhizobium*-inoculated soybean (Pioneer 95M82) was planted in the 2009 corn plots at a rate of 368,000 seeds ha^{-1} (resulting in a plant population of 304,000 plant ha^{-1}; 0.76 m row spacing) on May 19, 2010. Soybean matured and was harvested in October 2010. In 2011, the corn and soybean were rotated. Soybean received no fertilizer or biosolids treatment in both 2010 and 2011. To supplement rainfall and prevent crop failure due to drought, two applications of 2-cm irrigation each were provided in June and July 2009. Four applications of 2-cm irrigation were provided between June and August 2010.

Pest control was implemented according to standard Virginia Cooperative Extension recommendations (Virginia Cooperative Extension, 1992), including a pre-plant glyphosate-herbicide application before planting at 7.8 kg ha^{-1}. Insecticides included terbufos (20% a.i) for corn in the seed furrow at planting and lambda-cyhalothrin (22.8% a.i.) for soybean at moth egg threshold in August.

Measurements

Leaf Water Potential (WP) and Photochemical Efficiency (PE)

Midday leaf water potentials were measured from the earleaf blade or second fully developed leaflet using a pressure chamber (PMS-600; PMS Instruments Co., Corvallis, Oregon) at corn grain filling and soybean flowering stages. Briefly, the top half of a corn earleaf or soybean leaflet was removed with a sharp scissors and the sample was immediately placed in the chamber with the cut surface protruding through the chamber lid. Pressure in the chamber was increased slowly and the pressure value was recorded when water first appeared at the cut surface (Turner, 1988).

Photochemical efficiency was determined by measuring chlorophyll fluorescence with a dual wavelength fluorometer (OS-50II, Opti-sciences, Inc., Tyngsboro, MA) based on the ratio of variable fluorescence to maximum fluorescence at 690 nm (Fv690nm/Fm690nm; Zhang et al., 2009). These measurements were taken at the 11th leaf (~20 d before silking), silking, and grain filling stages (~20 d after silking). Three first fully developed leaves (11th leaf stage) and earleaf (silking and grain filling stages) were used for PE measurement. For soybean, three second fully developed young leaflets were used for PE measurement. The values of Fv/Fm were calculated based on an average of three measurements per plot.

The leaf content of three hormones (IAA, cytokinin as zeatin riboside, and ABA), proline, protein, and SOD activity were determined at the 11th leaf, silking, and grain filling stages for corn and flowering and pod fill for soybean. Leaf samples were collected from top 1/3 of earleaf tissue (4 cm long sections) and second fully developed young leaflets from soybean. Five leaves were collected from the central two rows per plot. The samples were placed in plastic bags, frozen with liquid N, and stored at −80°C for later hormone analysis.

Leaf Zeatin Riboside (ZR) Content

The ZR was extracted from leaf tissue (50 mg), purified, and determined using an indirect enzyme-linked immunosorbent assay (ELISA) procedure as described by Zhang and Ervin (2004).

Leaf IAA and ABA Content

Both IAA and ABA were extracted from the leaf tissues using the procedure (for IAA) as described by Zhang et al. (2009) and Man et al. (2011) with minor modifications. Briefly, leaf tissues (50 mg) were ground with a mortar and a pestle in liquid N and homogenized in 3 mL Na-phosphate buffer (50 mM pH 7.0) containing 0.02% sodium diethyldithiocarbamate as an antioxidant and the hormones were extracted by continuous shaking for 2 h at 4°C. An internal standard ($^{13}C_6$–IAA; 50 ng) was added into each sample. The samples were transferred into tubes after extraction and pH was adjusted to ~2.6 with 1.0 M HCL. The sample was slurried with Amberlite XAD-7 (150 mg) (Sigma, St. Louis, MO) for 30 min. After removal of the buffer, the XAD-7

Table 1. The attributes of Blue Plains (Washington, DC) lime-stabilized (LS) and Alexandria (VA) anaerobically digested (AD) biosolids applied at the field sites in spring 2009 to 2011.

	Lime-stabilized biosolids			Anaerobic digestion biosolids		
Attribute†	2009	2010	2011	2009	2010	2011
Solids (g kg^{-1})	421	322	335	282	263	271
Volatile solids (g kg^{-1})	546	629	609	627	635	642
Total Kjeldahl N (g kg^{-1})	30.4	42.2	40.0	60.0	76.5	56.8
NH_4–N (g kg^{-1})	1.1	2.6	2.3	17.2	21.4	14.7
Organic N (g kg^{-1})	29.3	39.6	37.7	42.8	55.1	42.1
Phosphorus (g kg^{-1})	10.2	10.9	13.6	37.5	33.5	35.9
Potassium (g kg^{-1})	1.8	2.0	2.3	1.5	1.7	1.7
Sulfur (g kg^{-1})	5.9	4.5	4.6	10.4	9.4	9.7
Calcium (g kg^{-1})	127	101	124	25	20	22
Magnesium (g kg^{-1})	3.0	2.5	2.4	3.5	3.3	3.1
pH	12.4	12.2	12.3	8.2	8.3	8.8
Calcium Carbonate Equivalent (CCE) (g Kg^{-1})	238	166	108			

†Indole-3-acetic acid was 14.2 μg g^{-1} in LS and 7.2 μg g^{-1} in AD as measured in 2009.

was washed two times with 1 mL 1% acetic acid before being slurried two times with 1 mL dichloromethane for 30 min. The combined dichloromethane fractions were reduced to dryness with N gas. After extraction and purification, samples were dissolved in 50 μL methanol and diluted to 1 mL with tris-phosphate buffer. Abscisic acid (ABA) content was determined using an indirect linked immunosorbent assay (ELISA; Walker-Simmons et al., 2000). Indole-3-acetic acid (IAA) was assayed using a Linear Ion Trap Quadrupole LC/MS/MS (3200 Q Trap model; Applied Biosystems, Foster City, CA) as described by Zhang et al. (2012). A recovery rate of 91% was obtained for IAA.

Leaf Proline Content and SOD Activity

Proline was extracted from leaf tissues and determined spectrophotometrically at 520 nm (Bates, 1973; Zhang et al., 2007). Briefly, frozen leaf tissue (50 mg) was crushed with liquid N and extracted in 4.5 mL 3% 5-sulfosalicylic acid. The homogenate was filtered with a filter paper (#2) and the filtrate was used for analysis. Leaf SOD activity was determined according to the method of Giannopolitis and Ries (1977) with some modifications (Zhang et al., 2012).

Determination of Grain Yield

Corn ears were collected in each treatment plot from 0.3 m lengths of the two center (record) rows, dried, shelled, and weighed to obtain yield (0.155 g moisture g^{-1} dry weight basis). Soybeans were harvested with a plot combine from the two rows of record, dried, and weighed to calculate yield (0.13 g moisture g^{-1} dry weight basis).

Data Analysis

The data were subjected to analysis of variance (ANOVA) with three factors (year, tillage, and biosolids treatments). If year × till × treatment interaction was significant, the data from each year were subjected to ANOVA. If till × treatment interaction was significant, the data at each tillage level were further analyzed. Mean separations were performed using a Fisher's protected least significant difference test at a 5% probability level (SAS Institute, 2009).

RESULTS

Biosolids Characterization

The attributes of the two types of biosolids are shown in Table 1. The AD biosolids contained about 1.5x as much TKN and a higher proportion of plant available nitrogen than the LS biosolids. The AD biosolids also contained higher concentrations of P and S and lower calcium carbonate equivalent (CCE), Ca and pH than the LS biosolids.

The IAA content of the LS was 97% greater than that of the AD biosolids. The biosolids application rates to NT were higher than those on CT to account for N volatilization loss; therefore, application rates of CCE and nutrients other than N were higher on the NT relative to the CT treatments. The high quality biosolids used in this study met USEPA pollutant ceiling limits and the concentrations of trace elements were much lower than the pollutant concentration limits (USEPA, 1993). The averaged soil pH value was 6.2 before treatment application, 6.5 following the addition of the AD and fertilizer treatments and 7.1 with LS biosolids treatment. The resulting soil pH from the LS biosolids should not have altered plant essential nutrient availability because of large buffering capacity of the soil.

Temperature and Precipitation

Total precipitation was greater than the 30-yr average in 2009 and 2011, but lower in 2010 (Table 2). The corn and soybean suffered severe drought stress in 2010 and mild drought stress in 2009 and 2011. Mean temperature was greater in 2010 and 2011 than the 30-yr average and slightly lower than the 30-yr average in 2009. The crops also experienced heat stress in 2010 as the monthly temperature was 1.3 to 2.5°C greater than the 30-yr average during the growing season from April through October.

Corn Responses

Leaf Water Potential (WP) and Photochemical Efficiency (PE)

Corn amended with biosolids had higher leaf WP than the control at silking (Table 3). Corn at the 1.0x and 1.5x N fertilizer rates also had higher WP than the control. A greater WP was found in soybean under CT relative to NT. The WP in corn and was greater in 2011 than 2010. The biosolids and 1.5x N fertilizer treatments increased corn leaf PE relative to the control at all three sampling dates (Table 3).

Leaf ZR Content

Biosolids and 1.5x N rate treatments increased ZR content when compared with the control and 0.5x and 1.0x N rate treatments at the 11th leaf and silking stages (Table 4). On average over the 3 yr, LS and AD treatments increased

Table 2. The recorded monthly temperature and precipitation means and departures at Sussex County, Virginia during 2009 to 2011.†

	Temperature					
	2009		2010		2011	
Month	Mean	Depart	Mean	Depart	Mean	Depart
	C°					
Jan.	2.8	0.0	1.9	–0.9	1.4	–1.4
Feb.	4.8	0.3	1.6	–3.0	5.7	1.2
Mar.	7.9	–1.2	10.1	0.9	8.7	–0.4
Apr.	14.6	0.4	15.9	1.7	15.7	1.4
May	20.1	0.9	20.5	1.3	19.7	0.6
June	24.1	0.3	26.3	2.5	24.8	1.0
July	24.4	-1.6	27.3	1.3	26.8	0.8
Aug.	26.2	1.6	26.2	1.6	25.3	0.8
Sep.	20.4	–0.7	23.3	2.2	22.2	1.1
Oct.	14.0	–0.3	15.6	1.3	14.6	0.3
Nov.	11.1	1.7	8.9	–0.4	11.2	1.8
Dec.	3.8	–0.8	0.2	–4.4	7.3	2.7
Annual	14.4	-0.1	14.8	0.3	15.3	0.8
	Precipitation					
	Total	Depart	Total	Depart	Total	Depart
	mm					
Jan.	48.3	–59.9	113.0	5.1	39.1	–69.1
Feb.	15.5	–69.3	72.6	–12.2	33.5	–51.3
Mar.	139.0	20.1	110.0	–9.4	112.0	–7.4
Apr.	35.8	–52.1	37.6	–50.3	47.0	–40.9
May	140.0	29.7	98.6	–11.4	83.6	–26.4
June	128.0	25.7	101.0	–1.8	175.0	72.4
July	59.7	–55.6	36.1	–79.2	143.0	27.9
Aug.	134.0	21.1	89.2	-23.4	234.0	121.0
Sep.	143.0	30.5	192.0	79.8	226.0	114.0
Oct.	46.7	–42.2	91.9	3.0	70.6	–18.3
Nov.	222.0	143.0	25.9	–53.3	153.7	74.4
Dec.	223.0	149.0	84.6	10.4	47.0	–27.2
Annual	1334.0	139.0	1053.0	–143.0	1365.0	169.7

†The data were obtained from the closest local weather station (Stony Creek 2 N), located in Stony Creek, Sussex County, VA, at 36°58′ N/77°24′ W and 32.0m (105′) above s/l, which belongs to the U.S. Department of Commerce National Climatic Data Center. (http://www4.ncdc.noaa.gov/cgi-in/wwcgi.dll?wwDI~StnSrch~StnID~20026946). Depart indicates the difference between the monthly mean and 30-yr average.

Table 3. Corn and soybean leaf water potential (WP), grain yield, and corn photochemical efficiency (PE) responses to biosolids and nitrogen fertilization under conventional tillage and no tillage systems.†

	WP		Grain yield	
Treatment	Corn	Soybean	Corn	Soybean
	MPa		Mg ha^{-1}	
	Silking	Pod filling		
Control	–1.51a	–2.23a	5.003d	1.814c
0.5x Ag N rate (fertilizer)	–1.39ab	–2.17ab	7.762c	1.891bc
1.0x Ag N rate (fertilizer)	–1.28c	–2.13abc	7.942c	1.931abc
1.5x Ag N rate (fertilizer)	–1.37b	–2.13abc	8.286bc	2.023abc
1.0x Ag N rate (LS)	–1.26b	–2.07bc	9.357a	2.091ab
1.0x Ag N rate (AD)	–1.29b	–2.01c	8.837ab	2.142a
Conventional tillage	–1.33x	–2.06y	7.548y	2.004x
No tillage	–1.37x	–2.19x	8.180x	1.960x
Year 2009			10.838x	–
Year 2010	–1.40x	–2.23x	4.245z	0.893y
Year 2011	–1.30y	–2.01y	8.510y	3.071x
	PE (Fv/Fm)			
	11th leaf	Silking	Grain filling	
Control	0.710b	0.611c	0.661d	
0.5x Ag N rate (fertilizer)	0.738ab	0.647b	0.692c	
1.0x Ag N rate (fertilizer)	0.739ab	0.678a	0.712b	
1.5x Ag N rate (fertilizer)	0.749a	0.692a	0.715ab	
1.0x Ag N rate (LS)	0.749a	0.694a	0.730a	
1.0x Ag N rate (AD)	0.762a	0.691a	0.708bc	
Conventional tillage	0.740x	0.661y	0.699x	
No tillage	0.741x	0.676x	0.707x	
Year 2009	0.700z	0.668y	0.754x	
Year 2010	0.785x	0.612z	0.618y	
Year 2011	0.737y	0.726x	0.737z	

†Means followed by same letters within each column for each data set are not significantly different at $P \leq 0.05$.

ZR content by 28% and 26%, respectively, when compared with the control at the silking stage. When compared with the 1.0x N rate, LS and AD biosolids increased ZR content by 13% and 11%, respectively, at the silking stage. Because year × till × treatment and till × treatment interactions were significant at the grain filling stage, ZR content was analyzed separately by year, tillage system, and treatment (Table 4). At grain filling, the two biosolids increased ZR content above those of the control, 0.5x N and 1.0x N rates in all 3 yr. No difference in ZR content was observed between CT and NT in 2010 or 2011. The ZR content was higher in 2009 than 2010 and 2011.

Leaf IAA content

The biosolids, 1.0x N, and 1.5x N rate treatments increased IAA content at the 11th leaf stage (Table 4). Both biosolids and all N fertilizer treatments increased IAA content at the silking stage. The LS and AD biosolids increased IAA content by 42% and 37%, respectively, when compared with the control at the 11th leaf stage. Similarly, the two biosolids increased IAA content by 56% and 53%, respectively, relative to the control at the silking stage. When compared with the 1.0x N rate, the LS and AD biosolids increased IAA content by 13% and 11%, respectively, at the silking stage. The biosolids treatments, however, did not significantly affect IAA content at the grain filling stage.

No difference in IAA content was found between CT and NT systems. The IAA content was lower in 2010 (dry year) than in 2009 and 2011.

Leaf ABA Content

Abscisic acid (ABA) content was higher in the LS biosolids than the control at the silking and grain filling stages. The AD biosolids also increased ABA content at grain filling (Table 4). No difference in ABA content was found between CT and NT. Leaf ABA content was higher in 2010 (dry year) and 2011 than in 2009 at the silking and grain filling stages.

Table 4. Corn earleaf zeatin riboside (ZR), indole-3-acetic acid (IAA), abscisic acid (ABA) responses to biosolids and nitrogen fertilization under conventional tillage and no tillage systems.†

Treatment	ZR				
			Grain filling		
	11th leaf	Silking	2009	2010	2011
	ng g^{-1} FW				
Control	52.1c	47.2d	60.6c	37.5d	38.0c
0.5x Ag N rate (fertilizer)	57.1b	50.8cd	64.5c	41.5cd	43.6bc
1.0x Ag N rate (fertilizer)	59.1b	54.2c	72.2b	42.3cd	45.3b
1.5x Ag N rate (fertilizer)	68.8a	59.3b	74.1b	44.6bc	48.2ab
1.0x Ag N rate (LS)‡	66.8a	64.1a	84.4a	50.1ab	52.5a
1.0x Ag N rate (AD)§	65.5a	62.4ab	83.8a	51.4a	51.2a
Conventional tillage	61.7x	55.5x	80.2x	45.3x	45.8x
No tillage	61.5x	57.1x	66.2y	43.9x	47.1x
Year 2009	67.9x	79.2x	67.9x		
Year 2010	58.9y	43.1z	58.9y		
Year 2011	57.9y	46.6y	57.9y		

Treatment	IAA			ABA		
	11th leaf	Silking	Grain filling	11th leaf	Silking	Grain filling
	ng g^{-1} FW					
Control	112.2b	79.1d	83.7b	30.0a	52.1b	27.4c
0.5x Ag N rate (fertilizer)	118.8b	97.2c	87.1b	28.5a	52.5ab	28.5c
1.0x Ag N rate (fertilizer)	160.4a	108.9b	97.8b	26.8a	53.8ab	29.3bc
1.5x Ag N rate (fertilizer)	148.9a	115.5ab	150.7a	28.2a	55.2ab	31.6ab
1.0x Ag N rate (LS)‡	159.7a	123.2a	131.6ab	27.9a	56.6a	31.9ab
1.0x Ag N rate (AD)§	153.5a	120.7a	114.7ab	25.7a	55.7ab	32.3a
Conventional tillage	144.0x	109.6x	116.3x	28.0x	53.4x	30.4x
No tillage	140.5x	105.2x	105.6x	27.7x	55.2x	29.9x
Year 2009	213.9x	142.5x	151.9x	34.2x	40.5y	16.5y
Year 2010	116.0y	50.1z	41.9y	24.3y	59.8x	36.5x
Year 2011	96.9z	129.6y	139.0x	25.0y	62.7x	37.5x

†Means followed by same letters within each column for each data set are not significantly different at $P \leq 0.05$.

‡Lime stabilized, (LS).

§Anaerobically digested, (AD).

Leaf Proline Content

Biosolids and 1.5x N rate treatments increased leaf proline content when compared with the control at all three sampling dates (Table 5). The LS and AD treatments increased leaf proline content by 51% and 56%, respectively, compared with the control, and 39% and 35%, respectively, compared with the 1.0x fertilizer N rate. No difference in proline content was found between CT and NT practices. Greater leaf proline content occurred in 2010 (dry year) compared with 2009 or 2011.

Leaf SOD Activity

The LS and AD biosolids increased SOD activity relative to the control at the 11th leaf and silking stages in all 3 yr and also at the grain filling stage in 2011. Lime stabilized (LS) and AD biosolids increased SOD activity by 60% and 69% compared with the control, and 29% and 36%, respectively, compared with the fertilizer at 1.0x N rate as measured at the silking stage (Table 5). There were significant year × tillage × treatment and tillage × treatment interactions for leaf SOD activity at the grain filling stage. LS and AD treatments increased SOD activity in 2010 and 2011 except for AD under NT in 2010 (Table 5).

Greater SOD activity was found under CT relative to NT at the 11th leaf stage (Table 5). In addition, a greater SOD activity was observed in 2010 than 2009 or 2011.

Grain Yield

The LS and AD treatments increased corn grain yield by 87% and 77%, respectively, compared with the control averaged across 3 yr (Table 3). The LS and AD biosolids increased grain yield by 18% and 11%, respectively, above the 1.0x N rate. The grain yield was greater under NT than CT and higher in 2009 and 2011 than during the dry year (2010).

Soybean Responses

Leaf WP and PE

Soybean grown in the soil amended previously with LS and AD biosolids had greater leaf WP relative to the control at the pod filling stage (Table 3). The biosolids treatments resulted in greater soybean PE at flowering compared with the control, 0.5x and 1.0x N rates (Table 6). No difference in PE was found between CT and NT. Soybean PE was greater in 2010 than 2011 at the pod filling stage.

Table 5. Corn earleaf proline and superoxide dismutase (SOD) responses to biosolids and nitrogen fertilization under conventional tillage and no tillage systems.†

Treatment	Proline			SOD activity	
	11th leaf	Silking	Grain filling	11th leaf	Silking
	mg g^{-1} FW			U mg^{-1} protein‡	
Control	0.31e	0.41c	0.30c	143.2c	239.3c
0.5x Ag N rate (fertilizer)	0.38d	0.44bc	0.36c	153.8c	265.9bc
1.0x Ag N rate (fertilizer)	0.45c	0.46bc	0.48b	153.0c	297.7b
1.5x Ag N rate (fertilizer)	0.52b	0.52b	0.57a	179.8a	298.0b
1.0x Ag N rate (LS)§	0.59a	0.64a	0.57a	171.4ab	383.9a
1.0x Ag N rate (AD)¶	0.55ab	0.62a	0.48b	157.4bc	404.4a
Conventional tillage	0.47x	0.50x	0.46x	165.2x	309.0x
No tillage	0.46x	0.53x	0.46x	154.4y	320.7x
Year 2009	0.43y	0.48y	0.42y	160.2y	292.0y
Year 2010	0.53x	0.58x	0.53x	183.5x	397.4x
Year 2011	0.44y	0.48y	0.42y	135.6z	255.2z

Treatment	SOD activity – Grain filling					
	2009		2010		2011	
	CT	NT	CT	NT	CT	NT
	U mg^{-1} protein					
Control	144.5b	188.1ab	203.3e	221.0c	130.5c	152.4b
0.5x Ag N rate (fertilizer)	191.6a	168.8bc	232.5d	235.6c	136.6c	161.5b
1.0x Ag N rate (fertilizer)	162.3ab	209.8a	265.7c	215.1c	230.3b	138.6b
1.5x Ag N rate (fertilizer)	194.1a	155.3bc	388.5a	252.3c	238.2b	206.2b
1.0x Ag N rate (LS)§	143.9b	147.0c	356.3b	664.6a	338.1a	290.0a
1.0x Ag N rate (AD)¶	182.3ab	177.9abc	360.3a	373.1c	249.9b	294.3a
Conventional tillage	169.7x		301.1x		220.6x	
No tillage	174.5x		326.9x		207.1x	
Year 2009			172.1z			
Year 2010			314.0x			
Year 2011			213.8y			

†Means followed by same letters within each column for each data set are not significantly different at $P \leq 0.05$.

‡U: unit for SOD activity.

§Lime stabilized, (LS).

¶Anaerobically digested, (AD).

Leaf ZR Content

Significant year × tillage × treatment and tillage × treatment interactions were found for leaf ZR content (Table 7). In both 2010 and 2011, biosolids treatments increased soybean leaf ZR content when compared with the control at the two sampling dates except for AD under CT and LS under NT in 2010 at the pod filling stage (Table 7). On average over the two tillage systems, LS and AD increased leaf ZR content by 12% and 14%, respectively, at the flowering stage, and 22% and 20%, at the pod filling stage, when compared with the control in 2010. Leaf ZR content was greater under CT than NT at the flowering stage in 2011. Leaf ZR content was greater in 2011 than 2010 at flowering, but was less at the pod filling stage.

Leaf IAA Content

Biosolids treatments increased leaf IAA content in 2010, but did not impact it in 2011 except for AD which increased IAA content under NT at flowering when compared with the control (Table 6 and Table 8). At the pod filling stage, LS and AD increased IAA content by 22% and 19%, respectively, relative to the control (Table 6). IAA content was higher under CT than NT at the flowering stage in 2010 (Table 8), but lower at the pod filling stage when averaged over years (Table 6). Leaf IAA was greater in 2011 than 2010 at both sampling dates.

Leaf ABA Content

Biosolids treatments did not impact leaf ABA content except for a greater amount under NT in 2010 relative to the control at the flowering stage (Table 6 and Table 8). No difference in leaf ABA content was found between CT and NT. Leaf ABA content was greater in the dry year (2010) than in 2011.

Leaf Proline Content

Leaf proline content was greater in soybean amended previously with the biosolids except for LS treatment at the flowering stage (Table 6). The LS and AD treatments increased proline content by 15% and 21%, respectively,

Table 6. Soybean leaf photochemical efficiency (PE), zeatin riboside (ZR), abscisic acid (ABA), proline, and superoxide dismutase (SOD) activity responses to biosolids and nitrogen fertilization under conventional tillage and no tillage systems.†

Treatment	PE (Fv/Fm) Flowering	PE (Fv/Fm) Pod filling	IAA Pod filling	ABA Pod filling
			ng g^{-1} FW	
Control	0.684c	0.708b	384.5c	62.8ab
0.5x Ag N rate (fertilizer)	0.676c	0.722b	356.6c	61.8b
1.0x Ag N rate (fertilizer)	0.716b	0.750a	395.3bc	63.0ab
1.5x Ag N rate (fertilizer)	0.720ab	0.742a	451.2ab	62.9ab
1.0 x Ag N rate (LS)‡	0.734a	0.744a	469.0a	63.9a
1.0x Ag N rate (AD)§	0.730a	0.746a	455.7a	62.9ab
Conventional tillage	0.706x	0.732x	392.8y	63.4x
No tillage	0.714x	0.738x	444.6x	62.4x
Year 2010	0.729x	0.751x	316.4y	76.3x
Year 2011	0.691x	0.720y	521.0x	49.5y
	Proline		**SOD activity**	
	Flowering	**Pod filling**	**Flowering**	**Pod filling**
	mg g^{-1} FW		U mg^{-1} protein¶	
Control	0.663b	0.744c	278.9c	239.7c
0.5x Ag N rate (fertilizer)	0.668b	0.796bc	297.8c	254.0bc
1.0x Ag N rate (fertilizer)	0.899a	0.769c	308.7c	265.6b
1.5x Ag N rate (fertilizer)	0.727ab	0.786c	373.3ab	266.6b
1.0 x Ag N rate (LS)‡	0.761ab	0.859ab	405.5a	312.7a
1.0x Ag N rate (AD)§	0.889a	0.903a	359.1b	292.1a
Conventional tillage	0.776x	0.805x	342.1x	270.6x
No tillage	0.759x	0.814x	332.4x	272.9x
Year 2010	0.837x	0.860x	218.5y	176.7y
Year 2011	0.699y	0.759y	455.9x	366.9y

†Means followed by same letters within same column for each data set are not significantly different at $P \leq 0.05$.

‡Lime stabilized (LS).

§Anaerobically digested (AD).

¶Unit for SOD activity (U).

relative to the control, and 12% and 17%, respectively, when compared with the 1.0x N rate. Tillage system did not impact leaf proline content in soybean. Greater proline content was found in 2010 compared with 2011.

Leaf SOD Activity

The biosolids treatments increased leaf SOD activity when compared with the control, 0.5x and 1.0x N rates at both sampling dates (Table 6). The LS and AD biosolids increased SOD activity by 45% and 29%, respectively, relative to the control, and 31% and 16%, respectively, when compared with the 1.0x N rate as measured at the flowering stage. No difference in SOD activity was observed between CT and NT. Leaf SOD activity was decreased due to drought stress in 2010 when compared with 2011.

Soybean Grain Yield

The LS and AD treatments increased grain yield by 15% and 18%, respectively, when compared with the control (Table 3). No yield difference was found between CT and NT. The grain yield in 2010 was only 29% of that in 2011.

Table 7. Soybean leaf zeatin riboside (ZR) responses to biosolids and nitrogen fertilization under conventional tillage (CT) and no tillage (NT) systems.†

Treatment	ZR Flowering CT	ZR Flowering NT	ZR Pod filling CT	ZR Pod filling NT
	ng g^{-1} FW			
	2010			
Control	27.8b	28.0c	26.8b	27.9c
0.5x Ag N rate (fertilizer)	28.5b	28.6c	27.5ab	28.7c
1.0x Ag N rate (fertilizer)	28.8b	28.2c	30.4ab	29.9bc
1.5x Ag N rate (fertilizer)	28.6b	29.1bc	31.6ab	30.7bc
1.0x Ag N rate (LS)‡	31.9a	30.8ab	33.7a	33.2ab
1.0x Ag N rate (AD)§	31.4a	32.3a	30.4ab	35.3a
Conventional tillage	29.5x		30.1x	
No tillage	29.5x		30.9x	
	2011			
Control	28.1c	26.1b	24.8b	17.9e
0.5x Ag N rate (fertilizer)	30.4bc	27.6b	26.8ab	22.3d
1.0x Ag N rate (fertilizer)	31.1b	32.7a	24.3b	27.1c
1.5x Ag N rate (fertilizer)	33.0b	34.7a	30.7a	30.8b
1.0x Ag N rate (LS)‡	37.1a	33.1a	29.7a	33.9a
1.0x Ag N rate (AD)§	37.5a	32.4a	29.6a	33.1ab
Conventional tillage	32.9x		27.6x	
No tillage	31.1y		27.5x	
Year 2010	29.5y		30.5x	
Year 2011	32.0x		27.6y	

†Means followed by same letters within same column for each data set are not significantly different at $P \leq 0.05$.

‡Lime stabilized, (LS).

§Anaerobically digested, (AD).

DISCUSSION

Concentrations of trace elements in the two high quality biosolids tested were much lower than even restricted USEPA Pollutant Concentration Limits (USEPA, 1993). Soil pH did not reach excessive levels, and no nutrient deficiency associated with high pH or toxicity associated with heavy metals was observed.

The results of this study indicated that land application of LS and AD biosolids improved plant water status and PE relative to the control in corn regardless of tillage system. This is in agreement with previous studies by Zhang et al. (2005, 2009) who showed that biosolids application increased PE and delayed leaf wilting of tall fescue under drought stress. The photosynthetic system is sensitive to drought stress. Biosolids treatments served to improve osmotic adjustment and plant water status by increasing N-containing osmoprotectants (e.g., proline). In addition, biosolids treatments resulted in greater contents of growth hormones (such as ZR and IAA) which were associated with delayed leaf senescence and sustained photosynthetic function (e.g., PE) under drought stress (Zhang et al., 2009).

In a companion manuscript concerning soil effects of these treatments it was found that application of LS and AD biosolids increased plant available water (PAW; $p = 0.027$) at the end of 3 yr relative to the control and 1x fertilizer N rate (Li et al., 2013). As measured in the top 10 cm of the tilled treatments, increases in PAW of this sandy soil due to biosolids treatments may have played a role in improving corn and soybean physiological function and yield gain during periodic drought stress. In the study herein, measures of consistently improved nitrogen and carbon (proline, SOD, ZR, IAA) metabolism due to the biosolids most likely played a more primary role.

The results of this study showed that the LS and AD treatments and 1.5x N rate increased leaf ZR content when compared with the control and 1.0x N rate under either CT or NT conditions. The biosolids treatments also increased leaf IAA content when compared with the control. This is supported by previous studies by Zhang et al. (2005, 2009). Leaf ZR is closely associated with drought tolerance in higher plants because of its antisenescence activity (Man et al., 2011; Zhang and Ervin, 2004; Zhang et al., 2009). Zeatin riboside (ZR) is synthesized in roots and translocated to shoots, with one function being to delay leaf senescence, especially under drought stress conditions. Cytokinins also promote chlorophyll biosynthesis and chloroplast development (Davies, 2010). Exogenous application of ZR enhanced PSII photochemical efficiency and drought tolerance in creeping bentgrass (Zhang and Ervin, 2004).

Auxin (IAA) can enhance root development and also delay plant senescence under drought stress conditions (Zhang et al., 2009). The AD and LS biosolids, which contained 7.2 to 14.2 μg IAA g^{-1}, respectively, most likely provided IAA directly to the soil and served as an organic matter source for soil microbial production of these hormones. Zhang et al. (2009) noted that soil amended with biosolids contained greater auxin than those without biosolids application after tall fescue was grown for 6 mo. Increase in soil IAA may enhance root growth. An actively growing root system cannot only absorb water and nutrients efficiently but also synthesize more hormones such as ZR. Our results suggest that biosolids application may improve corn drought tolerance by enhancing the content of growth hormones (ZR and IAA) in leaf tissues. Such boosted cytokinin and auxin levels most likely are associated with the delayed leaf senescence and improved photosynthetic function noted in these trials.

The results of this study indicated that the biosolids increased corn leaf ABA content, especially at the grain filling stage. Abscisic acid (ABA) can induce stomatal closure and reduce plant water loss via transpiration under drought stress. The improvement of corn leaf water potential due to biosolids application may have been associated with the increase in leaf ABA content under drought stress.

In this study, soybean was planted in rotation with corn that had been treated with biosolids or N fertilizer in the previous growing season; the soybean crop did not receive any supplemental fertilizer N or biosolids during its production cycle. Our results showed that biosolids treatments also improved soybean PE and leaf growth hormone contents

Table 8. Soybean leaf indole-3-acetic acid (IAA) and abscisic acid (ABA) responses to biosolids and nitrogen fertilization under conventional tillage (CT) and no tillage (NT) systems.†

	IAA		ABA	
	Flowering			
Treatment	CT	NT	CT	NT
	ng g^{-1} FW			
	2010			
Control	41.2b	36.2b	69.1ab	66.9c
0.5x Ag N rate (fertilizer)	45.3b	40.4b	71.4a	68.9bc
1.0x Ag N rate (fertilizer)	64.0a	39.2b	66.7bc	72.8ab
1.5x Ag N rate (fertilizer)	52.8ab	50.2ab	67.7b	69.7bc
1.0x Ag N rate (LS)‡	65.6a	59.5a	63.2c	74.2a
1.0x Ag N rate (AD)	62.7a	63.5a	71.7a	71.1ab
Conventional tillage	55.3x		68.3y	
No till	48.2y		70.6x	
	2011			
Control	216.2a	170.4c	49.9a	48.3b
0.5x Ag N rate (fertilizer)	217.4a	250.0ab	49.0a	49.3b
1.0x Ag N rate (fertilizer)	219.5a	186.3bc	52.0a	49.9b
1.5x Ag N rate (fertilizer)	230.4a	196.7bc	48.2a	51.6ab
1.0x Ag N rate (LS)‡	169.7a	228.0abc	50.0a	52.0ab
1.0x Ag N rate (AD)§	167.1a	292.9a	50.7a	53.9a
Conventional tillage	203.4x		50.0x	
No till	220.7x		50.8x	
Year 2010	51.7y		69.4x	
Year 2011	212.0x		50.4y	

†Means followed by same letters within same column for each data set are not significantly different at $P \leq 0.05$.

‡Lime stabilized, (LS).

§Anaerobically digested, (AD).

(IAA and ZR) when compared with the control. It is our speculation that addition of biosolids to the soil provides organic substrates for enhanced microbial production and subsequent plant uptake of these important growth hormones. Microbes may use biologically active substances (e.g., biosolids) as substrates, producing and releasing hormones into the soil (Frankenberger and Arshad, 1995). Barea et al. (1976) found that among 50 bacterial isolates obtained from the rhizosphere, 86, 58, and 90% produced auxins, gibberellins, and kinetin-like substances, respectively. Auxin production in soil is likely most active in the plant rhizosphere or at microsites where organic substrates and microorganisms are abundant (Arshad and Frankenberger, 1993). The majority of microorganisms that produce cytokinins, particularly diazotrophic bacteria, also synthesize auxins (Arshad and Frankenberger, 1993). This suggests that biosolids application may promote soil microbial production of hormones and increase plant endogenous hormone levels.

The results of our study also showed that drought stress (in 2010) reduced SOD activity when compared with the less stressful 2009 and 2011 seasons. However, even in 2010, biosolids treatments improved corn leaf SOD activity. These beneficial effects of biosolids have been reported previously (Lakhdar et al., 2010; Zhang et al., 2005, 2009). Lakhdar et al. (2010) noted that municipal solid waste compost and sewage sludge significantly increased activities of antioxidant enzymes including SOD, CAT, APX, and glutathione reductase in wheat (*Triticum aestivum* L.). Plants possess antioxidant defense systems to detoxify ROS induced injury under stress. Plants with greater SOD activity in leaf tissues have been shown to efficiently remove ROS and minimize or prevent ROS-induced damage. Biosolids may promote SOD activity by providing N and improving enzyme (protein) biosynthesis. Meanwhile, certain biologically active substances such as cytokinin may trigger up-regulation of SOD activity (Zhang and Ervin, 2004).

The growth hormones (ZR and IAA) and SOD are N-containing compounds and their levels are closely related to N metabolism. In this study, we found that application of the two biosolids increased leaf proline content in corn and subsequent soybean plants. This is in agreement with previous studies with tall fescue in greenhouse conditions (Zhang et al., 2009). In a companion study, Li et al. (2013) found that LS and AD biosolids increased spring soil nitrate N, plant tissue N at silking, and post-season corn stalk nitrate N. Zhang et al. (2009) noted that drought-stressed tall fescue amended with biosolids had greater leaf tissue proline levels, a result which was associated with less leaf firing and better drought tolerance.

Amino acids in the biosolids may be effectively absorbed and assimilated by treated plants. Proline is considered an osmoprotectant and also possesses antioxidant properties. It can stabilize cell membranes and scavenge toxic ROS under drought stress. Greater relative proline levels have been reported to be positively correlated with drought tolerance (Zhang et al., 2009). In this study, increased leaf proline content due to biosolids application may have contributed to better drought tolerance and grain yield increase in corn and soybean.

The results of this study indicated that tillage did not consistently impact leaf hormone and proline contents and SOD activity in corn and soybean. Biologically active substances in biosolids such as IAA and amino acids may be incorporated into soil after application regardless of tillage practices. This suggests that biosolids may have similar effects on crop plant metabolism and drought tolerance regardless of conventional tillage and no tillage systems.

Biosolids treatments increased grain yield when compared with the control and fertilizer treatments at 1.0x and 0.5x N rates. This is in agreement with a previous study by Bowden et al. (2010) who reported that organic amendments increased corn grain yield under field conditions. It is interesting to note that tillage significantly affected corn grain yield in 2011, with NT producing higher grain yield than CT. Although not directly measured in this study, the advantages of NT over CT may have been due to improved PAW which may have resulted in greater grain yield (Wagger and Cassel, 1993). It was also noted that grain yields increased with increasing fertilizer N rate and surpassed the expected yield for this soil (7.54 Mg ha^{-1}; Simpson et al., 1993) in two of the 3 yr (2009 and 2011). Our results suggest that the grain yield improvement provided by the biosolids was much more than a N nutrition effect as documented by improved soil and plant water status and increased leaf contents of growth hormones, SOD, and proline.

CONCLUSIONS

Previous anecdotal observations and greenhouse studies by our research group indicated that the use of biosolids as a fertilizer N source often resulted in greater drought tolerance and yield improvement in comparison to mineral fertilizer treatments that provided equivalent nutrient availability. Greater rooting and sustained leaf photosynthetic function (antisenescence effects) in these trials led to the hypothesis that the biosolids were directly and/or indirectly increasing endogenous cytokinin and auxin concentrations. Subsequent greenhouse testing supported this hypothesis as soil and plant concentrations of cytokinin and auxin were shown to increase significantly due to biosolids treatments (Zhang et al., 2009). The results herein provide multiple-year, field-based evidence to support our previous findings. Biosolids are more than a slow-release N source. They directly and/or indirectly increase endogenous growth hormone concentrations that are closely associated with improvements in biochemical and physiological responses to abiotic stresses such as drought and heat. Increases in soil PAW due to biosolids application also improved drought resistance and grain yield. Results that are consistent from greenhouse to multiple-year field trials warrant the pursuit of molecular and microbial research aimed at a clearer understanding of the mechanisms of action at work when biosolids are used for crop production.

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