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WYDZIAŁ
PRZYRODNICZO-TECHNOLOGICZNY

Rozprawa doktorska
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Wielokierunkowe wykorzystanie sorga cukrowego oraz ocena jego oddziaływania na środowisko

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Serdecznie dziękuję prof. dr hab. inż. Józefowi Sowińskiemu

mojemu naukowemu Mentorowi za nieocenioną pomoc w tworzeniu niniejszej rozprawy

„Nauka jest jak niezmierne morze.

Im więcej jej pijesz, tym bardziej jesteś spragniony.”

Stefan Żeromski

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RODZIAŁ 1

SYNTEZA

Niniejsza rozprawa doktorska została przygotowana zgodnie z art. 13 ust. 2 ustawy z dnia 14 marca 2003 r. o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz. U. z 2003 r. nr 65 poz. 595 z późń. zm) w oparciu o zbiór artykułów opublikowanych w czasopismach naukowych. W skład rozprawy wchodzą 4 artykuły naukowe, które zostały zamieszczone w zbiorczym opracowaniu, jako rozdziały 3-6.

Sorgo dwubarwne *Sorghum bicolor* (L.) Moench - jednoroczny gatunek należący do rodziny traw *Poaceae* pochodzący z Afryki Wschodniej to jedno z najbardziej znaczących zbóż w skali globalnej. Pod względem zajmowanego areału i produkcji ziarna w skali światowej jest 5 zbożem . Oszczędną gospodarkę wodną zawdzięcza niskiemu współczynnikowi transpiracji oraz większym możliwościom pobierania wody z gleby, wynikającymi z dużego zasięgu systemu korzeniowego i wykorzystania wody z głębszych warstw gleby. Jego szerokie zdolności adaptacyjne, niewielkie wymagania środowiskowe, krótki okres wegetacji, szybkie tempo wzrostu, wysoki potencjał plonowania oraz wszechstronność użytkowania czynią ten gatunek jako wysoce perspektywiczny w dobie globalnych zmian klimatu. Wzrost temperatury, nierównomierny rozkład opadów w ciągu sezonu wegetacyjnego, częsty ich deficyt, powoduje że rośnie zainteresowanie tym gatunkiem także w naszym kraju. Wprowadzenie sorga cukrowego – gatunku o małych wymaganiach siedliskowych – na gleby niższych klas bonitacyjnych; lekkie i bardzo lekkie, których udział w Polsce jest znaczący, może stanowić rozwiązanie problemu zagospodarowania gleb marginalnych, na których uprawa innych gatunków roślin jest zawodna. W obszarze klimatu umiarkowanego to gatunek relatywnie nowy i brakuje doniesień naukowych o wielu istotnych elementach uprawy i wykorzystania sorga.

Mając na uwadze, powyższe w niniejszej rozprawie w sposób transdyscyplinarny ujęto 2 tematy badawcze. Z jednej strony założono ukazanie wielokierunkowej możliwości uprawy sorga, a z drugiej skupienie się na wieloaspektowym wpływie na środowisko.

W obecnych, intensywnych systemach produkcji rolniczej jednym ze znaczących problemów jest niewłaściwe zarządzanie nawożeniem azotowym przejawiające się niską efektywnością wykorzystania azotu przez rośliny. Negatywne oddziaływanie na środowisko wynika ze strat azotu na drodze wymywania azotanów, emisji tlenków azotu i uwalniania amoniaku do atmosfery, co prowadzi do zanieczyszczenia powietrza i wód gruntowych. Nawożenie azotowe wpływa nie tylko na wielkość plonu, ale również istotnie determinuje jego jakość. Przenawożenie azotem jest podstawowym czynnikiem powodującym gromadzenie się azotanów w roślinach. Ilość tych związków wzrasta zwłaszcza w niekorzystnych warunkach środowiskowych, np. w okresie deficytu opadów i suszy. Ma to szczególne znaczenie w przypadku roślin przeznaczonych na paszę. Jakość paszy jest bowiem w dużej mierze zależna od zawartości azotanów, które stanowią szczególne zagrożenie dla zwierząt przeżuwających. Sorgo cukrowe cechujące się szybkim tempem wzrostu. Jako roślina cyklu fotosyntezy C4 ma wysoką tendencję do akumulowania azotanów, do poziomu przekraczającego wartości określone jako toksyczne.

Celem doświadczenia była ocena wpływu zróżnicowanego nawożenia azotowego na wielkość plonu biomasy sorga odmiany Sucrosorgo 304 oraz zawartość azotanów w biomasie jak i wytłokach. Wytłoki stanowią produkt powstający po wyciśnięciu soku, co jest pierwszym, przygotowawczym etapem produkcji etanolu w przypadku energetycznego wykorzystania sorga. W doświadczeniu testowano polimerowy nawóz otoczkowany Meister® LP70 jako alternatywę w stosunku do nawozów konwencjonalnych – mocznika i saletry amonowej. Nawozy stosowano w dwóch dawkach (90 i 180 $kg \cdot ha^{-1}$), aplikując je jednokrotnie lub w dawce podzielonej. Wykazano, że plony w latach badań znacząco się różniły i wynosiły od 9,1 do 14,8 t s.m. ha^{-1}. Zgodnie z przedstawionymi wynikami badań wykorzystanie nawozu otoczkowanego w dawce 90 kg ha^{-1} można uznać jako rekomendowane do otrzymania paszy o bezpiecznym dla zwierząt poziomie azotanów. W pracy podjęto ponadto próbę znalezienia prostego i nieinwazyjnego wskaźnika do oceny przydatności biomasy sorga do wykorzystania paszowego pod kątem zawartości azotanów. Dowiedziono, że za taki wskaźnik można uznać mierzenie indeksu zieloności liści (SPAD). Wykazano bowiem zależność pomiędzy wartością tego indeksu a ilością azotanów. Jest to sposób przewidywania jakości i przydatności biomasy z sorga cukrowego jako paszy.

W warunkach klimatu umiarkowanego, głównym kierunkiem wykorzystania sorga cukrowego są cele paszowe. Kiszonkę z sorga można uznać za wartościowe uzupełnienie kiszonki z kukurydzy będącej podstawową paszą objętościową w żywieniu bydła w naszym kraju. Obok dominującego w Europie przeznaczenia na paszę, coraz częściej artykułowany jest pogląd, że jest sorgo to gatunek o dużej przydatności do produkcji biopaliw – etanolu i biogazu. Zakładanie plantacji roślin energetycznych na gruntach odłogowanych, ubogich w materię organiczną i składniki pokarmowe wymusza jednak konieczność ich uzupełnienia. Zapotrzebowanie na składniki mineralne można w łatwy sposób zaspokoić aplikując nawozy mineralne. Jednak niska zawartość materii organicznej w glebie pozostaje czynnikiem limitującym funkcje produkcyjne gruntów ornych oraz sprzyja wymywaniu składników pokarmowych, zwłaszcza na glebach lekkich. Znaczne obniżenie obsady zwierząt w gospodarstwach rolnych w Polsce po roku 1990 oraz odejście od konwencjonalnego chowu ściółkowego doprowadziło do ograniczenia dostępności i stosowania obornika. Wobec deficytowych ilości obornika wzrasta w praktyce rolniczej, szczególnie w uprawie roślin niekonsumpcyjnych, znaczenie alternatywnych źródeł substancji organicznej, w tym produktów odpadowych, takich jak poferment czy osad ściekowy. Stosowanie w celach nawozowych produktów o charakterze odpadowym jest sposobem ich racjonalnego zagospodarowania w zgodzie ze zasadami zrównoważonego rozwoju. Zatem obok wykorzystania azotowego nawozu otoczkowanego w uprawie sorga w kolejnych badaniach ujętych w publikacji z rozdziału 4 zajęto się oceną przydatności do nawożenia produktów o charakterze odpadowym.

W doświadczeniu wykorzystano suszony w suszarni solarnej osad ściekowy w formie granulatu oraz ciecz pofermentacyjną, zwaną w skrócie pofermentem. W oparciu o wyniki analiz składu chemicznego osadu i pofermentu, oraz wyniki z poprzedniej serii badań (rozdział 3) ustalono ich dawki na podstawie założenia wprowadzenia 100 kg $N \cdot ha^{-1}$. Produkty odpadowe zaaplikowano jednokrotnie przed siewem, wprowadzając osad ściekowy w dawce 18,5 $t \cdot ha^{-1}$ i poferment w dawce 45 $m^3 \cdot ha^{-1}$. Przebadano 4 odmiany sorga cukrowego: (1) późna odmiana Goliath, (2) późna, fotoperiodycznie obojętna odmiana Sucrosorgo 506, (3) SuperSile20 odmiana późna oraz (4) średniej klasy wczesności mieszaniec Rona1. Odmiany pochodziły z różnych hodowli, odpowiednio: niemieckiej, amerykańskiej, francuskiej i węgierskiej. Badania wykazały, że plony wahały się od 10,5 do 23,6 ton suchej masy na hektar, odpowiednio dla nienawożonych roślin odmiany SuperSile 20 i dla Sucrosorgo 506 nawożonego osadem ściekowym. Zarówno osad ściekowy jak i poferment zgodnie z wynikami doświadczenia można uznać za substytut nawozu mineralnego pozwalający na otrzymanie plonu na zbliżonym poziomie do nawożenia mocznikiem.

W aspekcie oddziaływania uprawy sorga cukrowego na środowisko oceniono emisję gazów cieplarnianych. Produkcja rolnicza ma istotny wpływ na zmiany klimatu. Podaje się, że obiecującym sposobem obniżenia emisji gazów cieplarnianych z sektora rolniczego jest precyzyjne rolnictwo, poprawa zarządzania nawożeniem, uprawa roślin cechujących się wyższym potencjałem sekwestracji węgla, a także zastępowanie nawozów mineralnych ich organicznymi alternatywnymi substytutami. Wobec tego w publikacji rozdziału 5 oceniono wpływ wykorzystania do nawożenia osadu ściekowego i pofermentu w porównaniu do konwencjonalnie używanego mocznika na emisję gazów cieplarnianych. Emisje określono zgodnie z metodologią IPCC (*Intergovermental Panel on Climate Change*) do obliczeń wykorzystując kalkulator BioGrace rekomendowany przez dyrektywy unijne. Emisje podzielono na 2 kategorie: (1) zewnętrzne związane z produkcją i transportem produktów koniecznych do prowadzenia uprawy (nasiona, nawozy, herbicydy) i (2) wewnętrzne, związane z prowadzeniem uprawy od przygotowania gleby aż do zbioru. Emisji na etapie produkcji biopaliw nie brano pod uwagę. Wykazano, że aplikacja mocznika miała największy udział w zewnętrznej emisji gazów cieplarnianych odpowiadając za 54% tej kategorii emisji. Emisja ogólna była mniejsza o odpowiednio 14 i 11%, gdy zastosowano osad ściekowy i poferment. Przedstawione wyniki świadczą o tym, że wykorzystanie produktów odpadowych jako substytutów nawozów mineralnych jest szansą na obniżenie emisji CO_2 z produkcji sorga na cele energetyczne.

Uprawa sorga na cele energetyczne w warunkach klimatu umiarkowanego wciąż jest nierozpowszechniona, a głównym kierunkiem użytkowania jak wspomniano pozostają cele paszowe. W ostatnich latach obserwuje się jednak wzrost zainteresowania jego uprawą z przeznaczeniem na produkcję „zielonej energii". W dobie ciągłego wzrostu znaczenia na arenie międzynarodowej energii wytwarzanej ze źródeł odnawialnych, głównym wyzwaniem wciąż pozostaje opracowanie metod efektywnie obniżających koszty jej produkcji oraz sposobów optymalizacji procesów technologicznych.

Zrównoważona uprawa roślin energetycznych wymaga ciągłej poprawy wskaźników bilansu i efektywności energetycznej. W rozdziale 5 zamieszczono publikację ujmującą porównanie energetycznej efektywności uprawy sorga jako surowca do produkcji bioetanolu i biogazu. Określono ilości otrzymywanego metanu i etanolu z tony suchej masy a także z jednostki powierzchni. Ponadto wyliczono uzysk energetyczny dla obu biopaliw. Obliczenia obejmowały etap uprawy i przygotowania biomasy sorga. Konwersja świeżej biomasy sorga do metanu generowała 76,6 – 179,5GJ ha^{-1}, natomiast w przypadku etanolu było to 22,6 – 70,5GJ ha^{-1}. Zastosowanie pofermentu skutkowało uzyskaniem najwyższego współczynnika efektywności energetycznej w produkcji etanolu dla obu badanych odmian – Sucrosorgo 506 i Rona 1 (odpowiednio 5,3 i 7,3), a w przypadku produkcji metanu dla odmiany Rona 1 (15,5).

Badania wskazują, że sorgo w warunkach klimatu umiarkowanego powinno być uprawiane w przeznaczeniem na produkcję biogazu.

W aspekcie wielokierunkowego wykorzystania sorga w niniejszej rozprawie osobny rozdział poświęcono wykorzystaniu sorga cukrowego jako rośliny, która może mieć znaczenie w zrównoważonej kontroli zachwaszczenia. Oddziaływanie chwastów to najbardziej uciążliwy dla roślin stres biotyczny, który odpowiada za 34% strat plonu. Konwencjonalne metody zwalczania chwastów opierają się na użyciu herbicydów. Jednak w obliczu problemu zwiększającej się ilości pozostałości pestycydów w agroekosystemie oraz pojawiania się co raz to nowych przypadków uodparniania się chwastów, alternatywne metody kontroli zachwaszczenia, w tym wykorzystanie zjawiska allelopatii zyskują na znaczeniu.

Na podstawie licznie zgromadzonej literatury w publikacji rozdziału 6 wykazano, że sorgo posiada właściwości allelopatyczne, wynikające z obecności kwasów fenolowych i ich aldehydowych pochodnych oraz głównego związku allelopatycznego – sorgoleonu wydzielanego przez korzenie. Z punktu widzenia praktyki rolniczej istotne są sposoby jak można wykorzystać potencjał allelopatyczny sorga w uprawie. Najlepiej przebadanym sposobem jest produkcja wodnego ekstraktu z nadziemnych części sorga. Inne możliwości to włączenie sorga do płodozmianu, jego uprawa jako rośliny okrywowej, zaorywanie resztek pożniwnych. Najbardziej wymagającą metodą lecz i najbardziej obiecującą jest wytworzenie alleloherbicydu.

RODZIAŁ 2

SPIS PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

W skład niniejszej rozprawy doktorskiej wchodzą 4 artykuły naukowe:

1. Sowiński J., Głąb L. 2018. The effect of nitrogen fertilization management on yield and nitrate contents in sorghum biomass and bagasse. Field Crops Research 227: 132-143. doi: 10.1016/j.fcr.2018.08.006.
2. Głąb L., Sowiński J. 2019. Sustainable production of sweet sorghum as a bioenergy crop using biosolids taking into account greenhouse gas emission. Sustainability 11: 3033. doi:10.3390/su11113033
3. Głąb L., Sowiński J., Chmielewska J., Prask H., Fugol M., Szlachta J. 2019. Comparison of the energy efficiency of methane and ethanol production from sweet sorghum (*Sorghum bicolor* (L.) Moench) with a variety of feedstock management technologies. Biomass Bioenergy 129: doi: 10.1016/j.biombioe.2019.105332
4. Głąb L., Sowiński J., Bough R., Dayan F.E. 2017. Allelopathic Potential of Sorghum (*Sorghum bicolor* (L.) Moench) in Weed Control: A Comprehensive Review. Chapter II Advances in Agronomy 145: 43-95. doi: 10.1016/bs.agron.2017.05.001

RODZIAŁ 3

Wpływ nawożenia azotowego na plon sorga i zawartość azotanów w biomasie i wytłokach

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The effect of nitrogen fertilization management on yield and nitrate contents in sorghum biomass and bagasse

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ABSTRACT

Improved nitrogen (N) management for sorghum production on sandy soils is necessary to maximize N use efficiency, increase yield and mitigate N losses contributing to environmental contamination. A three-year field experiment was conducted on Brunic Arenosols soil in the southwestern region of Poland in a moderate tem-perate climate to evaluate the effects of varied N fertilization management methods on sweet sorghum hybrid (Sucrosorgo 304) yield and nitrate (NO_3^-) contents in biomass and bagasse. Sorghum was grown under two levels of N supply – 90 and 180 kg N ha^{-1}, once or split applied as enhanced-efficiency N fertilizer – polymer coated urea and as common N sources – ammonium nitrate and urea. The experimental design included control treatment without N input. Sweet sorghum biomass yields did not significantly differ between N sources. The split application of conventional N sources did not improve sorghum biomass yield. No significant differences were observed in biomass yield averaged across years and N rates in response to the application strategy in the case of all N sources. Highly variable weather conditions during the three sorghum growing seasons resulted in significantly varied biomass yields, ranging from 9.1 to 14.8 Mg dry mass ha^{-1}. Nitrate content tended to be higher in biomass within each fertilizer compared with that in bagasse. This study demonstrates that polymer coated urea at the rate of 90 kg N ha^{-1} provides biomass with a safe level of NO_3- and can be recommended in sustainable sweet sorghum production for forage. In addition, in this study an indirect strategy based on Soil Plant Analysis Development (SPAD) readings measured during growing season was proposed to predict NO_3- level in biomass at harvest. Results showed that this non-invasive method could provide valuable information on potential NO_3^- accumulation and animal poisoning risk. However, further research is needed to establish the quantitative relationship between SPAD readings and NO_3^- level in relation to environmental factors and varied N supply.

1. Introduction

Modern intensive crop production systems are based on high ni-trogen (N) fertilizer inputs to maximize crop yields in order to meet the demand of the growing world population (Le Noë et al., 2017). The world agricultural use of N fertilizers in 2015 was over 109 million tonnes and according to forecasts this amount will increase over the coming years (FAOSTAT, 2017). Improper N management, especially over-application of N fertilizers, has led to low N use efficiency limiting crop yields (Zhang et al., 2015). Low N recovery has resulted in N losses through ammonia (NH_3) volatilization, nitric oxide (NO) and nitrous oxide (N_2O) emissions, nitrate (NO_3^-) leaching and surface runoff and has consequently contributed to air pollution, groundwater con-tamination and problems connected with the eutrophication of surface waters (Benoit et al., 2015; Demurtas et al., 2016; Pan et al., 2016; Yan et al., 2015). Therefore, more sustainable N management is necessary to increase low N use efficiency in modern agricultural systems and mi-tigate the environmental pollution resulting from N losses (Zhang et al., 2017). Sustainable N management is particularly crucial in environ-ments prone to N leaching such as coarse-textured (sandy) soils with a poor sorption complex and limited retention capacity (Herrera et al., 2016). Split fertilizer application is considered one such improved N management practice providing higher crop uptake efficiency due to minimizing the length of time that inorganic N is present in the soil solution prior to uptake by the plant (Grant et al., 2012; Kilcer et al., 2002). However, the split application system has some drawbacks. It requires an extra field operation and, as a result, is more time- and labor-consuming than single fertilizer use (Trenkel, 2010). Moreover, studies on the benefits of N rate splitting have strongly inconsistent results. In brown midrib (BMR) sorghum (Sorghum bicolor (L.) Moench)

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× Sudangrass (Sorghum sudanense (Piper) Stapf.) hybrid production in the northwestern USA and in grain sorghum production in Ethiopian highland Vertisols, split application has shown advantages as compared to the single application of full N rate (Kilcer et al., 2002; Melaku et al., 2017). However, under the growing conditions of northern Ethiopia splitting the N application does not enhance the biomass yield of sweet sorghum (Gebremedhin et al., 2016).

Applying slow- or controlled-release fertilizer, such as polymer coated urea, which more closely matches N release to uptake by crops, is a promising alternative practice to split fertilizer application (Trenkel, 2010; Du et al., 2006). Many recent studies have indicated that the application of controlled-release fertilizer significantly in-creases N use efficiency and crop yield and decreases the risk of N loss (Kabała et al., 2017; Sowiński et al., 2016b; Yan et al., 2015; Yang et al., 2017). In contrast, outcomes of studies conducted in northeastern Australia have shown that urea coated with a nitrification inhibitor does not provide substantial improvements in sorghum grain yield (De Antoni Migliorati et al., 2016; Lester et al., 2016).

In recent years, researchers have made significant efforts to devise N management strategies for maximizing N use efficiency, taking into account the 4R nutrient management principles (right source, right rate, right time, and right placement) (IPNI, 2018). Keeping this in mind, monitoring of crop N status and in-season N dynamics plays a pivotal role in delivering correct N recommendations. The Soil Plant Analysis Development (SPAD) meter (also known as a chlorophyll meter) is a well-known tool for determining crop N status that can be useful in the adjustment of fertilizer level during the growing season (Herrera et al., 2016). In this study, we evaluated the utility of SPAD measurements as a tool to predict NO_3^- content in sorghum biomass and bagasse.

N fertilization management significantly affects not only crop yield, but also plant chemical composition, thus influencing quality. Applying N fertilizer at rates exceeding crop demand decreases forage quality as a consequence of NO_3^- accumulation (Liu et al., 2016). Many sorghum hybrids, the related Sudangrass and hybrids between these two species are important forage crops, particularly in warm, dry regions (Pino and Heinrichs, 2016). However, feeding with sorghum or Sudangrass can pose the threat of inadvertent NO_3- poisoning (Bhatti et al., 2011). The fast growing C4 grasses from the sorghum genus (Sorghum Moench) tend to accumulate allelochemicals (Głąb et al., 2017), as well as toxic NO_3^- levels, particularly when grown under stress conditions such as low temperature, drought or high humidity (Bhatti et al., 2011; Bolan and Kemp, 2003; Sidhu et al., 2011). Moreover, the problem of NO_3^- accumulation can escalate as a result of an increasing frequency of drought.

Very few studies have included measurements of the response of NO_3^- accumulation in crop biomass to controlled-release fertilizer or split N fertilizer application. Furthermore, the outcomes of these studies are inconsistent (Gagnon et al., 2016; Payne et al., 2015; Shapiro et al., 2016). In maize (Zea mays L.) production in the midwestern United States on loamy sand soil, polymer coated urea application resulted in greater stalk NO_3-N as compared with urea ammonium nitrate (Shapiro et al., 2016). Under similar climatic and soil conditions, both polymer coated urea and urea amended with urease and nitrification inhibitors decreased the amount of NO_3-N in maize stems as compared with a split application of urea (Maharjan et al., 2016). It is also worth mentioning that so far there is a lack of such studies conducted in the temperate climate of Central Europe. Until now, to the best of our knowledge no reports are available on the NO_3^- content in sorghum bagasse; an important by-product in the sweet sorghum-based ethanol industry which remains after extraction of juice from sorghum biomass and is considered a promising alternative livestock feed resource (Rao et al., 2013).

Given this, the objectives of this study were to assess the forage yield and NO_3^- content in sorghum biomass and bagasse in response to: (i) polymer coated urea and conventional N fertilizers: non-coated urea and ammonium nitrate, (ii) N rate and (iii) N fertilizer application strategy. Based on a determination of the relationship between SPAD readings and NO_3- content, the possibility of using SPAD as a quick, early method for forecasting NO_3^- content in biomass at harvest was evaluated.

The results of this study will help to devise sustainable N fertiliza-tion management in the production of sweet sorghum with safe NO_3^- levels under moderate climatic conditions.

2. Materials and methods

2.1. Study site and experimental materials

A three-year (2013–2015) field experiment to test the impact of N fertilization management on sweet sorghum yield and NO_3^- accumu-lation in sorghum biomass and bagasse was conducted at the Agricultural Research Station belonging to the Institute of Agroecology and Plant Production of Wroclaw University of Environmental and Life Sciences. The study site (51°10′25″N and 17°07′02″E) is located in the Lower Silesia region of southwestern Poland. The area is characterized by a temperate and moderate climate, with oceanic influences from the west and continental weather masses from the east (Dubicki et al., 2002). The annual mean air temperature is 9.0 °C, with a monthly minimum of −0.4 °C in January and a maximum of 18.8 °C in July. The annual mean precipitation is 583 mm. The growing season is char-acterized by daily mean temperatures ≥5 °C for 237 days (Tomczyk and Szyga-Pluta, 2016). The monthly rainfall and temperature at the experimental site during the period of the experiment were recorded at 10-minute intervals using an AsterMet automatic meteorological station (temperature sensor and hygrometer HMP-155; rain gauge station TPG-

Table 1

Monthly temperature and precipitation for site where sorghum trial was conducted during three growing seasons (2013–2015). Data were obtained from automatic weather station (Agricultural Research Station of Wroclaw University of Environmental and Life Sciences, southwestern Poland).

Month	$T_{average}$ (°C)				Precipitation (mm)			
	2013	2014	2015	Long-term average (1981–2010)	2013	2014	2015	Long-term average (1981–2010)
April	9.2	10.6	8.9	8.9	42.7	55.2	15.8	30.5
May	14.6	13.3	13.5	14.4	135.9	101.4	21.0	51.3
June	17.7	16.6	16.6	17.1	171.7	40.2	73.3	59.5
July	20.5	21.2	20.3	19.3	36.3	52.9	55.6	78.9
August	19.0	17.3	22.7	18.3	68.2	75.0	5.6	61.7
September	12.9	15.5	15.1	13.6	105.8	72.2	23.2	45.3
October	10.8	10.7	8.4	9.1	7.8	59.4	20.0	32.3
Mean/sum for period Apr.-Oct.	14.9	15.0	15.1	14.4	568.4	456.3	191.3	359.5

The long-term (30-yr) average weather data (1981–2010) was obtained from the meteorological station of Wroclaw University.

034-NH) (https://monitoring.a-ster.net/login.php). In 2013, 2014 and 2015, the mean air temperature during the growing season was higher than the long-term average by 0.5, 0.6 and 0.7 °C, respectively (Table 1). Mean temperatures in the period from August to October 2015 were 1.7 °C higher than the long-term average. The total pre-cipitation in the period from August to October strongly varied between years (Table 1). In 2013 and 2014, the total precipitation during the growing season was 208.9 and 96.8 mm, respectively, higher than the multiyear average. In 2015, the total precipitation for the growing season was extremely low and represented only 53% of the long-term average for the growing season. A significant water deficit occurred in particular in the second part of the growing season, from August to October 2015, when the total precipitation was 90 mm lower then the long-term average.

Sorghum trials were carried out on coarse-textured (sandy) soils (3% clay, 9% silt and 87% sand) originally classified as Brunic Arenosols soil (IUSS Working Group WRB, 2014), currently converted into Gleyic Phaeozems soil (Anthric, Arenic, Brunic) as a result of long-term, intense cultivation (Kabała et al., 2011; Łabaz and Kabała, 2016). The groundwater table is at the depth of 75–85 cm. Soils have a thick (28–32 cm) humus horizon and are close-neutral-subalkaline in pH (6.8–7.8). Prior to planting, the main properties for the top soil layer (0–30 cm) at the study site were as follows: medium–high content of organic carbon (0.5–2.0%), high content of available phosphorus, medium content of available potassium and magnesium, and highly varied content of nitrogen (Table 2). A detailed description of the physico-chemical soil properties has been presented in previous studies (Gałka et al., 2016; Sowiński et al., 2016a).

Meister® LP70 polymer coated urea (containing 42% N, release longevity in water of 25 °C – 70 days) served as the controlled release urea in the present study. The enhanced efficiency fertilizer was man-ufactured by Chisso Asahi Fertilizer Co., Ltd., Tokyo, Japan. The coating material was a hydrophobic organic polymer compound, polyolefin (a monomer of carbon and hydrogen), which is subject to slow degradation as a result of solar radiation influence and then wetting and drying of prills causing cracking and mechanical destruc-tion (Trenkel, 2010). This fertilizer will be called throughout this study as coated urea. The common N fertilizers used were commercial non-coated urea containing 46% N and ammonium nitrate containing 34% N. K fertilizer was potassium salt (60% K_2O) and P fertilizer was triple super phosphate (46% P_2O_5).

A sweet × sweet sorghum hybrid (Sucrosorgo 304; Sorghum Partners – Chromatin, Inc., USA) recommended for silage production was used for the experiment. This is a medium to full-maturity,

Table 2
Basic physical and chemical properties of soils (0–30 cm depth) at the experi-mental site.

Parameter	minimum	maximum
Sand[a] (%)	84.5	90.0
Silt[a] (%)	7.0	11.0
Clay[a] (%)	2.0	3.0
pH (H_2O)[b]	6.8	7.8
Soil organic matter (mg_kg^{-1})	4.6	20.9
	0.25	0.85
C:N ratio	17	19
Available phosphorus (mg kg^{-1})	127	460
Available potassium (mg kg^{-1}) —	67	166
	18	23

The soil parameters were analyzed in the mixed, air dried, grounded and sieved (2 mm) composite sample resulted from the blending of ten single soil surface (20 cm depth) samples, which were randomly collected from ten spots across the experimental field.

[a] The soil texture was defined as sand, 0.05–2.0 mm; silt, 0.002–0.05 mm, and clay, < 0.002 mm diameter.
[b] Soil to water content 1:2.5.

photoperiod insensitive sweet sorghum characterized by a high content of soluble sugars and high biomass yield potential, 55–59 Mg ha^{-1} in the weather conditions of southwestern Poland (Szydełko-Rabska and Sowiński, 2014).

2.2. Experimental design and treatments

The experiment was conducted using the split-split-plot design (SSP) with four replicates (each experimental plot with an area of 7 m length × 2.1 m width). In this study, there were thirteen treatments: a control with no N application and twelve N management treatments. The analyzed variance factors were: (i) N fertilizer source; (ii) rate of N application; and (iii) timing of N fertilizer application. Three N rates were tested for their effect on yield and NO_3^- content: 0, 90 and 180 kg N ha^{-1}. The rate of 90 kg N ha^{-1} was chosen as an optimal N dose for sorghum cultivation in Polish conditions, based on the results of a previous study (Sowiński and Szydełko-Rabska, 2013). The rate of 180 kg N ha^{-1} was tested to examine ways for decreasing NO_3^- con-tent under heavy N application.

Tested N fertilizers were applied either in full at the 2–3-leaf stage of sorghum (at 4, 5, 3 June and 27, 30, 26 days after sowing in 2013, 2014 and 2015, respectively) or as equally split applications at the 2–3-leaf stage and at 7–8-leaf stage (at 22, 16, 9 July and 75, 71, 62 days after sowing in 2013, 2014 and 2015, respectively) (45/45 and 90/90 kg N ha^{-1}). Fertilizer treatments were compared to the control without N application. Phosphate and potassium fertilizers in all treatments were broadcast just before sowing at 90 kg ha^{-1} (P_2O_5) and 120 kg ha^{-1} (K_2O). The previous crop had been oats (Avena sativa L.) in 2013.

Seeds were sown using a Wintersteiger self-propelled plot drill at a depth of 3–5 cm and at a density of 200,000 seeds ha^{-1} with a row spacing of 70 cm on 8 May 2013, 6 May 2014, and 8 May 2015. In 2013, the sowing operation had to be repeated (16 June 2013) due to poor plant density affected by adverse weather conditions, in particular low temperature at the beginning of the growing season.

Lumax® 537.5 SE (terbuthylazine 187.5 g L^{-1} + mesotrione 37.5 g L^{-1} + s-metolachlor 312.5 g L^{-1}; Syngenta, Switzerland) was applied to control a wide spectrum of dicot and monocot species of weeds. Herbicide was applied without a safener; therefore, a lower rate (2 l ha^{-1}) than that recommended for maize (3.5–4 l ha^{-1}) was used di-rectly after sowing, pre-emergence to avoid injuries to sorghum plants caused by the phytotoxic effects of s-metolachlor.

2.3. Sampling, measurements and chemical analyses

SPAD values (SPAD units) were taken from the uppermost, fully expanded leaves using a chlorophyll meter (Minolta SPAD-502, Japan). Three SPAD readings were collected across the adaxial surface and averaged as a mean SPAD value for each leaf. A total of ten re-presentative plants were measured in each plot. SPAD data were col-lected twice during each growing season. The first measurement was taken during the second half of N rate application (at 7–8-leaf stage; at

22, 16, 9 July and 75, 71, 62 days after sowing in 2013, 2014 and 2015, respectively), and the second SPAD measurement was performed 60 days later at booting stage.

The same (uppermost, fully expanded) leaves were taken for total leaf N analysis at both times of SPAD value measurements. Three leaves were sampled per plot, oven-dried at 70 °C for 24 h, ground using a knife mill (LMN-100C, TestChem, Poland) and passed through a 1 mm mesh. Total leaf N content was determined using the Kjeldahl method according to the procedure of the Association of Official Agricultural Chemists (AOAC, 1990; Büchi Distillation Unit K-350, Switzerland). The crop was harvested mechanically from 14.7 m^2 plots using a brush cutter (Stihl FS400 C, Germany) at milky dough stage: on 10 October 2013, 15 October 2014, and 8 October 2015 (153, 162 and 155 DAS, respectively) leaving a stubble height of 0.2 m. Sorghum plant samples were weighed during the harvest operation for fresh yield (Mg

ha^{-1}) estimation. Following sorghum harvest each year, the stubble was tilled and remaining crop residues were reincorporated into the soil.

A sample of four representative plants within each plot was col-lected and the aggregate sample was cut to an average particle size of 7 mm using a bowl chopper (Krag). A subsample of shredded biomass was taken for juice extraction (piston press – perforated cage of 80 mm diameter, 30 bar pressure; Hydropras Skalar, Poland). Another sub-sample of biomass, as well as a sample of the bagasse (solid lig-nocellulosic residue material remaining after extraction of soluble su-gars), was weighed and oven-dried at 70 °C until reaching constant mass. The moisture content was gravimetrically determined and the dry matter ratio was calculated. Subsamples of fresh biomass and bagasse were taken directly for NO_3^- analysis. Nitrate content was analyzed by the Bremner microdistillation method according to the Starck mod-ification after extraction in 2% acetic acid solution (Nowosielski, 1988). All chemical analyses were carried out in triplicate and the mean values are presented on an oven-dried basis.

2.4. Statistical analyses

Means and the standard errors of the mean (SEM) for each para-meter were calculated across four plot replicates. One-way analyses of variance (ANOVAs) for each individual year's data were conducted. A mean comparison was performed using Tukey’s multiple range test at the $P < 0.05$ level. Data were combined across the years for pooled analysis. In pooled analysis, four-way ANOVA was performed to assess the interactions of year, N fertilizer, N rate and N application strategy.

A linear model was used to describe the relationship between SPAD chlorophyll meter readings and NO_3^- content, as well as total N and NO_3^- concentrations. The coefficients of determination R^2 were cal-culated. The Statistica (version 13.1 StatSoft, Poland) software package was used to carry out all statistical analyses and devise figures.

3. Results

3.1. ANOVA analysis

The effects of the variables year, fertilizer, rate and application strategy on biomass and bagasse yields and NO_3^- contents in biomass and bagasse were analyzed for significance at $P < 0.05$, $P < 0.01$ or $P < 0.001$. Significant effects of year were observed on all measured response variables ($P < 0.001$), while fertilizer, N rate and interaction of year × fertilizer exerted significant effects on either yield of bagasse, NO_3^- contents in biomass or bagasse (Table 3). No significant effects on biomass and bagasse yields were revealed as a result of the appli-cation strategy. Similarly, significant effects of each of the possible interactions were observed only for NO_3^- contents in biomass and bagasse ($P < 0.001$).

3.2. Yield response to N source, N rate and fertilizer application strategy

Significant yearly differences in sweet sorghum biomass and ba-gasse yields were reported in this study. In 2014, the yield, when averaged across all the treatments, was 24.3% and 38.5% higher than in 2013 and 2015, respectively (Table 4). Except for the ammonium ni-trate-90-single treatment, all other N fertilizer treatments resulted in significant improvements in biomass yield over the control in 2013. However, in 2014 and 2015, no N fertilizer treatments significantly enhanced sorghum biomass yield. Among all the treatments, the highest biomass yield occurred in urea-90-split, urea-180-single and coated urea-180-single treatments in 2013, 2014 and 2015, respectively (Table 4).

Biomass yield averaged across N rates and application strategies was significantly affected by N fertilizer source in 2013 alone, while sig-nificant effects of N fertilizer on bagasse yields were observed in 2013 and 2014. Coated urea produced a statistically equal biomass yield averaged across N rate and application strategy compared to urea and ammonium nitrate in each experimental year (Table 4). Increasing N rates from 90 to 180 kg ha^{-1} had no significant effect on biomass yield. Sorghum biomass and bagasse yields averaged across N fertilizers and their rates were not significantly affected by the application strategy in any experimental year.

Based on averaging across years and N application strategies, in-creasing N levels from 90 to 180 kg N ha^{-1} in the case of all fertilizers had no significant effect on biomass yield (Fig. 1A). At the nitrogen dose of 90 kg N ha^{-1}, coated urea produced almost the same yield of sorghum biomass as urea applied at the same rate (Fig. 1A). No sig-nificant differences were observed in biomass yield averaged across years and N rates in response to application strategy in the case of all N fertilizers (Fig. 1B).

3.3. Nitrate contents in sweet sorghum biomass and bagasse

Both the NO_3^- contents in biomass and bagasse averaged across all treatments significantly varied across experimental years. In 2014, NO_3^- content in biomass was 2.9 and 3.5 times lower than in 2013 and 2015, respectively (Table 5). Significant differences between the treatments were found for NO_3^- level in both biomass and bagasse in each year. Among all thirteen treatments, ammonium nitrate-180-split,

Table 3

Summary of the analysis of variance of sweet sorghum for year, fertilizer, N rate, N application strategy, and their possible interactions on biomass and bagasse dry matter yields, and NO_3^- contents (pooled of 2013, 2014 and 2015).

Effect	df	Biomass yield (Mg DM ha^{-1})	Bagasse yield (Mg DM ha^{-1})	NO_3^- content in biomass (mg kg DM^{-1})	NO_3^- content in bagasse (mg kg DM^{-1})
year	2	***	***	***	***
fertilizer	2	ns	ns	***	***
rate	2	*	***	***	***
application	1	ns	ns	***	***
year × fertilizer	4	ns	***	***	***
year × rate	4	ns	*	***	***
fertilizer × rate	2	ns	ns	***	***
year × application	2	ns	ns	***	***
fertilizer × application	2	ns	ns	***	***
rate × application	1	ns	ns	***	***
year × fertilizer × rate	4	ns	ns	***	***
year × fertilizer × rate × application	4	ns	ns	***	***

DM: dry matter.
ns: No significant effects; * Significant effect at P < 0.05 level; *** Significant effect at P < 0.001 level.

Table 4

The sweet sorghum biomass and bagasse yields (Mg dry matter ha^{-1}) as affected by different N treatments for 2013, 2014 and 2015. Average values for each factor and overall average values for year are listed in the bottom section of the table. Each value represents the mean of four replications ± standard error of the mean (SEM).

Treatment	Biomass					Bagasse				
	2013 Mg DM ha^{-1}	2014		2015		2013		2014	2015	
Control	7.3a ± 0.3	12.2	± 2.0	8.7	± 1.74.7a ± 0.2			8.5a ± 1.4	5.6	± 1.1
AN90-single	9.7ab ± 0.7	16.7 ± 0.6		9.6	± 0.87.8b ± 0.6			14.2bcd ± 0.7	6.5	± 0.6
AN90-split	11.5bc ± 0.4	15.1	± 0.8	9.9	± 1.18.3b		± 0.3	12.0abc ± 0.6	6.1	± 0.7
AN180-single	11.6bc ± 0.5	14.2 ± 0.5		10.2 ± 1.6		8.5b ± 0.3		14.9 cd ± 0.6	8.0	± 1.3
AN180-split	11.0bc ± 0.2	15.8	± 1.0	10.1 ± 1.6		8.6b ± 0.2		16.7d ± 1.2	8.5	± 1.4
U90-single	12.0bc ± 0.5	14.8	± 0.9	8.0	± 0.79.1b		± 0.4	11.6abc ± 0.7	5.4	± 0.5
U90-split	12.7c ± 1.0	13.3	± 1.0	8.6	± 1.29.9b		± 0.8	11.0abc ± 0.7	6.0	± 0.8
U180-single	12.4bc ± 0.8	17.0	± 1.1	8.5	± 2.69.7b ± 0.5			13.1bcd ± 0.8	5.4	± 1.7
U180-split	11.9bc ± 0.8	16.0	± 1.1	8.4	± 1.59.5b		± 0.7	13.0bcd ± 0.8	5.4	± 1.0
PCU90-single	11.9bc ± 0.4	14.9	± 1.2	8.7	± 1.48.5b ± 0.2			11.9abc ± 0.9	5.9	± 0.9
PCU90-split	10.6bc ± 0.4	14.0	± 1.3	8.9	± 1.48.6b ± 0.3			10.3ab ± 0.9	5.9	± 0.9
PCU180-single	12.2bc ± 0.4	15.4 ± 1.1		11.2 ± 2.5		9.3b ± 0.4		12.8abcd ± 0.8	7.1	± 1.6
PCU180-split	10.3bc ± 0.7	13.2	± 0.8	8.4	± 1.57.9b ± 0.4			11.3abc ± 0.8	6.0	± 1.0
Average for factors:										
Fertilizer*										
AN	11.0a ± 0.3	15.5 ± 0.4		9.9 ± 0.6		8.3a ± 0.2		14.5b ± 0.6	7.3 ± 0.5	
U	12.3b ± 0.4	15.3 ± 0.6		8.4 ± 0.8		9.6b ± 0.3		12.2a ± 0.4	5.5 ± 0.5	
PCU	11.2ab ± 0.3	14.3 ± 0.6		9.3 ± 0.2		8.6a ± 0.8		11.6a ± 0.4	6.2 ± 0.5	
N rate**										
0	7.3a ± 0.3	12.2 ± 2.0		8.7 ± 1.4		4.7a ± 0.2		8.6a ± 1.4	5.6 ± 0.9	
90	11.4b ± 0.3	14.8 ± 0.4		9.0 ± 0.4		8.7b ± 0.2		11.9b ± 0.4	6.0 ± 0.3	
180	11.5b ± 0.3	15.3 ± 0.4		9.5 ± 0.7		8.9b ± 0.2		13.6c ± 0.5	6.7 ± 0.5	
Application strategy***										
single	11.6 ± 0.3	15.5	± 0.4	9.4	± 0.78.8 ± 0.2			13.1 ± 0.4	6.4	± 0.5
split	11.3 ± 0.3	14.6	± 0.4	9.1	± 0.58.8 ± 0.2			12.4 ± 0.5	6.3	± 0.4
Average for year****										
2013	11.2b ± 0.2					8.5b ± 0.2				
2014	14.8c ± 0.3					12.4c ± 0.4				
2015	9.1a ± 0.4					6.3a ± 0.3				

For each N treatment, individual factor and year, means followed by a different letter within the same column are significantly different at $P < 0.05$ based on analyses by one-way ANOVAs followed by Tukey's multiple range tests. No lowercase letters indicate non-significant differences within a column.
DM: dry matter; AN: ammonium nitrate; U: urea; PCU: polymer coated urea.

* Denotes that values were averaged across two N fertilizer rates (90 and 180 kg ha^{-1}) and two strategies of application (single and split).

** Denotes that values were averaged across three N fertilizers: ammonium nitrate (AN); urea (U); polymer coated urea (PCU) and two strategies of application (single and split).

*** Denotes that values were averaged across three N fertilizers (AN, U, PCU) and two N fertilizer rates (90 and 180 kg ha^{-1}).

**** Denotes that values were averaged across all treatments.

ammonium nitrate-180-single and urea180-split treatments resulted in highest NO3- content in sorghum biomass, in 2013, 2014 and 2015, respectively.

Among the three fertilizer sources, ammonium nitrate contributed the highest NO_3^- accumulation in biomass and bagasse when averaged across rate and application treatment. Coated urea resulted in sig-nificantly lower NO_3^- content in biomass than the ammonium nitrate application in each experimental year and the urea application in 2015 (Table 5). Both NO_3^- contents in biomass and bagasse averaged across fertilizers and application strategies showed increasing trends as application rates of N were increased. However, the rate of 90 kg N ha^{-1} did significantly increase in the NO_3^- content in biomass and bagasse when compared with the control in any experimental year. With the application of 180 kg N ha^{-1}, the NO_3^- content in biomass was significantly increased (44.8, 66.8 and 48.4%) over the application of the lower rate, and 83.7, 91.3 and 76.6% higher than the control in 2013, 2014 and 2015, respectively (Table 5). The application of 180 kg
N ha^{-1} increased NO3- content in bagasse significantly: 42.6, 56.1 and 74.3% in 2013, 2014 and 2015, respectively, compared with the use of a lower N rate. Split application resulted in significantly higher NO_3^-

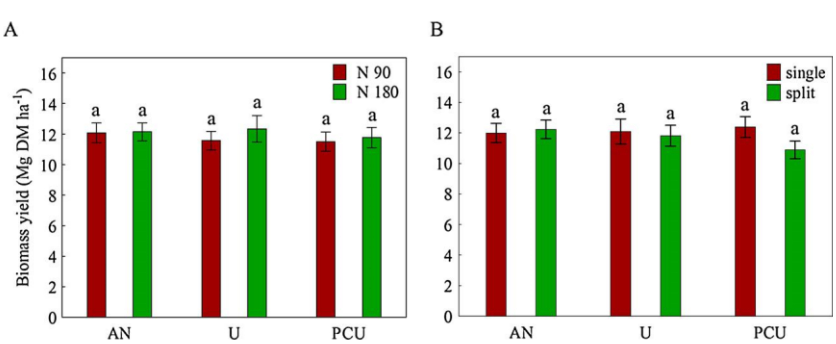

Fig. 1. (A) Effects of N fertilizer: ammonium nitrate (AN), urea (U), polymer coated urea (PCU) and N rate (90, 180 kg N ha^{-1}) on biomass yield. Data averaged across two N applica-tion strategies (single, split) and three experimental years. (B) Effects of fertilizer and application strategy on biomass yield. Data averaged across two N rates and three experimental years. Whiskers represent standard errors. The different small letters indicate significant differences within (A) N rates or (B) N fertilizer application strategies at $P < 0.05$ level.

Table 5
Nitrate contents in sorghum biomass and bagasse (mg kg^{-1} dry matter) as affected by different N treatments for 2013, 2014 and 2015. Average values for each factor and overall average values for year are listed in the bottom section of the table. Each value represents the mean of four replications ± standard error of the mean (SEM).

Treatment	Biomass			Bagasse		
	2013 mg kg DM^{-1}	2014	2015	2013	2014	2015
Control	1425a ± 18	306a ± 18	2361a ± 48	877a ± 7	716a ± 3	879a ± 42
AN90-single	1894ab ± 27	816ab ± 20	10799c ± 256	937a ± 9	1210c ± 2	3630d ± 72
AN90-split	10239f ± 118	3232c ± 92	5178b ± 113	4827g ± 31	3018e ± 69	2366b ± 52
AN180-single	9208e ± 131	6220e ± 191	17424d ± 341	5270h ± 57	3828g ± 51	4755e ± 100
AN180-split	16195h ± 68	5318d ± 162	11306c ± 716	8245i ± 64	5860h ± 53	5113e ± 23
U90-single	6769d ± 154	656a ± 50	4680b ± 209	2779d ± 31	819ab ± 8	1183a ± 61
U90-split	5272c ± 86	1363b ± 66	5332b ± 180	3692e ± 93	1084bc ± 51	1289a ± 81
U180-single	5444c ± 59	3813c ± 19	11564c ± 448	2798d ± 49	2569d ± 116	13188f ± 198
U180-split	11190g ± 52	3715c ± 88	18869d ± 552	4441f ± 18	3480f ± 69	24656g ± 226
PCU90-single	2213ab ± 38	500a ± 65	3123ab ± 126	1266b ± 4	987abc ± 16	2807bc ± 29
PCU90-split	2626b ± 40	405a ± 14	2208a ± 183	1241b ± 5	862ab ± 7	2411b ± 29
PCU180-single	5115c ± 195	1322b ± 34	1616a ± 65	1895c ± 24	1319c ± 11	3283cd ± 33
PCU180-split	5434c ± 309	599a ± 24	4764b ± 49	3057d ± 22	1142bc ± 20	2295b ± 31
Average for factors						
Fertilizer*						
AN	9384b ± 1568	3897b ± 645	11177b ± 1347	4820b ± 802	3479b ± 516	3966 ± 322
U	7169ab ± 739	2386ab ± 433	10111b ± 1773	3428ab ± 214	1988ab ± 337	10079 ± 3003
PCU	3846a ± 450	706a ± 113	2928a ± 369	1865a ± 227	1077a ± 53	2699 ± 120
N rate**						
0	1425a ± 10	306a ± 10	2361a ± 28	877a ± 4	716a ± 2	879a ± 24
90	4835a ± 736	1162a ± 241	5220a ± 676	2457a ± 355	1330a ± 189	2281a ± 209
180	8764b ± 990	3498b ± 494	10093b ± 1532	4284b ± 514	3033b ± 397	8882b ± 1942
Application strategy***						
single	5107a ± 624	2221 ± 520	8201 ± 1368	2491a ± 351	1789 ± 265	4807 ± 960
split	8493b ± 1119	2439 ± 442	7943 ± 1384	4250b ± 524	2574 ± 439	6355 ± 2035
Average for year****						
2013	6386b ± 677			3179ab ± 334		
2014	2174a ± 320			2069a ± 247		
2015	7632b ± 912			5220b ± 1042		

For each N treatment, individual factor and year, means followed by a different letter within the same column are significantly different at P < 0.05 based on analyses by one-way ANOVAs followed by Tukey's multiple range tests. No lowercase letters indicate non-significant differences within a column.
DM: dry matter; AN: ammonium nitrate; U: urea; PCU: polymer coated urea.

* Denotes that values were averaged across two N fertilizer rates (90 and 180 kg ha^{-1}) and two strategies of application (single and split).
** Denotes that values were averaged across three N fertilizers: ammonium nitrate (AN); urea (U); polymer coated urea (PCU) and two strategies of application (single and split).

*** Denotes that values were averaged across three N fertilizers (AN, U, PCU) and two N fertilizer rates (90 and 180 kg ha^{-1}).
**** Denotes that values were averaged across all treatments.

content in biomass averaged across three fertilizers and their two rates in 2013 compared with single fertilizer application. In contrast, in 2015, a decreasing NO_3^- content in biomass was recorded as a result of splitting N, but the difference was not significant. With the split ap-plication, the rates of NO_3^- content in bagasse averaged across ferti-lizers were 41.4, 30.5 and 24.4% higher than for single application in 2013, 2014 and 2015, respectively, but the difference was significant only in 2013 (Table 5).

Nitrate content in sweet sorghum biomass averaged across both N application strategies and all experimental years was analyzed for responses to increasing N application rates for each fertilizer, as shown in Fig. 2A. The response of biomass NO_3^- content to N application rate was greater when ammonium nitrate or urea was applied. Urea and ammonium nitrate at the rate of 180 kg N ha^{-1} increased NO_3^- content significantly, i.e. the contents were 51.0 and 55.9% higher, respec-tively, compared with application of urea and ammonium nitrate at the rate of 90 kg N ha^{-1} (Fig. 2A). In the case of coated urea, NO_3^- in biomass also exhibited an upward trend with increasing N application rate; however, NO_3^- content did not increase significantly. Coated urea at the rate of 90 kg N ha^{-1} resulted in the lowest NO_3^- content when

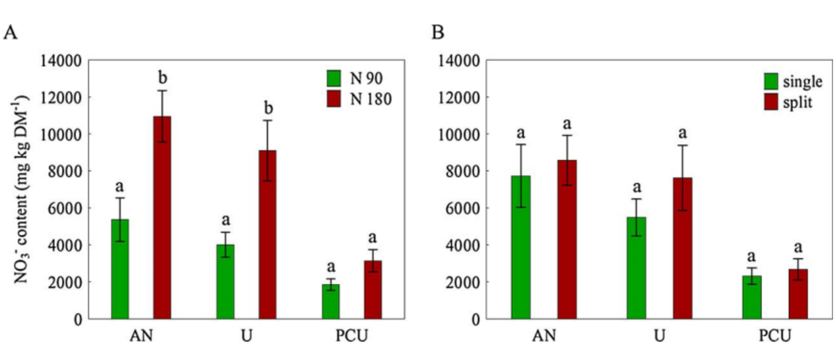

Fig. 2. (A) Effects of N fertilizer: ammonium nitrate (AN), urea (U), polymer coated urea (PCU) and N rate (90, 180 kg N ha^{-1}) on NO3- content in sorghum biomass. Data averaged across two N application strategies (single, split) and three experimental years. (B) Effects of fertilizer and application strategy on NO3- content in sorghum biomass. Data averaged across two N rates and three experimental years. Whiskers represent standard errors. The different small letters indicate significant differences within (A) N rates or (B) N fertilizer application strategies at P < 0.05 level.

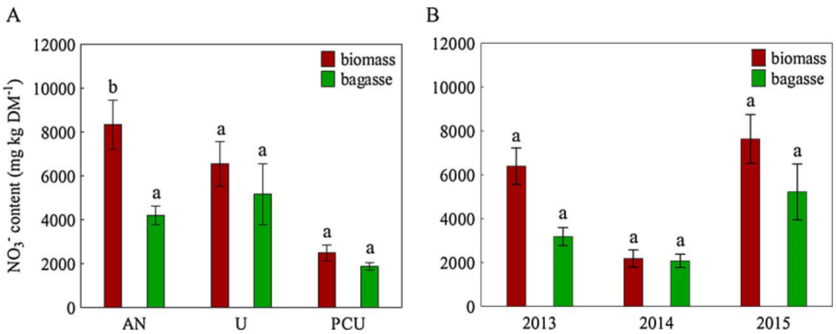

Fig. 3. The comparison of NO_3^- contents in sorghum biomass and bagasse. (A) Effects of N fertilizer: ammonium nitrate (AN), urea (U), polymer coated urea (PCU). (B) Effects of growing season: 2013, 2014, 2015. Whiskers represent stan-dard errors. The different small letters indicate significant differences within biomass and bagasse at P < 0.05 level.

averaged across application strategies and all years, while 180 kg N ha^{-1} applied as ammonium nitrate produced the highest NO_3^- content. There were no significant responses in NO_3^- content averaged across two N application rates and all years to N application strategy for any fertilizers (Fig. 2B). However, N rate splitting of ammonium nitrate, urea and coated urea insignificantly increased biomass NO_3^- content by 9.9, 28.0 and 13.4%, respectively, over the single application at full rate.

Nitrate contents in biomass and bagasse averaged across N rates, both N application strategies and all experimental years were com-pared, as shown in Fig. 3A. Nitrate content tended to be higher in biomass within each fertilizer compared with that in bagasse. The NO_3^- content in bagasse was 49.6% lower than that in biomass for AN application. Nitrate content in bagasse averaged across N fertilizers, N rates and N application strategies tended to be lower in each experi-mental year, but no significant difference was found between biomass and bagasse NO_3^- contents in any year (Fig. 3B).

3.4. Relationship between biomass and bagasse NO_3^- contents and SPAD readings and leaf N content

The relationships between SPAD readings and biomass and bagasse NO_3^- contents were plotted and linear regression equations were fitted (Fig. 4). The adjusted coefficients of determination (R^2) were resolved to describe goodness-of-fit for the regression equations. The NO_3-contents were positively related to SPAD readings. In each experimental year, linear regression equations generated by SPAD readings measured at 7–8-leaf stage were poorer fits as compared with the data measured at booting stage. The correlation of SPAD readings measured at the 7–8 leaf and boot stages with both biomass and bagasse NO_3^- contents was highly significant in 2013. In 2015, the relationship between them was not significant (P > 0.05). In 2014, the biomass and NO_3^- contents had a significantly positive linear relationship with SPAD readings measured at booting stage, while for SPAD readings from the first measurement the relationship was not significant (Fig. 4).

The R^2 values between total leaf N content at both stages of plant development and biomass and bagasse NO_3^- contents are given in Table 6. Biomass and bagasse NO_3- contents had positive linear re-lationships with total leaf N content. There was a general trend for linear regression equations generated by total leaf N content measured at 7–8-leaf stage to be a poorer fit as compared with the data measured at booting stage. Only in 2013 were both biomass and bagasse NO_3^- content significantly correlated with both total leaf N content (at the 7–8 leaf and booting stages). In 2014, the linear relationships between total leaf N content measured at 7–8-leaf stage and biomass and bagasse NO_3- contents were not significant. The total leaf N contents both at the 7–8 leaf and booting stages were significantly positively correlated to biomass NO_3- content alone in 2015. The R^2 values indicated that biomass and bagasse NO_3- contents were not correlated with biomass and bagasse yields (P > 0.05; data not presented).

4. Discussion

4.1. Seasonal difference

The biomass and bagasse yields, which were averaged across all treatments, varied significantly across experimental years, presumably because of the highly variable weather conditions. Both biomass and bagasse yields were lower in 2015, and this is likely because of the severe drought that occurred from August to October.

Plant NO_3^- content is highly dependent on weather conditions (Ćustić et al., 2003). In this study, significant seasonal differences in biomass and bagasse NO_3^- contents were observed, with the highest NO_3- content recorded in 2015, which was associated with the strong water-deficit stress. These results are in accordance with the findings from Neilson et al. (2015), who revealed under controlled conditions that water deficiency resulted in significantly higher foliar NO_3^- con-tent in sorghum seedlings. These findings were confirmed by the later report of Gleadow et al. (2016). Similarly, in a study on maize in the midwestern United States, water-stress conditions contributed to higher stalk NO_3^- level as compared to full irrigation treatments (Maharjan et al., 2016). In the current study, under extremely adverse weather conditions occurring in the second part of the growing season in 2015, plants tended to NO_3- accumulation probably as a result of reduced growth rates which are associated with decreasing rates of NO_3^- re-duction to NH_4^+ and its further assimilation into organic compounds. Similarly, based on field experiments with Sudangrass carried out by Sunaga et al. (2008) in Japan, it was reported that poorer rates of crop growth and development under adverse climatic conditions were major factors contributing to higher NO_3-N level in Sudangrass biomass.

4.2. Response of sorghum yield to different N fertilization management

In the current study, only in 2013 did N application result in sub-stantially greater biomass yield as compared to the control (with no N applied). These findings may be due to the high N content in soil before the experiment was established. However, these results may indicate that sweet sorghum has a very high efficiency at extracting soil N, which is consistent with outcomes of studies conducted by Adams et al. (2015), who reported that sorghum is capable of extracting high quantities of soil N even when grown under N-limiting conditions of marginal sandy soils on relatively low fertilizer N inputs. The outcomes of this work are consistent with previous studies conducted under temperate conditions in Poland, which have indicated that increasing N rate beyond 100 kg N ha^{-1} does not significantly improve sorghum yield (Księżak et al., 2012; Sowiński and Liszka-Podkowa, 2008). Si-milarly, based on the results of a study conducted on semi-arid marginal land in Inner Mongolia it can be concluded that N fertilizer demand of sorghum is low and the rate of 60 kg N ha^{-1} is recommended for sweet sorghum production for forage (Tang et al., 2018).

Based on the results of this study, split N application does not in-crease sorghum biomass yield. Conversely, in Ethiopian highland Vertisols, Melaku et al. (2017) reported an increase in sorghum grain

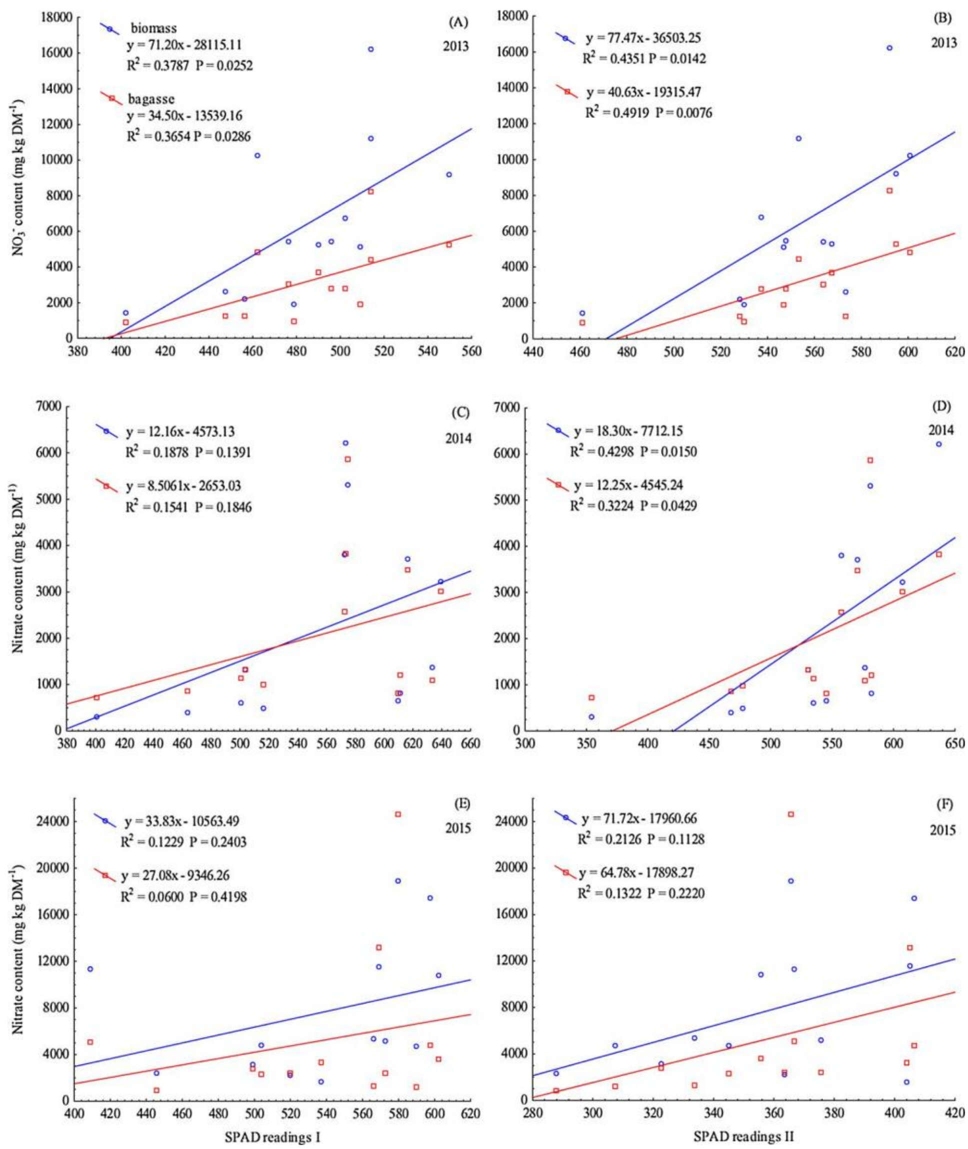

Fig. 4. Relationship between SPAD readings and biomass and bagasse NO_3^- contents in 2013 (A, B), 2014 (C, D) and 2015 (E,F). SPAD readings I (A, C, E) denotes SPAD readings measured during second half of N rate application at 7–8-leaf stage and SPAD readings II (B, D, F) denotes SPAD readings measured at booting stage.

yields over a single dose application with split N fertilizer application. Varmazyari et al. (2012) recommended the rate of 180 kg N ha^{-1} split into six equal doses applied during the growing season in forage sorghum under the semi-arid climate conditions of Iran.

The outcomes of the current study show that a slow release fertilizer does not contribute to increased sorghum biomass yield compared to conventional N fertilizers. It is worth mentioning that this study did not include treatments with limited N supply, only an optimal and a very high level of N were tested. It can be assumed that some benefits of a slow release fertilizer or splitting application can occur with the use of a lower rate of N. In Poland, knowledge about N fertilization manage-ment in sorghum is still in its initial stages of development. Similarly, studies on split or time release application is considered pioneering, and therefore further research is needed to evaluate the effect of varied N rates, including treatment with limited N supply.

Similarly, Grant et al. (2012) found that, under growing conditions across varied regions in western Canada, controlled-release fertilizer or split applications of N at the optimal rate did not provide any im-provements in crop yields or N use efficiency as compared to one ap-plication of non-coated urea. Other studies have also indicated no or only little response of maize grain yield to enhanced-efficiency urea as compared with conventional N fertilizers (Chilundo et al., 2016; Mello et al., 2017; Sistani et al., 2014).

The results of research conducted in subtropical cropping systems on Oxisols have shown that maize and wheat (Triticum aestivum L.) grain yield, biomass production and N recovery are not significantly affected by DMPP – (3,4-dimethylpyrazole phosphate) treated urea (De Antoni Migliorati et al., 2014). In contrast, Amal et al. (2007) reported that slow release N fertilizer significantly increased grain and straw yield of grain sorghum compared with conventional N sources – urea, ammonium nitrate and ammonium sulfate.

Based on results indicating improvements in maize yield, reducing N loss and greater profitability of maize production, Yang et al. (2017) compared slow-release urea with conventional urea, at common rates for the growing conditions of China (195 kg N ha^{-1}), and they re-commended matrix-based urea use in maize production to reduce N loss and improve grain yield. Similarly, studies conducted by Bartholomew et al. (2017) indicated some benefits of polymer coated urea application in improving crop yield and quality compared to uncoated urea. An-other study found that a mixture of coated urea and untreated urea resulted in 6.8–9.8% higher maize grain yield over uncoated urea treatments (Geng et al., 2017). Research in China has shown that

Table 6

Relationship between total leaf N content and biomass and bagasse NO_3^- contents in 2013, 2014, 2015.

Season	Parameter	Total leaf N content I	Total leaf N content II
2013	Biomass NO_3^- content	y = 6102.11x −14,134.66 R^2 = 0.4527, P = 0.0117	y = 19,082.00x − 41,930.33 R^2 = 0.5714, P = 0.0028
	Bagasse NO_3^- content	y = 2844.30x −6386.58 R^2 = 0.4043, P = 0.0195	y = 8436.43x −18,182.87 R^2 = 0.4590, P = 0.0109
2014	Biomass NO_3^- content	y = 1583.30x −3026.22 R^2 = 0.2911, ns	y = 4643.47x −9795.12 R^2 = 0.3537, P = 0.0321
	Bagasse NO_3^- content	y = 1044.67x −1362.64 R^2 = 0.2124, ns	y = 3418.15x −6742.23 R^2 = 0.3212, P = 0.0434
2015	Biomass NO_3^- content	y = 8956.48x −19,553.72 R^2 = 0.5028, P = 0.0067	y = 23,499.68x − 39,511.33 R^2 = 0.3462, P = 0.0344
	Bagasse NO_3^- content	y = 7349.41x −17,088.79 R^2 = 0.2581, ns	y = 24,524.48x − 43,980.37 R^2 = 0.2874, ns

ns: not significant.

Total leaf N content I was measured during the second half of N rate application at 7–8-leaf stage and total leaf N content I was measured about 60 days later at booting stage.

combining controlled-release urea and conventional urea improved crop yields under a wheat-maize double cropping system (Zheng et al., 2016). Studies in the central United States on silt loamy soil have shown that coated urea produces higher maize grain yield compared with untreated urea (Gordon, 2014).

4.3. Nitrate content: effects of differing N fertilization management

The ability to accumulate nitrate in cell vacuoles prior to its as-similation can be recognized as a beneficial trait in crop breeding, providing excess uptake of NO_3^-, when plant-available N is in plentiful supply, for later use during further crop development (Worland et al., 2017). During the reproductive phase, N uptake and assimilation of nitrate into amino acids and then proteins decline, whereas grain yield is highly reliant on efficient remobilization of nitrogenous compounds (Bahrami et al., 2017; Kant, 2017). However, excessive doses of ni-trogen fertilizer are considered as the one of the most significant factors leading to NO_3^- build-up in crops (Anjana and Iqbal, 2007). The findings of the current study showed that N rate had a marked effect on NO_3- contents in sorghum biomass and bagasse. Based on the present results, it can be concluded that NO_3- contents in both biomass and bagasse significantly increased as application rates of N were increased when common N fertilizers were applied. These results are in agree-ment with those reported by Abo-Zeid et al. (2017), who tested four N rates under the growing conditions of Egypt, starting from a low rate of 120 kg N ha^{-1}, through 180 and 240 kg N ha^{-1} to a very high N rate of 300 kg N ha^{-1}. They indicated that NO_3- content in Sudangrass biomass exhibited a rising trend with increasing N application rate. Similarly, Neilson et al. (2015) reported that foliar NO_3- concentration in sorghum was significantly affected by N rate and increased with increasing N rate. They tested three levels of N (low, moderate and high) in a greenhouse experiment.
In our study, there was an overall trend to produce biomass with higher NO_3- content as a result of the application of split fertilizers. These outcomes are consistent with those of previous research conducted by Maharjan et al. (2016), who compared the effect of two rates (180, 225 kg ha^{-1}) of nitrogen applied as urea and coated urea on stalk nitrate nitrogen content in maize. The N rate of 225 kg ha^{-1} is considered a high N rate in sorghum production in the mid-western United States. Increasing N rates from 180 kg ha^{-1} to 225 kg ha^{-1} caused a significant increase in NO_3^--N content. The split application of urea at the rate of 180 kg ha^{-1} produced maize biomass with a 10-fold higher stalk NO_3^--N content compared to pre-plant applied urea (Maharjan et al., 2016).
According to Bartholomew et al. (2017), the quality of crop yield characterized by a high protein content and low non-toxic nitrate level could be considered a function of steady, controlled N release. This study demonstrates that PCU decreased NO_3^- levels in sorghum bio-mass and bagasse. This lower level of NO_3- build-up in sorghum tissues is due to improving synchronization of N supply with crop demand during all growing stages. Decreasing NO_3^- concentration in sorghum fertilized with coated urea is associated with slow, controlled N release that prevents excessive amounts of N uptake. These results are in ac-cordance with the findings that coated urea leads to a 2000 mg kg^{-1} decrease in potato (Solanum tuberosum L.) petiole tissue NO_3- con-centration compared with urea (Bartholomew et al., 2017). Outcomes of the study conducted by Connell et al. (2011) suggest some ad-vantages of splitting enhanced-efficiency urea fertilizer into two equal applications for lowering NO_3^- levels in forage grass production. In another study, polymer coated urea blended with urea resulted in de-creased NO_3- concentration in biomass of bermudagrass (Cynodon dactylon (L.) Pers.) (Payne et al., 2015).

In contrast, Shapiro et al (2016) found that slow-release urea treatment resulted in significantly greater maize stalk NO_3^- con-centration compared with urea treatment. The results of the current study clearly indicated that ammonium nitrate produced biomass with the highest NO_3^- concentration. Some previous studies have also in-dicated that ammonium nitrate application contributes to relatively high NO_3^- levels in crop tissues compared with other N sources in-cluding enhanced efficiency N fertilizers, untreated urea, urea ammo-nium nitrate, ammonium sulfate and slow release urea blended with common urea (Connell et al., 2011; Payne et al., 2015; Teutsch and Tilson, 2005).

4.4. Implications for livestock feeding

Sorghum is a species that shows an intrinsic ability to accumulate NO_3^- (Sidhu et al., 2011). High NO_3^- levels in forage crops cause chronic or acute NO_3^- poisoning of stock (i.e. methemoglobinemia), which is particularly hazardous for ruminants (Bolan and Kemp, 2003). Therefore, NO_3^- content in biomass is a crucial indicator of forage quality. Acute toxicity and livestock poisoning have been noted when NO_3^- level in forage exceeds 5.0 g kg DM^{-1} (1.1 g NO_3-N kg DM $^{-1}$) (Bhatti et al., 2011). Forage with NO_3^- concentration exceeding 8.8 g kg DM $^{-1}$ (2.0 g NO_3-N kg DM $^{-1}$) should not be fed to pregnant ani-mals. The toxic level of NO_3^- for cattle is 18.0 g NO_3^- kg DM $^{-1}$ (4.0 g NO_3-N kg DM $^{-1}$) (Vough et al., 2006).
Data averaging across application strategies and years showed that urea at the rate of 90 kg N ha^{-1} and coated urea at 90 and 180 kg N ha^{-1} produced biomass with acceptably low NO_3^- levels, which were below the acute toxicity threshold (5.0 g kg DM^{-1}). Ammonium nitrate at both rates contributed to NO_3^- accumulation at toxic levels for ru-minants. Coated urea at 180 kg N ha^{-1} in 2013 produced biomass with NO_3- exceeding the acute toxicity threshold.
Sorghum bagasse, which is a solid lignocellulosic agro-industrial by-product, has gained importance in ruminant feed as an alternative that compensates for fodder loss (Rao et al., 2013). There was an overall trend indicating that bagasse contained lower NO_3^- levels compared with fresh sorghum biomass for each N source and in each year. These results may be due to possible displacement of most of the soluble NO_3^- to liquid phase during the biomass fractionation process (Chmielewska et al., 2014). However, some treatments, such as am-monium nitrate-180-single, ammonium nitrate-180-split and urea-180-split, produced bagasse with NO_3- concentrations exceeding the acute toxicity threshold (5.0 g kg DM^{-1}).

Appropriate N management is essential for lowering NO_3^- con-centration in sorghum. Based on our results, polymer coated urea-90-

single treatment can be recommended for sustainable sorghum pro-duction for forage with safe NO_3^- levels, which allow the avoidance of NO3- poisoning of ruminants. These results indicated some benefits of coated urea in the prevention of NO_3^- accumulation at toxic levels in sorghum biomass and bagasse. Ammonium nitrate application should be avoided in sorghum production for forage, because of the high risk of NO_3^- intoxication.

4.5. SPAD as a tool to predict NO_3^- concentration

The identification of the NO_3^- concentration in forage crops such as sorghum is necessary to prevent overfeeding of NO_3^-, particularly when crop growth is poor because of adverse weather conditions. In this study, SPAD readings have been used to provide information about the sorghum N status during the growing season as well as to predict NO_3^- concentration at harvest. To measure SPAD readings a quick, non-destructive strategy is used to reveal crop N status in situ and this is included in strategies to increase NUE (Jinwen et al., 2009; Xiong et al., 2015).

Based on the results of this study, SPAD readings could provide valuable information about potential NO_3^- accumulation much earlier than at harvest and enable the planning of appropriate biomass man-agement. However, SPAD readings can be affected by environmental factors, in particular light intensity (Muñoz-Huerta et al., 2013). Our results indicated that the possibility of the use of SPAD readings for estimating NO_3^- accumulation risk could be strongly limited in years with extremely adverse weather conditions. In all likelihood, the main reason for the weak relationship between the SPAD readings and NO_3^- at harvest that was detected in 2015 was the occurrence of severe drought during the growing season. Our data clearly showed that SPAD readings measured at booting stage had a greater usefulness for esti-mating NO3- than those measured at 7–8 leaf stage. The 7- leaf stage was probably too early to provide a reliable prediction of the risk of NO_3^- accumulation at the end of the growing season.

Another approach was suggested by Sunaga et al. (2005), who proposed an analysis of juice squeezed from a part of the stem as a simple method to estimate NO_3^- level in Sudangrass biomass. An al-ternative strategy to promote sustainable production of Sudangrass with low NO_3^- content was shown in further study, where the re-lationship between soil available N levels and NO_3^- concentration in Sudangrass was investigated (Sunaga et al., 2008). According to the results of the study mentioned above, available N content in soil can be regarded as an effective indicator for Sudangrass production with safe NO_3^- levels.

5. Conclusion

The enhanced-efficiency N fertilizer used in this study did not im-prove sweet sorghum biomass yield. The results indicated that in sweet sorghum production for forage particular care should be taken to re-duce the risk of inadvertent NO_3^- poisoning. Our findings report a significant response of NO3- content in crop tissues to water-stress conditions and N managements. Taking this into consideration, the development of a sustainable sweet sorghum cropping system would help to reduce the NO_3^- toxicity threat. Based on the results of the current study, a single application of polymer coated urea at the rate of 90 kg N ha^- can produce sweet sorghum biomass and bagasse with safe levels of NO_3^- and this method can be recommended for sustainable sweet sorghum production for forage. Nevertheless, further research is needed to determine the effect of a greater range of N rates and varied soil conditions. Ammonium nitrate application at the rate of 90 kg ha^{-1} and higher should be avoided in sweet sorghum production for forage. In this study, a simple indirect strategy based on SPAD readings mea-sured during the growing season has been proposed as an indicator of NO3- level in sorghum biomass and bagasse at harvest. The results in-dicated that there appears to be an opportunity for application of the proposed method by farmers. However, our present knowledge about relationships between environmental factors and SPAD readings and plant nitrate content is still in its infancy. Thus, further research is in-dispensable to develop the technology for estimating NO_3^- accumula-tion risk in forage based on SPAD readings. Moreover, the interest of future research should be focused on the determination of the effect of a wide-range of N rates on SPAD readings and nitrate content.

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Zrównoważona uprawa sorga cukrowego nawożonego produktami odpadowymi w aspekcie emisji gazów cieplarnianych

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Sustainable Production of Sweet Sorghum as a Bioenergy Crop Using Biosolids Taking into Account Greenhouse Gas Emissions

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Abstract: Currently, little data are available on greenhouse gas (GHG) emissions from sweet sorghum production under temperate climate. Similarly, information on the effect of bio-based waste products use on the carbon (C) footprint of sorghum cultivation is rare in the literature. The aim of this study was to evaluate the agronomical and environmental effects of the application of biosolids as a nitrogen source in the production of sweet sorghum as a bioenergy crop. The yield of sorghum biomass was assessed and the GHG emissions arising from crop production were quantified. The present study focused on whether agricultural use of sewage sludge and digestate could be considered an option to improve the C footprint of sorghum production. Biosolids—sewage sludge and digestate—could be recognized as a nutrient substitute without crop yield losses. Nitrogen application had the greatest impact on the external GHG emissions and it was responsible for 54% of these emissions. CO_{2eq} emissions decreased by 14 and 11%, respectively, when sewage sludge and digestate were applied. This fertilization practice represents a promising strategy for low C agriculture and could be recommended to provide sustainable sorghum production as a bioenergy crop to mitigate GHG emissions.

Keywords: GHG emissions; carbon footprint; sweet sorghum; fertilization management; digestate; sewage sludge

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1. Introduction

Sustainable agricultural systems should be economically profitable, but they should also provide food, feed, and biofuels and prevent or even enhance ecosystem services [1]. In recent years, the agricultural sector has become increasingly heavily dependent on mainly non-renewable energy sources. One of the challenges for sustainable crop production is to decrease the external energy inputs [2]. Among the key issues for agricultural sustainability is greenhouse gas (GHG) emissions as well as their effect on climate [3]. Agricultural production has a significant impact on climate change [4]. Emissions from the agricultural sector in the European Union were estimated at 432 million tons of

CO_2 equivalents (CO_{2eq}) in 2017 and this was responsible for emitting 10% of the total amount of European GHG emissions [5]. Effective methods (i.e., methods which have the potential to mitigate emissions in agriculture) need development, because current decreases in these areas of emission levels are insufficient [6]. However, development of the following methods for carbon (C) reduction in agriculture are promising: precision farming; improved fertilization management; cultivation of crops with a higher potential for C sequestration (i.e., C4 photosynthesis cycle crops); and lastly, but not all-inclusively, the implementation of organic fertilizers and alternative soil amendments to replace synthetic fertilizers [7,8].

Varied management practices have different impacts on GHG emissions and crops cultivation and therefore they should be examined [9]. The detailed estimation of GHG emissions from the

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agricultural sector allows for the identification of hot-spots, which provide information about which input causes the most significant effect on climate change due to the release of GHG [10]. The amounts of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) emissions from various sources are converted to one unit, such as kilograms of CO_{2eq}, emitted to the atmosphere and this is defined as the C footprint [11]. The C footprint can be quantified on a land-area basis as a spatial C footprint, on an output basis defined as per yield unit of produced biomass C footprint or on a produced energy basis [12,13].

In the European Union in 2009, legislation in the form of Renewable Energy Directives provided the criteria for biofuels [14,15]. These two legislative acts state that dedicated energy crops cultivation is one of the three main stages of biofuels production which should be taken into account during GHG emission quantification [16]. Carbon footprint calculators, which take into account the LCA approach represent useful tools for estimating GHG emissions from cultivation of crops for energy purposes. A detailed overview of a wide range of these calculators was provided by Peter et al. [10]. One such calculator—BioGrace (Biofuel Greenhouse Gas Emissions in Europe)—was used in the present study to quantify GHG emissions from energy crops such as sweet sorghum (*Sorghum bicolor* (L.) Moench) at farm stage [17]. This calculation tool is approved and recommended by the two key European Directives as a method to harmonize calculations of biofuel GHG emissions and support implementation of European directives [14,15] into national laws [16]. BioGrace was developed by economic operators for consultants and politicians to improve decision-making and for implementation of national programs aimed at decreasing GHG emissions [10,16]. It is also a useful tool for farmers, who can check how different management practices affect the carbon footprint of biofuel production [10]. The method of GHG estimation offered by the BioGrace tool is based on standard conversion values, which are mainly emission factors, and also other data that are needed to convert some agricultural inputs into emissions [16]. The Tier 1 approach with national or global standard values was used in this study. The country specific emission values allow improvement of these calculations according to the Tier 1 approach [18].

Greenhousegasemissionscanbedividedintoexternalandon-farmemissions[11]. Theseemissions are a result of production processes and application of agricultural inputs, such as pesticides, fertilizers, seeds, and combustion of diesel oil during farm operations [8,19]. Production and application of fertilizers is a significant contributor to the emissions of GHG from arable crop production [11,12,20,21]. Crop production should take into account the C footprint of the whole biomass energy production chain, in particular at the farm stage. A more sustainable approach to nitrogen (N) fertilization management has a large potential to decrease GHG emissions from energy crop cultivation [10]. There is a great need to focus on more sustainable improvement of soil fertility and optimal use of synthetic

N fertilizers. Application of bio-based by-products represents a sustainable waste management method and it provides recycling nutrients for crop growth, which is in line with the European policy for a circular economy [2,22–24]. Results of studies concerning the environmental impact of digestate vary significantly throughout the literature. Agricultural use of digestate, which is a stable organic waste material, has the potential to decrease soil CO_{2eq} emissions [25]. However, there is an environmental risk associated with increasing N_2O volatilization [26]. Some authors have noted especially high N_2O emissions, when liquid digestate is applied in moderately wet soil [27,28]. This significant loss of N_2O can negate any benefits from replacing synthetic fertilizers with digestate [28]. Pezzolla et al. [29] obtained different results and suggest that the digestate can be used as a fertilizer to grow crops without any harmful effect on the agroecosystem, including GHG emissions. However, data about the impact of sewage sludge application as a soil amendment on the C footprint of crop cultivation is still lacking.
Biomass use for production of heat, electricity, and fuel has significantly increased in recent years [30]. Using energy crops with a high C sequestration potential such as sweet sorghum for energy purposes represents an almost-closed CO_2 cycle [8,10]. It is believed that the biofuels production processes give the same amount of CO_{2eq} as was fixed in the biomass through photosynthesis at the production stage of the raw material [31]. However, crop growth generates a certain amount of GHG and therefore it is not the ideal carbon neutral production chain [10,31]. Sweet sorghum represents a promising bioenergy feedstock in the temperate climate of Central Europe [32]. It is still not well understood how energy crop cultivation systems affect GHG emissions. Moreover, so far, there are a limited number of studies on GHG emissions from sorghum production. Storlien et al. [9] examined the effect of various N fertilizer rates, crop rotation, and crop residue managements on GHG emissions from sorghum production for bioenergy purposes. According to their results, N addition significantly increased N_2O emissions, and incorporation of half of sorghum residues increased CO_2 emissions [9]. Davis et al. [33] recommended the perennial grasses switchgrass (*Panicum virgatum* L.) and miscanthus (*Miscanthus* × *giganteus* Greef et Deuter) for ethanol instead of maize (*Zea mays* L.) as a cellulosic feedstock for ethanol production. The production of biofuel from these crops characterized by low N demand allows significant mitigation of GHG emissions at the farm stage [33].
Given the aforementioned considerations, the response of sweet sorghum dry matter (DM) yield to biosolids fertilization was evaluated. This study focused on evaluating how sewage sludge and digestate application affect the C footprint of the sorghum production system, compared with conventional fertilization management. This study improves knowledge on the environmental impact of sorghum production with different fertilization managements in regards to the C footprint. It provides insight into the necessity of finding the most sustainable and cleanest methods of crop production for energy purposes.

2. Materials and Methods

2.1. Study Site Description

This study on N fertilization management of sorghum yield and GHG emissions was carried out in the period 2016–2018. It was conducted under field experiment conditions at the Agricultural Research Station of Wroclaw University of Environmental and Life Sciences. This experimental site is located in Central Europe (southwestern Poland; $51^{\circ}10^{0}25^{00}$ N and $17^{\circ}07^{0}02^{00}$ E). The climate in the study site is classified as temperate [34]. The annual average temperature is 9.0 °C and the annual average rainfall is 583 mm. The monthly temperature and precipitation of the area of research at the time the experiment was carried out were recorded every 10 min using an 'AsterMet' meteorological station (A-STER s.c., Krakow, Poland).

Ten soil samples (0.1 kg) from a layer of 30 cm depth were randomly taken from 10 spots across the experimental area (PN-ISO 10381-2). The soil samples were collected before sowing, prior to the field experiment was established. Then, individual samples were mixed to receive one composite sample, air dried and sieved with a mesh size of 2.0 mm. Then, physio-chemical properties of the topsoil (layer of 0–30 cm depth) were assessed. Particle-size distribution was determined by the sieve method (sand fraction) and hydrometer method (silt and clay fractions) after sample dispersion with hexametaphosphate. The soil texture was defined as sand, 0.05–2.0 mm; silt, 0.002–0.05 mm, and clay, <0.002 mm diameter (PN-R-04033 I USDA). pH was measured in distilled water and 1 mol dm^{-3} KCl solution, at the soil:solution ratio 1:2.5 with a pH meter (Omega Engineering, Inc., Norwalk, USA).

Mineral forms of nitrogen (soluble and exchangeable NH_4^+ and NO_3^-) were determined after extraction of fresh soil (stored in a fridge until extraction at −20 ºC temperature) with a 1% solution of K_2SO_4, at the solution to soil ratio of 5:1 and a shaking time of 1 h. Then, suspensions of each derived sample were prepared by filtration through Whatman 2 filter paper (Whatman International Ltd, Maidstone, UK), followed by storage at −20 °C until analysis. In the filtrate, concentrations of mineral nitrogen forms were measured colorimetrically: N-NO_3 with phenoldisulfonic acid, and N-NH_4 with potassium sodium tartrate and Nessler's reagent (UV-Vis spectrometer, Cintra 4040, GBC Scientific Equipment, Braeside, Australia) [35]. The contents of plant-available phosphorus and potassium were analyzed by ICP-OES after Egner–Riehm extraction with calcium lactate (spectrometer Varian Inc. (Part A)—Vista MPX Simultaneous ICP-OES) [35].

2.2. Experimental Materials

Sucrosorgo 506—a late-maturing photoperiod insensitive triple-cross hybrid of sweet sorghum developed by Sorghum Partners Inc. (USA)—was used in this experiment. Stems of this hybrid have a relatively higher concentration of soluble sugars. It is well adapted for Central European conditions and produces a high amount of biomass in moderate climates [36]. Medium maturing, triple-cross hybrid Rona 1 with juicy stems recommended for silage is the result of a Hungarian breeding program (Gabonakutató) [37]. French late-maturing hybrid SuperSile 20 was received from Caussade Semences [38]. Late-maturing Goliath, mainly used for forage, was developed by Saaten Union GmbH (Germany) [39]. These hybrids have been registered in the EU Common Catalogue and were chosen based on the results of previous long-term studies [36].

Commercial urea characterized by 46% N was used in this study. Triple superphosphate with 46% P_2O_5 content was used as a phosphorus (P) source and potassium salt with 60% K_2O content as a potassium (K) fertilizer. Solar dried sewage sludge sourced from a municipal sewage treatment plant in Klodzko operating in EXPOVAL technology was used. Methanogenic post-digestion liquid digestate (termed digestate in this paper) was obtained from a mesophilic biogas plant in Strzelin (Südzucker Polska Inc.) fed with beet pulp. The chemical characteristics of biosolids used for fertilization are given in Table 1.

Table 1. Characteristics of biosolids used as fertilizers in the field experiment.

Parameters/Chemical Elements with Limit Value for Organic Fertilizer and Organic Soil Improver	Unit	Digestate	Sewage Sludge	Methods

pH		7.6	7.4	PN-EN 12176:2004
DM (1)	%	2.8	42	PN-EN 12880:2004
Organic compounds	% DM	71	31.5	PN-EN 12879:2004
Total nitrogen (N)		8	1.29	KJ-I-5.4-179
Ammonia nitrogen N-NH4		2	<0.10	PN-EN 14671:2007
Total phosphorus (P)	$mg\ kg^{-1}$ DM	0.54	1.63	PN-EN ISO 1185:2009
Calcium (Ca)		2.99	4.11	
Magnesium (Mg)		1.02	0.60	PN-EN ISO 1185:2009;
Potassium (K)		1280	n.a. (2)	PN-EN 13657:2006
Copper (Cu), 200 (3)		49.6	239	PN-EN ISO 1185:2009
Zinc (Zn)		170	777	
Lead (Pb), 120 (3)		6.13	94	
Cadmium (Cd), 3 (3)		2.78	0.71	
Chromium (Cr)		11.2	32.9	
Nickel (Ni), 50 (3)		11.6	24.7	
Mercury (Hg), 1 (3)		0.050	0.540	KJ-I-5.4-36
Salmonella bacteria:				PB/BB/7/F:20.03.2014
no *Salmonella* species in 25 g sample (3)		0	0	

The results received from Südzucker Polska S.A. and Wodociagi Klodzkie sp. z o.o.; (1) DM dry matter; (2) n.a. not analyzed; and (3) the maximum permissible concentrations of contaminants in organic soil improver in the framework of the Fertilizing Product Regulation Proposal for a Regulation on the making available on the market of CE marked fertilizing products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 [22].

2.3. Field Treatments and Experimental Design

The experiment had a two-factorial split-plot design including sorghum hybrids and four fertilization managements. Treatments were arranged in a randomized, complete block with four replications. Experimental plots were 12.6 m^2 6 × 2.1 m (length × width). The research used four sweet sorghum hybrids: Sucrosorgo 506, Rona 1, SuperSile 20, and Goliath.

Primary tillage was done with a moldboard plow in the fall and with leveling and an aggregate seedbed preparation in the spring. The seeds of sweet sorghum hybrids were sown on 6 May 2016, 5 May 2017, and 15 May 2018. The annual N input of 100 kg N ha^{-1} was provided by broadcast application before sowing of (1) 19 t ha^{-1} sewage sludge, (2) 45 m^3 ha^{-1} digestate, and (3) 220 kg ha^{-1} urea. Unfertilized plots were also included in the experimental work. The rates of potassium and phosphorus were as follows: 100 kg ha^{-1} in the form of K_2O and 70 kg ha^{-1} of P_2O_5. These fertilizers were provided by single broadcast pre-sowing application and then were mixed with topsoil using a rotary harrow. Additional surrounding plots were set to minimize boundary effects through edges of the experimental field. Lumax 537.5 SE (s-metolachlor 312.5 $g \cdot dm^{-3}$ + mesotrione 37.5 $g \cdot dm^{-3}$ + terbuthylazine 187.5 $g \cdot dm^{-3}$) at the dose of 2.0 $dm^3 \cdot ha^{-1}$ was post-sowing, pre-emergency applied for weed control. Safener Concep III (oxabetrinil 700 g kg^{-1}) was used to avoid injuries to sorghum plants caused by the phytotoxic effects of s-metolachlor.

Before harvest, five representative plants from the middle row within each plot were collected and the aggregate sample was cut using a bowl chopper (Krag). The sample of shredded biomass was weighed and oven-dried at 105 °C until reaching constant mass. The moisture content was gravimetrically determined and the DM ratio was calculated (PN-EN 12880:2004).

The plants were mechanically harvested with a brush cutter (Stihl FS400 C, Germany) on 23 September 2016, 20 September 2017, and 11 October 2018. To estimate the fresh matter yield, the sorghum biomass was weighed just after harvest and the biomass production per hectare was extrapolated (Mg ha^{-1}). Harvesting losses were also included in calculations.

2.4. CO_2 Emission Determination and Carbon Footprint Calculation

The system boundaries for the carbon footprint calculation within the scope of this study are presented in Figure 1.

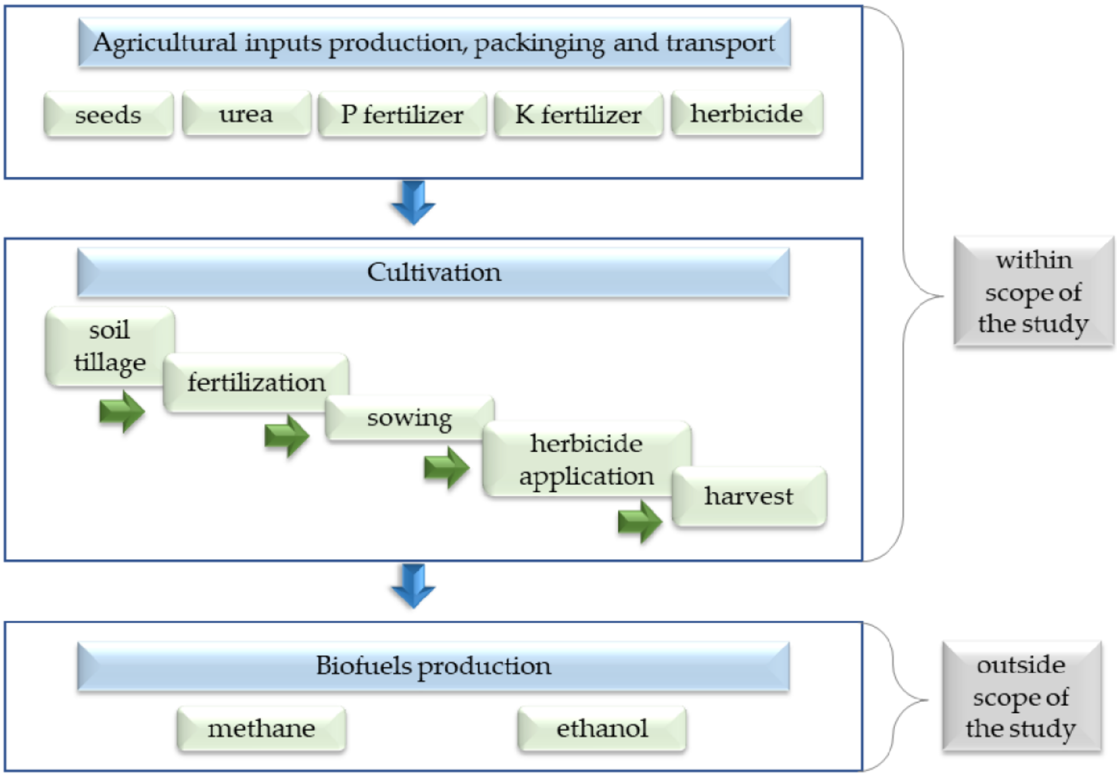

Figure 1. System boundaries for the carbon footprint calculation.

Calculations were performed based on Intergovernmental Panel on Climate Change methodology [18,40,41]. The main assumptions are given in Table 2. The quantification of GHG emissions was made according to ISO TS 14067 [42]. The freely available BioGrace Excel GHG calculation tool was used to estimate the C footprint of sorghum production [17]. Standard values containing conversion factors and LHV (lower heating values) from the database developed by IPCC were used for computing GHG emissions [43]. Other sources of emission factors are included in Table 3. The climate was classified as cold temperate and dry, and soil conditions were classified as sandy, in accordance with IPCC methodology. The environmental impact of different fertilization managements was assessed by estimating the spatial- and yield-scaled C footprint, expressed as kg CO_{2eq} ha^{-1} and kg CO_{2eq} Mg^{-1} DM produced, respectively. The assessment covers the major greenhouse gas emissions (CO_2, N_2O, and CH_4) generated during all major processes: from input materials for crop production, through the on-farm crop cultivation, to the field-gate. Analysis included both direct and indirect N_2O emissions. The following sources of direct N_2O emissions were incorporated into the analysis: N synthetic fertilizer, organic N applied as soil amendments (digestate and sewage sludge), and N in sorghum residues.

Indirect N_2O was related to the atmospheric deposition of N volatilized from leaching and runoff, and CO_2 from urea fertilization.

Table 2. The main assumptions of the study.

Rule	Description
Scope of the study	Calculate the GHG emissions during sweet sorghum production for biofuels (methane and bioethanol) production.
System boundary	Farm stage—including external and on-farm greenhouse gas emissions.
Functional unit	1 ton of sorghum biomass.
Time reference	One growing season (as an average of three seasons).
Data collection—cultivation	The following agricultural operations were included: soil tillage, sowing, fertilization, herbicide application, and harvest.
Carbon footprint calculation:	
Calculator	BioGrace Excel GHG calculation tool [17]
Methods	IPCC 2006 [18,40,41]
Norm	ISO14067 [42]

The table structure was based on [21].

The GHG emissions were divided into two categories: (1) external emissions associated with production and transport of farm inputs, such as fertilizers, pesticides, and seeds and (2) on-site emissions including tractors and machinery fuel consumption during farm practices, and direct and indirect N_2O emissions. The GHG emissions from diesel oil consumption included all the operations of farm machinery used for various crop production activities, such as tillage, fertilizer and herbicide application, and sowing and harvesting. Annual CO_{2eq} emissions from urea application associated with the loss of CO_{2eq} during the industrial production process were calculated in the present study. Emissions related to soil C stock changes were included in the total C footprint of the farm. Quantification of GHG emissions was computed with emission factors according to the values shown in Table 3. Greenhouse gas emissions generated outside the farms (in wastewater treatment plants and during the biogas production process) were not considered. However, the analysis included emissions related to the application of sewage sludge and digestate and direct N_2O emissions due to N losses from soil fertilized with these kinds of organic amendments. According to IPCC methodology, it was assumed that there is no net accumulation of biomass C stocks. The change in biomass was not estimated, because for annual crops the increase in biomass stocks in a single year is equal to biomass losses from harvest and mortality in this year [41].

Conversion from N_2O-N emissions to N_2O was done by multiplication by 44/28. Emissions of CO_2, CH_4, and N_2O were quantified taking into account their 100-year global warming potentials (GWP), i.e., 1 for CO_2, 28 for CH_4, and 265 for N_2O. As recommended by IPCC, the most recent values of the 100-year time horizon GWP relative to CO_2 were used in this study, adapted from IPCC Fifth Assessment Report [44].

Table 3. Greenhouse gas emission factors for agricultural inputs and correlated coefficients used in the estimation in this study

Description of Emission Factor	Unit	Default Value	References
Emission factor for N_2O emissions from N inputs	kg N_2O–N kg^{-1} N input	0.01	[18]
$Frac_{GASF}$ fraction of synthetic fertilizer N that volatilizes as NH_3 and NO_x, kg N volatilized	%	10	
$Frac_{GASM}$ fraction of applied organic N fertilizer materials that volatilizes as NH_3 and NO_x, kg N volatilized		20	
Emission factor for N_2O emissions from atmospheric deposition of N on soils and water surfaces		1	
$Frac_{LEACH-(H)}$ fraction of all N added to/mineralized in managed soils in regions where leaching/runoff occurs that is lost through leaching and runoff,		30	
Emission factor for N_2O emissions from N leaching and runoff		0.75	
Energy factor for urea production		20	
Fuel density $_{(diesel)}$	kg m^{-3}	832	[17]
LHV $_{(diesel)}$ (1)	MJ kg^{-1}	43.1	
Emission factor for combustion of Diesel: $CO_{2\ diesel}$	kg TJ^{-1}	74100	[40]
Emission factor for combustion of Diesel: $CH_{4\ diesel}$		4.15	
Emission factor for combustion of Diesel: $N_2O_{\ diesel}$		28.6	
Energy factor for mesotrione	MJ kg^{-1} a.i. (2)	691	[31]
Energy factor for tetrabulazine and atriazine		208	
Energy factor for metolachlor and metazachlor		388	
Energy factor for pesticide	kg CO_{2eq} MJ^{-1}	0.069	
Energy factor for P fertilizer production	kg CO_{2eq} kg^{-1} fertilizer	0.26	
Energy factor for K fertilizer production	kg CO_{2eq} kg^{-1} fertilizer	0.25	
Emission factor for sorghum seeds	g $CO_{2\ eq}$ kg^{-1}	0.86	[45]

(1) LHV lower heating value; (2) a.i. active ingredient.

2.5. Statistical Analyses

The normal distribution of the obtained data was checked with the Shapiro–Wilk test [46]. An analysis of variance (ANOVA) was performed to assess the statistical significance of the sorghum biomass yield and C footprint. The parameter averages were estimated across four plot replications. Treatmentaveragesseparationwascarriedoutusingthe Tukeytestattheprobabilitylevelofα=0.05[47]. The four tested fertilization managements were considered fixed factors and the four replications were entered as random effects [48]. The Levene test at P level < 0.05 was used for evaluation of the variance homogeneity. The Statistica software package (version 13.1 StatSoft, Poland) was used to carry out statistical analyses [49].

3. Results

3.1. Weather and Soil Conditions

In 2016, 2017, and 2018, the average temperatures during the sweet sorghum growing season were higher than the 30-year average: 0.8, 0.2, and 1.9 °C, respectively (Table 4). The period from August to September 2018 was characterized by the highest average temperature, which was 2.3 °C higher than the multiyear mean. In each experimental year, the total rainfall during the vegetation period was lower than the 30-year average: 74.6, 12.5, and 81.9 mm, respectively, for 2016, 2017, and 2018 (Table 4). Extremely adverse weather conditions with drought occurred at the beginning of the sorghum vegetation period in May 2016, when total precipitation was 90% lower than the 30year average. In 2017, both average temperature and precipitation were close to the long-term average temperature and rainfall sum.

Table 4. Monthly temperature and rainfall in the research area in the period of 2016–2018.

Month	$T_{average}$ (°C)				Rainfall (mm)			
	2016	2017	2018	Long-Term Average 1986–2015	2016	2017	2018	Long-Term Average 1986–2015
May	15.3	14.2	17.1	14.4	5.3	24.1	54.3	54.1
June	18.6	18.5	18.8	17.3	44.6	52.5	36.6	67.4
July	19.5	19.0	20.1	19.6	114.3	112.2	79.1	78.9
August	17.9	19.4	21.1	18.6	27.1	43.6	20.3	65.3
September	16.4	13.3	15.8	13.7	44.7	65.7	38.4	44.9
Average temperature or rainfall sum in the period from May to September	17.5	16.9	18.6	16.7	236.0	298.1	228.7	310.6

The experimental site is composed of loamy sand textured soils, originally classified as Brunic Arenosols soil (IUSS Working Group WRB, 2014). The soil was characterized by a thick (30–34 cm) humus layer and slightly acidic pH (6.0). The soil has a high content of nitrate and ammonium nitrogen, available phosphorus, and plant available form of potassium (Table 5). The soil is well-drained with the water table at 130 cm depth.

Table 5. Soil properties (topsoil layer 0–30 depth) at the study site.

Soil Texture			pH	NO_3-N	NH_4-N	$P_{available}$	$K_{available}$
%				g kg^{-1}		mg kg^{-1}	
sand: 87	silt: 5	clay: 8	6.0	0.79	0.55	337.5	154.0

3.2. Sorghum Biomass Yield

The results of sorghum biomass DM yield and C footprint are shown in Table 6. The analysis of variance showed that the DM yield production was not significantly affected by interaction between experimental factors considered in the study. The average sorghum DM yields ranged from 10.5 for non-fertilized SuperSile 20 to 23.6 Mg ha^{-1} for Sucrosorgo 506 fertilized with sewage sludge. In the case of each sorghum hybrid, the lowest biomass yield was produced by control plants. Hybrids produced significantly different biomass yields. Sucrosorgo 506 was characterized by the highest productivity in the study area. Dry matter yield of Sucrosorgo 506 was 35% higher than the yield of SuperSile 20, which produced the lowest yield of the four tested hybrids (Table 6). Both sewage sludge and digestate application significantly increased the yields across the hybrids, which were 45% and 28% higher than that for the control. The yields of sweet sorghum biomass were significantly different in the experimental period. In 2017, DM yield was 24.3% and 41.6% higher than in 2016 and 2018, respectively.

Table 6. Dry matter yield and carbon footprint of sorghum production as affected by different fertilization managements (pooled from 2016, 2017, and 2018).

Sorghum Hybrid	Fertilization Treatment	Dry Matter Yield Mg ha^{-1}	Spatial Carbon Footprint kg CO_{2eq} ha^{-1}	Yield-Scaled Carbon Footprint kg CO_{2eq}Mg^{-1}
Sucrosorgo 506	control	17.0	1731 ^{b}	88
	Urea	18.9	2742 ^{f}	130
	sewage sludge	23.6	2736 ^{f}	111
	digestate	19.0	2498 ^{def}	117

Rona 1	control	10.9	1414 [a]	96
	Urea	15.0	2528 [def]	141
	sewage sludge	15.3	2282 [cd]	126
	digestate	15.8	2303 [cd]	125
Goliath	control	12.2	1286 [a]	109
	Urea	17.9	2621 [ef]	135
	sewage sludge	19.6	2446 [cde]	118
	digestate	16.9	2340 [cde]	120
SuperSile 20	control	10.5	1412 [a]	96
	Urea	13.1	2472 [def]	147
	sewage sludge	14.8	2301 [cd]	124
	digestate	12.6	2180 [c]	131
		Average for factors Hybrid *		
Sucrosorgo 506		19.6 [c]	2427 [b]	111 [a]
Rona 1		14.2 [ab]	2132 [a]	122 [ab]
Goliath		16.6 [b]	2163 [ab]	120 [ab]
SuperSile 20		12.7 [a]	2091 [a]	125 [b]
		Fertilization treatment **		
control		12.6 [a]	1461 [a]	97 [a]
urea		16.2 [b]	2590 [c]	138 [c]
sewage sludge		18.3 [b]	2441 [b]	119 [b]
digestate		16.1 [b]	2330 [b]	123 [b]
		Average for years ***		
2016		15.3 [b]	2019 [a]	133 [b]
2017		20.2 [c]	2282 [b]	114 [a]
2018		11.8 [a]	2265 [b]	115 [a]

Each value is the average of four replicates. [a,b,c] For interaction of factors analysis, each individual year and factor, averages with different letters in the same column are significantly different at $p < 0.05$ according to the results of ANOVA and Tukey's test. The columns without letters indicate that significant differences were not observed. * Values were averaged across four fertilization treatments and three years. ** Values were averaged across four hybrids and three years. *** Values were averaged across four hybrids and four fertilization treatments.

3.3. Carbon Footprint of Sorghum Per Area and Per Mg of Biomass

Averaged over the three years, a significant effect of both tested factors on spatial C footprint was reported in this study. The cultivation of sorghum without N fertilizer application resulted in the lowest CO_{2eq} per unit of area and per unit of biomass yield; in particular, in the case of the three hybrids: Rona 1, Goliath, and SuperSile 20. Irrespective of fertilization treatments, the highest yielding hybrid, Sucrosorgo 506, emitted the highest amount of GHG per hectare. Emissions from field production of this hybrid were 16% higher than those from cultivation of SuperSile 20, which was characterized by the lowest yield and the lowest area-scaled C footprint. The spatial C footprint was significantly affected by fertilization management systems. The application of waste products caused a decrease in GHG emissions compared to conventional fertilization. The application of sewage sludge and digestate resulted in 6% and 10% lower CO_{2eq} emissions per ha, respectively, compared with the use of urea. The control sorghum plants produced the lowest amount of GHG. The spatial C footprint varied greatly during the period of the experiment. In 2017 and 2018, this was at a comparable level, which was on average 12.5% higher than in 2016.

The yield-scaled C footprint expressed as a CO_{2eq} per Mg DM of yield produced presented a quite narrow range from 88 to 147 kg CO_{2eq} Mg^{-1} DM, without significant differences between the interactions of tested factors. Greenhouse gas emissions per Mg of biomass were the highest from SuperSile 20 hybrid cultivation, which was characterized by the lowest biomass yield. Across the hybrids, the effect of fertilization treatment was the same as for spatial C footprint, with the highest value for urea application. Application of sewage sludge and digestate provided, respectively, 14% and 11% lower emissions of GHG than from the use of urea. Across all hybrids and fertilization treatments, differences between years were also significant. In 2016, sorghum cultivation emitted higher amounts of GHG per Mg of produced biomass compared to 2017 and 2018.

3.4. Structure of Inputs Share of Carbon Footprint

Across all the hybrids and years, the share of varied inputs in the C footprint is presented for each fertilization treatment in Figure 2. When the emissions were averaged across all hybrids, the largest contributor to the total amount of GHG emissions was the combination of direct and indirect N_2O emissions, which ranged from 56% to 63% of the total emissions and from 10% to 17%, respectively, for direct and indirect N_2O emissions. An increase in direct N_2O emissions was observed when sorghum was fertilized with sewage sludge and digestate (Figure 2C,D). When urea was applied as the N source, it was responsible for 13% of the total GHG emission and this was the second largest contributor to the C footprint. Diesel combustion during various farming operations (such as soil tillage, sowing, herbicide spraying, fertilizer application, harvesting, etc.) was responsible for relatively high GHG emissions and its contribution to the C footprint of sorghum production was estimated at 13–22%. Carbon dioxide equivalents emissions related to the use of P and K fertilizers, seeds and herbicide had the lowest contribution to the total GHG emission and on average they all accounted for from 5% to 9% (Figure 2A–D).

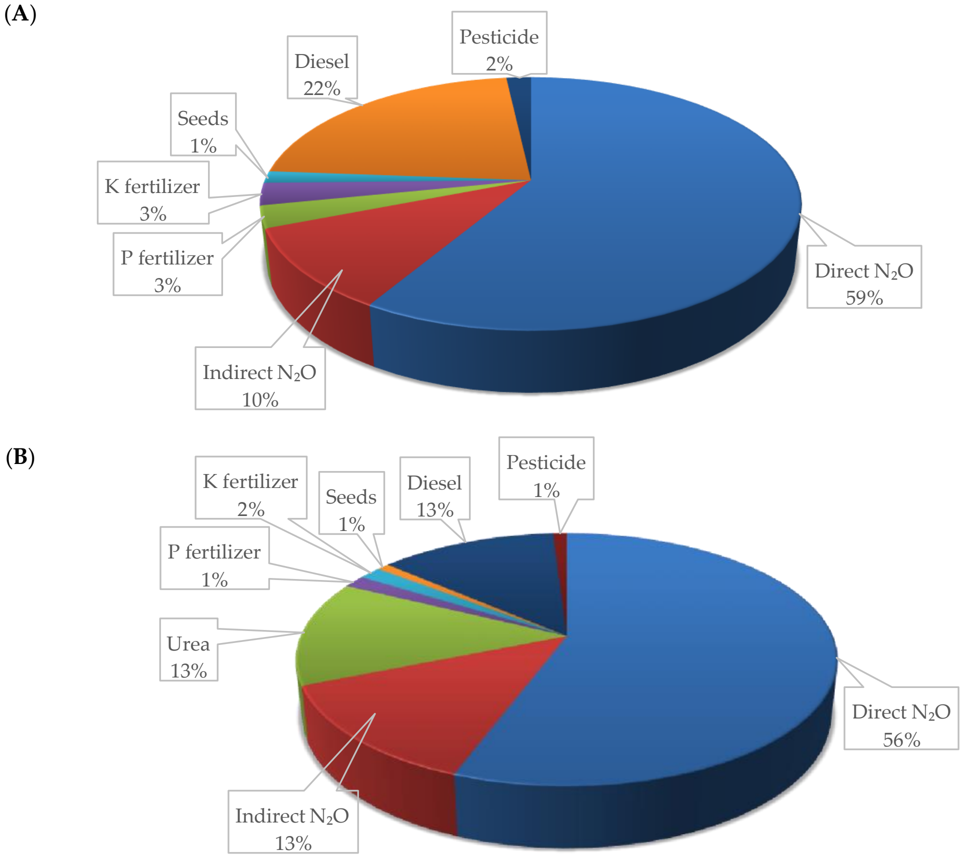

Figure 2. *Cont.*

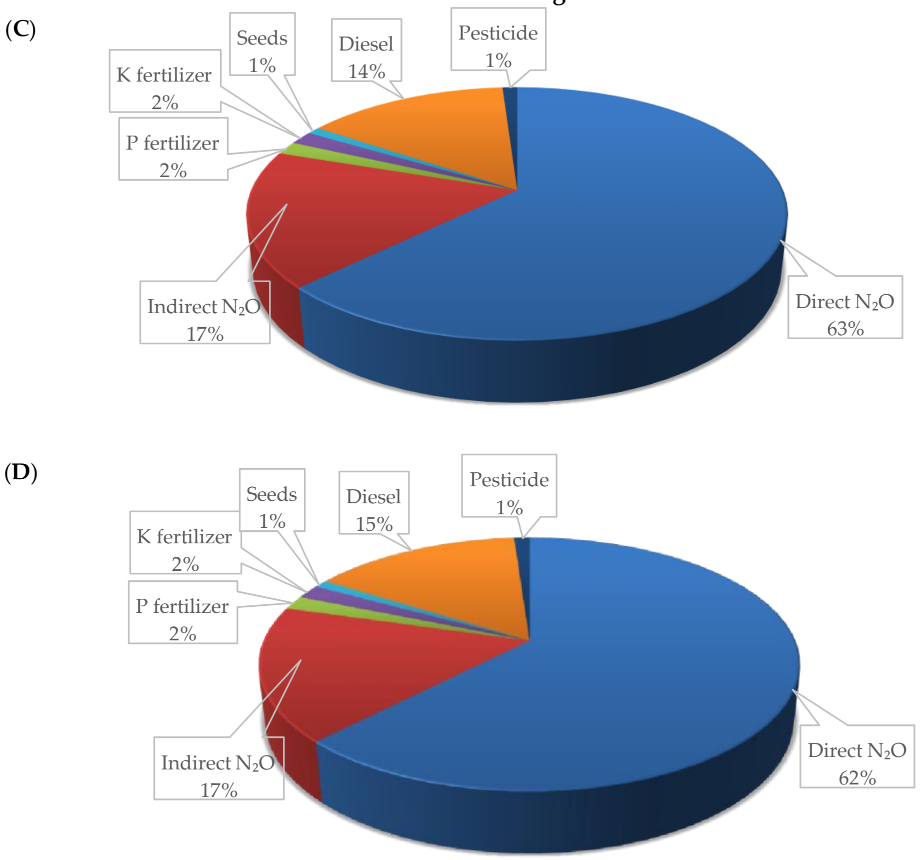

Figure 2. Structure of inputs share of carbon footprint in sorghum production (**A**) without nitrogen fertilization, fertilized with (**B**) urea, (**C**) sewage sludge, and (**D**) digestate (pooled from 2016, 2017, and 2018).

3.5. External and On-Site Emissions

The sources of external GHG emissions are shown in Figure 3. In the analysis, the following sources of external emission were included: manufacture, transportation, storage, and delivery of agricultural inputs to the farm gate. The use of N fertilizer was responsible for 54% of external emissions and dominated in terms of the external GHG emissions. Consequently, the external emissions from sorghum fertilized with urea were 2.2 times higher than emissions from treatments fertilized with sewage sludge and digestate (Figure 3).

As an average of four hybrids and three years, on-site emissions made a significantly higher contribution to the total GHG emission, because these were from 8 to 17 times higher than external emissions (Figures 3 and 4). Direct N_2O was responsible for the largest amount of emitted GHG in each fertilization treatment. Averaged over three years, diesel consumption was the second largest contributor to the C footprint at the farm level. On-farm emissions from unfertilized sorghum were 27%, 19%, and 22% lower compared to those from sorghum fertilized in a conventional way and by application of sewage sludge and digestate as soil amendments, respectively. On-site emissions were similar between fertilization treatments that used synthetic N or biosolids as a source of nutrients (Figure 4).

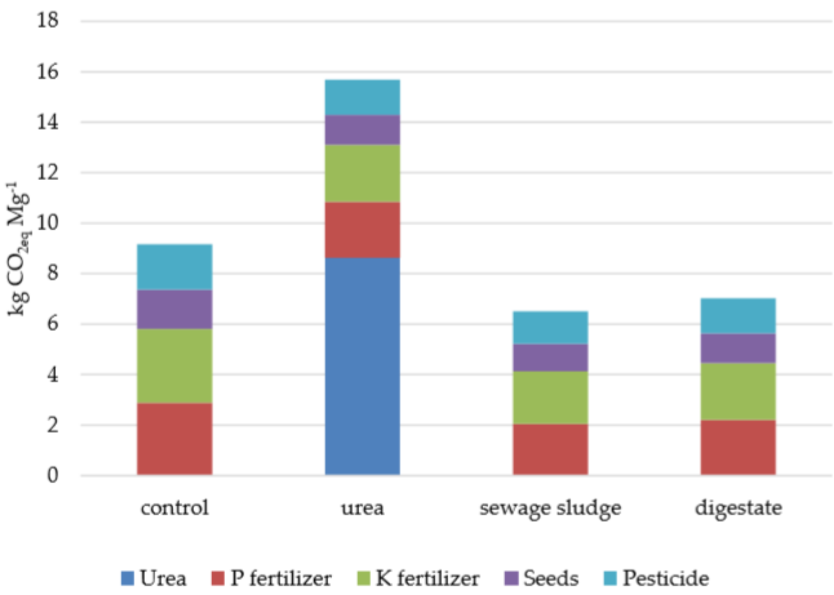

Figure 3. External emissions of CO_2 related to agricultural inputs, such as N, P, and K fertilizers, seeds, and pesticide, as affected by different fertilization managements. Values correspond to the annual average for the 2016–2018 period.

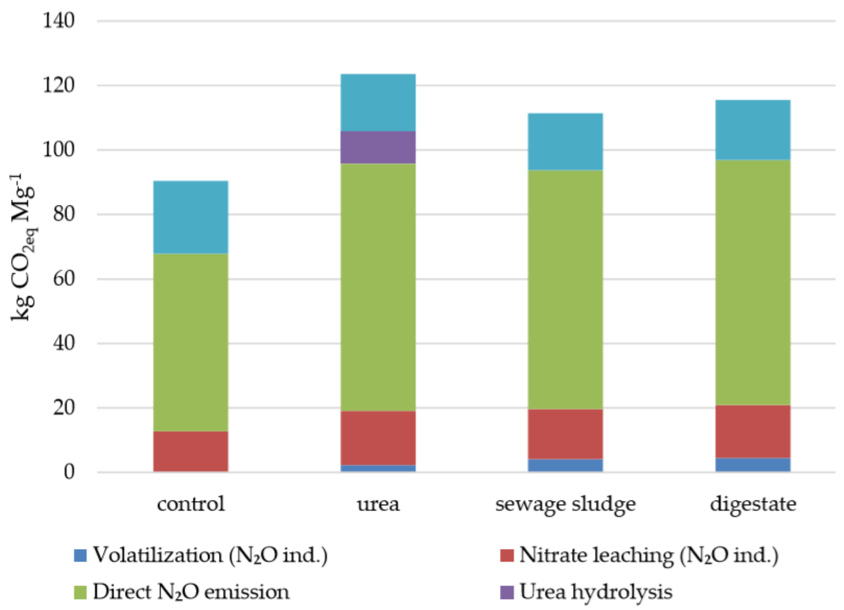

Figure 4. On-site emissions related to N_2O direct and indirect emissions, fuel combustion, and urea hydrolysis from sorghum production as affected by different fertilization managements. Values correspond to the annual average for the 2016–2018 period.

4. Discussion

4.1. Biomass Yield

The DM biomass yield of Rona 1 fertilized with urea recorded in this study (15.0 Mg ha^{-1}) was similar to that reported in a previous study carried out under the same environmental conditions (15.2 Mg ha^{-1}) [36]. In previous research at the same experimental site, a lower DM yield for sweet sorghum hybrid Sucrosorgo 304 fertilized with urea compared to the yield of Sucrosorgo 506 was reported [49]. Irrespective of the hybrids, application of bio-based waste products provided a biomass yield statistically comparable to that for crops fertilized in a conventional way with urea. These observations are in line with the findings of Kołodziej et al. [23], who reported that application of sewage sludge enhanced yields of Sucrosorgo 506 and Rona 1. Similarly, Akdeniz et al. [50] recorded an increase in sorghum DM yield as a response to sewage sludge application. Our findings are congruent with the results of Verdi et al. [51], who found no differences between the yield of crops fertilized with digestate and urea. In another study conducted in southwestern Germany, it was found that digestate could be an adequate substitute for mineral fertilizer in sweet sorghum production [52]. The same reaction of sorghum plants to biosolids application was noted by Sigurnjak et al. [53] in a study carried out in the Czech Republic under similar weather conditions. Digestate can be considered a synthetic N substitute without crop yield losses [54]. The biomass yields were significantly different across the years of the experiment. This was probably associated with the varied weather conditions.

4.2. Carbon Footprint

Results indicated that greenhouse gas emissions varied considerably between the fertilization treatments. Both the CO_{2eq} emitted to produce a metric ton of biomass and emissions per area unit decreased when sewage sludge and digestate were applied. Styles and Jones [55] reported that production of miscanthus biomass for energy purposes resulted in GHG emissions of 1938 kg CO_{2eq} per hectare. This lower value can be associated with the lower N demand of miscanthus [33].

The application of synthetic fertilizer is the main source of external GHG emissions from sorghum production. Similar results were obtained in a study conducted by Plaza-Bonilla et al. [11], who reported the great impact of N fertilization on external emissions. Most of the C footprint is associated with N fertilizer production and use [19]. Storlien et al. [9] reported a significant impact of N fertilization on CO_2 and N_2O emissions from sweet sorghum, especially at the beginning of the growing season in each year of the experiment.

Lower emissions from the production of sorghum using biosolids were associated with the lower reliance on the external input of synthetic fertilizer. Considering the CO_{2eq} quantity emitted into the atmosphere for synthetic fertilizers production, partial or total fertilization with digestate provided lower CO_{2eq} emissions [25]. Application of digestate had a relatively lower impact on the emissions of CO_2 and CH_4 compared to urea [56]. Cumulative N emissions via volatilization showed that digestate could be a promising method of sustainable fertilization management to decrease N losses [51]. However, these research projects pointed out differences between gases emitted by the two kinds of fertilizers: digestate emitted 23% more N_2O than urea, but urea emitted 66% more ammonia than digestate [51]. These results are congruent with the findings of Dendooven et al. [26], who reported that emissions of CH_4 and CO_2 were not affected by fertilization treatments; however, digestate application increased emissions of N_2O. It was revealed that combining fresh and more stabilized sewage sludge enabled a decrease in N_2O emissions [44,57]. The emissions intensity of GHG from digestate amended soils was lower compared to the use of untreated manure and was at a similar level to those for synthetic fertilizer. However, the researchers pointed out that the agronomic and environmental results related to the impact on crop yield and C footprint cannot simply be predicted on this basis; rather, specific soil and digestate physio-chemical characteristics should also be taken into account [57].

Diesel combustion during farming operations is a significant contributor to global warming potential [12]. Pesticide application accounts for the lowest GHG contribution and this is associated with the low demand of sorghum for plant protection chemicals used for weed, disease, and insect control. Only a low rate of herbicide is needed in sorghum cultivation, which is a new crop in the Central European region and does not yet have natural enemies. Findings of the present study are in line with Plaza-Bonilla et al. [11], who found that emissions related to pesticides represented only 1% of external emissions, as an average of the tested hybrids and years. Sweet sorghum can be recognized as a high-yielding biofuel feedstock with minimal impact on net GHG emissions [58].

5. Conclusions

This study was performed because there is limited information on the allocation of agricultural residues for sweet sorghum as well as for digestate, which is a sub-product of anaerobic digestion. Application of bio-based by-products (sewage sludge and digestate) provided a sorghum biomass yield close to that obtained when conventional synthetic fertilizer was used. Combined direct and indirect CO_{2eq} emissions on the basis of both per unit area and per unit of biomass yield were lower when bio-based waste products were used compared with the application of urea. The present study indicated that the lower GHG emissions resulted from the reduced reliance on synthetic N fertilizers due to their replacement by alternative nutrient sources, such as sewage sludge and digestate. It can be concluded that this fertilization practice can be considered a promising sustainable strategy for low carbon agriculture, which allows the recycling of nitrogen and other nutrients as an element of the circular economy. Biosolids should be recommended for providing sustainable sorghum production as a feedstock for bioenergy to mitigate GHG emissions and global climate change processes. Further research is needed to confirm the suitability of the alternative fertilization management proposed in the present study. This future work should be focused on comparing results based on other available calculation tools. There is a great need to develop local emission factors, which will provide better characteristics of national conditions. The system boundaries can be extended to the production process of bioethanol and methane from sorghum. Moreover, data thus obtained could be compared with results from direct measurements of GHG emissions from soil using chambers placed in the field.

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ROZDZIAŁ 5

Porównanie efektywności energetycznej produkcji metanu i etanolu wytwarzanego z sorga cukrowego z uwzględnieniem zróżnicowanych technologii przygotowania surowca

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Research paper

Comparison of the energy efficiency of methane and ethanol production from sweet sorghum (Sorghum bicolor (L.) Moench) with a variety of feedstock management technologies

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ABSTRACT

This study investigated the energy outputs from methane and ethanol production from sweet sorghum (Sorghum bicolor (L.) Moench). Different methods of sorghum biomass management were compared. The effect of bio-based waste products – sewage sludge and digestate replacing urea – on the energy output of biofuels produced from two different hybrids of sweet sorghum was evaluated. Conversion of fresh sorghum biomass into methane generated 76.6–179.5 GJ ha^{-1}, while the gross energy output from ethanol was only 22.6–70.5 GJ ha^{-1}. Application of digestate allowed the highest energy efficiency ratio to be obtained in terms of ethanol production for both tested hybrids and in terms of methane for Rona 1. Sorghum should be used as biogas feedstock in the temperate climate of Central Europe. The application of waste – sewage sludge and digestate – in feedstock management increased the energy efficiency of biofuel production.

1. Introduction

Biomass and waste currently accounts for almost two-thirds (123 million Mg of oil equivalent, 63.1%) of all renewable energy con-sumption in the European Union (EU) [1]. The total supply potential of energy crops for all EU members is estimated to increase from 39 million Mg of oil equivalent in 2010 to 131 million Mg of oil equivalent in 2030 [1]. In 2015 51% of biogas produced in the EU came from dedicated energy crops [2]. Germany, the leader in the European biogas sector, generates 93% of its biogas volume from agricultural crops, predominantly maize (Zea mays L.) silage [3].

In the EU, production of bioethanol is based on crops used for food production – in Germany and France the key feedstock is wheat (Triticum aestivum L.), while maize is mainly used in Central Europe [3]. In 2012, total agricultural land used for bioenergy crops in the EU was about 4.5 Mha. The area which might become available for production of energy crops is estimated to be 24 Mha in 2030 (18% of total arable land representing 12% of agricultural area) [1]. However, increasing land demand for biofuel feedstock cultivation is associated with severe competition with food production. To reduce the risk of indirect land use change, the EU (Directive EU 2015/1513 of the European Parlia-ment and Council of 9 September 2015) endorsed a mandatory cap of 7% of biofuels to be sourced from crops grown on agricultural land and used in transport fuel consumption in 2020. Therefore, today the cru-cial challenge for the sustainable production of agricultural biomass as biofuel feedstock is the choice of crops that can be promoted within the framework of new policy regulations within the EU.

Consequently, a sustainable approach to land management can be realized through the use in energy crop production of agricultural areas that are less favorable for food crops. Sweet sorghum (Sorghum bicolor (L.) Moench) - lignocellulosic grass species represents a 2nd generation biofuel feedstock [4]. One of the crucial factors in the sustainable production of energy crops is improvement of the energy balance and efficiency [5,6]. Sweet sorghum is characterized by wide adaptability to marginal growing conditions, resilience, and, due to its efficient C_4 photosynthetic pathway, generation of high biomass yield [7,8]. Therefore, it exhibits lower input and better energy efficiency than maize [5,9], and also than alfalfa (Medicago sativa L.) with Timothy grass (Phleum pratense L.) [10]. Sorghum gives a better energy yield and net gain than sugar beet (*Beta*

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vulgaris L.) [6], and has a higher net energy balance than grain sorghum and maize [11].

Nitrogen demands contribute to the highest proportions of total energy consumption of all energy inputs for feedstock management [5,6,10,12]. The recycling of biowastes such as biogas digestate or sewage sludge through agricultural use as an alternative to synthetic N fertilizers represents a sustainable solution and leads to a decrease in energy input at the feedstock production stage [12]. Use of biowastes allows nutrient recirculation, which is in line with the European policy for a circular economy [13]. Commercial biomass conversion technol-ogies for second generation biofuels are still under development [14]. Currently, anaerobic digestion technology for methane production is recognized as a more efficient method of energy generation from lig-nocellulosic biomass than anaerobic fermentation for ethanol produc-tion [10,11].

Given the aforementioned considerations, the present study in-vestigated the suitability of sweet sorghum for the production of biogas and bioethanol in the temperate Central Europe (CE) climate. In Poland and other CE countries sweet sorghum is a relatively new crop and a promising alternative to maize that can contribute to the diversification of feedstock supplies [4]. There is still very little information available concerning energy inputs for sorghum feedstock production for either anaerobic digestion or ethanol fermentation.

Thus, this research sought to: (i) determine the biogas – methane and ethanol – production on fresh and dry matter of sorghum biomass,

B evaluate the effect of replacing urea – a synthetic fertilizer – with biogas digestate or sewage sludge on energy inputs of sorghum as biofuels feedstock, (iii) compare land use efficiency expressed as gross methane and ethanol energy outputs based on lower biofuel heating values, and (iv) calculate the energy efficiency ratio.

This study provides practical data required for the determination of whether sustainable management in feedstock production based on recycling biowastes could be considered beneficial from an energy balance perspective. Furthermore, the results represent an interesting insight into energy balances at the field level that will help in the choice of a more energy efficient technology for biomass conversion.

2. Materials and methods

2.1. Field experiment

Biomass of two different maturing sweet sorghum hybrid types (Rona 1 and Sucrosorgo 506) was obtained as a feedstock from an ex-perimental site located in the southwestern Poland (Central Europe; N 51°10′25″ and E 17°07′02″). Two-factorial field experiment included two sorghum hybrids - Sucrosorgo 506 and Rona 1- and four fertiliza-tion managements. Treatments were replicated four times.

Sucrosorgo 506 is a late-maturing triple-cross sweet sorghum hybrid (Sorghum Partners Inc., USA). According to previous research, this hybrid gives high biomass yield in the moderate climate of Central Europe [15]. The second hybrid used in the present study was Hun-garian hybrid Rona 1 recommended for silage production (Gabonaku-tató) [16].

The treatments consisted of: (1) control fertilized exclusively with potassium and phosphorus and three treatments including the same ratios of potassium (70 kg ha^{-1} K_2O) and phosphorus (100 kg ha^{-1} P_2O_5) fertilizers and different sources of nitrogen (100 kg N ha^{-1}): such as (2) 220 kg ha^{-1} urea, (3) 19 t ha^{-1} solar dried sewage sludge sourced from a municipal sewage treatment plant and d 45 m^3 ha^{-1} methanogenic post-digestion liquid digestate (termed digestate in this paper). Each of fertilizer was applied just before sowing. Sorghum seeds were sown on 6 May 2016. Plants were har-vested on 23 September 2016 using brush cutter developed by Stihl in Germany (model FS400C). For total solids (TS) estimation, 5 sorghum plants per each plot were harvested with bowl chopper (Krag), weighed to reach constant mass and total solids (TS) ratio was calculated according to Polish norm PN-R-04013:1988 [17]. Samples of sorghum biomass for ethanol and biogas production were taken from each treatment as an aggregate sample and then the whole biomass included stalks, leaves and panicle were cut for 3–4 particles size. After harvest operation, biomass from area of 12.6 m^2 (2.1 × 6.0 m) was weighed and then yield per ha was extrapolated. In biomass production calcu-lations, losses during harvest were also included.

2.2. Analytical methods

The chemical composition of biowastes (solar dried sewage sludge and digestate) used for satisfying sorghum nitrogen demands was de-termined according to the following methods: total solids - PN-EN 12880:2004 [18], volatile solids - PN-EN 12879:2004 [19], pH value - PN-EN 12176:2004 [20], total nitrogen (N) –PN-EN 13342:2002 [21], ammonia nitrogen N–NH_4 – PN-EN 14671:2007 [22], potassium (K) – PN-EN ISO 11185:2009 [23]; PN-EN 13657:2006 [24], total phos-phorus (P) PN-EN 13965-1:2004 [25], calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni) – PN-EN ISO 11185:2009 [23]; KJ-I-5.4-174 [26], mercury (Hg) - KJ-I-5.4-36 [27], Salmonella bacteria –PN-EN ISO 6579-1:2016–04 [28], number of live eggs of intestinal parasites - KJ-I-5.4–59 M 03 version [29].

Specific information concerning the chemical composition of bio-wastes used as nutrient sources is shown in Table 1. Dried municipal sewage sludge contained 42% TS and 1.29% of TS of total nitrogen. The sewage sludge was characterized by relatively higher heavy metal contents compared to values indicated by other authors [30,31]. However, the heavy metals and mercury contents were significantly below the maximum permissible values, as regulated by the EU [13]. Digestate had a TS content of 2.8% and total nitrogen content of 8% TS. The mean content of heavy metals in digestate was relatively low and did not exceed the maximum permissible values [13].

Total nitrogen content in shredded plant material was determined

Table 1
Characteristics of the wastes biomass used as fertilizers in field experiment.

Parameter/chemical element with limits value for organic fertilizer and organic soil improver	Unit	Digestate	Sewage sludge
pH		7.6	7.4
TS[(1)]	%	2.8	42
organic compounds	% TS	71	31.5
total nitrogen (N)		8	1.29
N–NH_4		2	< 0.10
total phosphorus (P)		0.54	1.63
calcium (Ca)		2.99	4.11
magnesium (Mg)		1.02	0.60
potassium (K)	mg kg^{-1} TS	1280	n.a.[(2)]
copper (Cu) - 200[(3)]		49.6	239
zinc (Zn)		170	777
lead (Pb) - 120[(3)]		6.13	94
cadmium (Cd) - 3[(3)]		2.78	0.71
chromium (Cr)		11.2	32.9
nickel (Ni) - 50[(3)]		11.6	24.7
mercury (Hg) - 1[(3)]		0.050	0.540
Salmonella bacteria	no Salmonella species in 25 g sample[(3)]	0	0

The results received from Südzucker Polska S.A. and Wodociagi Klodzkie sp. z o.o.

[(1)] TS total solids.
[(2)] n. a. not analysed.
[(3)] The maximum permissible concentrations of contaminants in organic soil improver in the frame of the Fertilizing Product Regulation Proposal for a Regulation on the making available on the market of CE marked fertilizing products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/ 2009 (COM, 2016).

using the Kjeldahl method according to the procedure of the Association of Official Agricultural Chemists (AOAC) [32] (Büchi Dis-tillation Unit K-350, Switzerland). Total solids, volatile solids (VS) and ash contents of the tested sorghum biomass were analysed according to standard methods: DIN DEV 38 414 part 2 [33] and DIN DEV 38 414 part 3 [34]. To determine the neutral detergent fiber (NDF), acid de-tergent fiber (ADF) and acid detergent lignin (ADL) concentrations, the van Soest method was used; PN-EN ISO 13906:2009 [35,36]. Cellulose and hemicellulose contents were calculated based on data on fiber fractions and ash contents according to the following equations [37]:

$$\% \text{ Cellulose} = \% \text{ ADF} - (\% \text{ lignin} + \% \text{ ash}) \quad (1)$$

$$\% \text{ Hemicellulose} = \% \text{ NDF} - \% \text{ ADF} \quad (2)$$

The calcium content was determined by flame photometry [38]. The colorimetric method was used to measure magnesium and phosphorus concentration [39]. pH was determined by use of a basic pH meter (type MP220, Mettler-Toledo).

2.3. Biofuel experimental design

2.3.1. Batch anaerobic digestion process

Production of biogas and methane from sorghum samples was evaluated in a batch anaerobic digestion (AD) at lab-scale. The ex-perimental design was identical to that for ethanol fermentation. Experimental equipment complied with the standard method – DIN 38 414 – S8 (1985) – based on VDI 4630. Tests were carried out in three replicates in mesophilic conditions (38 °C) in unstirred eudiometer batch digesters of 1000 mL total capacity containing 400 g of substrate and inoculum. The digestate collected from agricultural biogas plant was separated and the liquid fraction was used as an inoculum in the assays. Representative samples of inoculum were taken for analyses of TS and VS content. The inoculum had a TS content of 5.25% ± 0.05, a VS content of 65.46% TS ± 1.78 and a pH value of 8.0 ± 0.4 (average value over three replicates and standard deviation). The digesters were filled with substrates and inoculum to achieve 8.0% TS concentration. Substrates were not supplemented with nutrients and pH value was not adjusted. Inoculum without substrate was tested as a control. To maintain the homogeneity of substrates and provide for easy biogas collection, each fermenter was mixed for approximately 30 s (initially, every day and then every two or three days). The AD process was conducted until the daily biogas production decreased to a value less than 1% of the total biogas volume. The anaerobic digestion process was monitored through evaluation of daily volumes of produced biogas. The results were normalized to standard conditions comprising dry gas, a temperature of 0 °C and a pressure of 1013 hPa. The resulting data were recalculated per tonne of sorghum biomass dry matter (described in this study as methane production, Nm^3 Mg^{-1} TS) and hectare (de-scribed as methane yield, Nm^3 ha^{-1}).

Biogas qualitative analyses were initially performed every day and then every two or three days with a portable gas analyzer from Geotech (Biogas 5000). This equipment has been certified: ATEX, IECEx, CSA, MCERTS and UKAS calibration (ISO/IEC17025). The concentrations of methane, carbon dioxide, oxygen, hydrogen, ammonia, hydrogen sul-fide and other volatile substances (the fraction comprising mainly ni-trogen, carbon monoxide and volatile organic compounds, which are often called 'balance' in the literature) were determined.

2.3.2. Ethanol fermentation

Fresh shredded sorghum biomass was pretreated and hydrolyzed in a double step. First, the substrate was pretreated using microwave pulses for 5 min in a microwave digestion apparatus (speed wave™ MWS-2, Berghof Instruments GmBH, Germany). The microwave power was set at 800 W. Afterwards, biomass (25% w/w) was added into 250-mL Erlenmeyer flasks containing 2% v/v dilute sulfuric acid (H_2SO_4). The mixtures were incubated at 150 °C for 1 h. The pH value of acidic hydrolysates was adjusted to 5.0 with 30% sodium hydroxide. A com-mercial hydrolytic enzymes cocktail – Cellic CTec2 (Novozymes, Denmark) – was used at a concentration of 3% w/w for enzymatic hydrolysis. Samples were incubated in a WNB7-47 water bath (Memmert GmBH, Germany) with shaking at 150 rpm for 72 h at 50 °C.

After pretreatment and hydrolysis, 100 g of enzymatic hydrolysates samples was inoculated with Saccharomyces cerevisiae (Strain DF 639 SIHA® Active Yeast 6, Eaton, Germany) cultures with a cell con-centration of 0.2 g of TS of yeast per kg (approximately 1×10^7 cells/ mL). The fermentation assays were performed in a CWE-2 incubator (Poland) at 30 °C. The process was terminated after 72 h. After fer-mentation, all samples were distilled (BÜCHI Distillation Unit K-355, Switzerland). Ethanol concentration in the distillate was determined using a DMA 4500 M Density Meter (Anton Paar, Austria). In this ex-periment densitometry was used instead of GC/HPLC method, because it is recommend by PN-ISO 5725-1:2002. All assays were carried out in triplicate. The analysis of the by-products content, forming during pre-hydrolysis and fermentation as well as supplementation of fermentation media with additional nitrogen compounds, were not the subject of this work. Results were recalculated per tonne of dry matter of sorghum feedstock (described in this study as ethanol production, L Mg^{-1} TS) and hectare (described as ethanol yield, L ha^{-1}).

2.4. Energy analysis procedures

2.4.1. Energy input in feedstock production

Production of biofuel feedstock includes various field technology operations (e.g. tillage, sowing, fertilization, harvest), requiring ap-propriate energy inputs. The energy inputs were divided into direct and indirect energy flux. The direct energy items included consumption of fuel for tractors and machines during various field operations and labor, whereas indirect energy items comprised energy embodied in ma-chinery, fertilizers, pesticides and seeds.

Four categories of energy inputs based on energy fluxes were con-sidered, such as: labor, fuel, farming machinery operations and mate-rials (including seeds, fertilizers and pesticides). Input data, such as fuel consumption, amount of herbicides, fertilizers and seeds are based on the results of the field experiment. Similarly, machinery and all agro-technical operations included in the analysis were specified for sweet sorghum cultivation in weather and soil conditions of the research area. The data were converted into a common energy unit by conversion with energy equivalence for various energy inputs according to the metho-dology developed by Institute for Building, Mechanization and Electrification of Agriculture (IBMER) [40]. This research institute is a part of Institute of Technology and Life Sciences under the Polish Minister of Agriculture and Rural Development ordinance. Country data were used in the analysis as a recommended practice, because they describe specific energy efficiency of production systems in the region, where present research was carried out. Only energy equivalence for sorghum seeds was based on other source [41]. Sweet sorghum is still new species in Central Europe and therefore there is a lack of energy equivalence for its seeds.

Energy inputs associated with production of sorghum biomass were calculated both per unit of production (MJ Mg^{-1} TS) and per area unit (MJ ha^{-1}). To compare all the treatments on an equal basis, the same field technology operations were assumed. The energy equivalents of agrotechnical inputs included in the analysis were listed in Table 2. Consumption of diesel was estimated based on the typical consumption per hour of a given tractor and its characteristics, e.g. working time during each field operation.

Indirect energy input of machinery was calculated from the annual use of the accompanying machine and tractor, and their nominal life-time and weight. Data for technical parameters of farm machinery used in sorghum production process are shown in Table 3. The working time for each farming operation was estimated based on the performance of the machine in question, according to producer data.

Table 2
Energy equivalents of agrotechnical inputs in sweet sorghum production.

Input	Unit	Energy equivalent	Reference
Diesel oil	MJ dm^{-3}	48	[39]
Tractors	MJ kg^{-1}	125	
Machines		110	[39]
Labor	MJ h^{-1}	80	
Herbicide	MJ kg^{-1} a.i.[(1)]	300	
Fertilizers	MJ kg^{-1}		
N		77	
P_2O_5		15	
K_2O		10	
Sweet sorghum seeds		44	[38]

** a.i. active ingredient.

In the analysis, the 1000 seed weight, the germination capacity of using sowing material from sorghum hybrids and the energy equivalence of 44 MJ kg^{-1} were calculated [41].
Sewage sludge and digestate were not considered as energy inputs since they are waste products. The energy inputs associated with the application of sewage sludge and digestate included transport to the field (distance 10 km) and spreading. Data for energy inputs not ac-counted for are those for electricity and buildings.

2.4.2. Biofuel energy output

It is worth noting that in the present study the assessment of energy output is related to methane and ethanol produced from sweet sorghum as a biofuel feedstock. For conversion of yields of biofuels (ethanol and methane) produced from sorghum to energy units (net energy output), the lower heating values (LHV) of ethanol (21.26 MJ dm^{-3}) and methane (35.89 MJ m^{-1}) were used [42]. The LHV of ethanol and
methane feedstock produced by different methods was calculated. Net energy output expressed in GJ ha^{-1} was determined as the product of LHV (MJ Mg^{-1} TS) and sorghum dry matter yield (Mg TS ha^{-1}). The energy efficiency ratio was calculated according to the following equation:

$$\text{Energy efficiency ratio} = \frac{\text{Energy output } [GJ\ ha^{-1}]}{\text{Energy input } [GJ\ ha^{-1}]}$$

where:

- energy output refers to energy output of biofuels produced from sweet sorghum feedstock,
- energy input refers to energy input exclusively at the field level feedstock

In this study the energy analysis did not include the energy input at the stage of the technological processes of biofuel production.

2.5. Statistical analytical methods

The mean values for sorghum chemical composition, dry matter yield of sorghum biomass, ethanol and methane production per tonne and yields per hectare, energy values of ethanol and methane and en-ergy output of ethanol and methane were processed by two-way ana-lysis of variance (ANOVA). The methods of sorghum management were entered as a fixed effect in the analysis and replications were considered random effects. Mean values were compared by Tukey's test at the significance level of P ≤ 0.05. All statistical analyses were carried out using the Statistica (version 13.1 StatSoft, Poland) software package. The same software was used for graphs .

Table 3
Technical parameters of agricultural machines, their performance and fuel consumption in the sorghum production process.

Field operations	Tractor			Parameters of operative machine				Fuel consumption (L ha^{-1})	Notes
	Power (kW)	Weight (kg)	Service life (h)	Type Size	Weight (kg)	Service life (h)	Performance (ha h^{-1})		
Stubble treatment	66.19	4120	11000	Stubble cultivator 2.6 m	940	1095	2.10	5.20	
Moldboard ploughing	66.19	4120	11000	Plough 1.5 m	740	920	0.60	18.20	
Harrowing	34.60	2880	5100	Spike-tooth harrow 5 m	530	1230	3.00	1.90	
Pre-sowing cultivation	66.19	4120	11000	Seedbed cultivator 3 m	770	1460	2.40	4.55	
Mineral fertilizer spreading	34.60	2880	5100	Pneumatic fertilizer spreader 18 m 1400 dm^3	260	1530	4.00	1.43	NPK or PK[(1)]
Digestate application	88.00	4800	12000	Liquid manure spreader 7.5 m 12000 dm^3	2000	2000	2.06	6.41	45 m^3 ha^{-1}[(2)]
Sewage sludge application	66.19	4120	11000	Manure spreader 4 m 5000 kg	2000	1290	1.40	7.80	19 Mg ha^{-1}[(3)]
Sowing	34.60	2880	5100	Pneumatic seeder 3 m 750 dm^3	620	1800	1.05	5.44	20 seeds m^{-2} [(4)]
Chemical weeding	66.19	4120	11000	Sprayer 1500 dm^3 18 m	375	2200	7.00	1.56	2 dm^3 ha^{-1} Lumax[(5)]
Harvest	66.19	4120	11000	Forage harvester 2 row	550	3300	0.90	12.13	
Transport	88.00	4800	12000	3 farm trailers 14 000 kg max m^3	4640	2480	0.45	29.33	

A NPK treatment - mineral fertilizer as a source of 100 kg N ha^{-1}: 220 kg urea ha^{-1}; PK - triple superphosphate (70 kg P_2O_5 ha^{-1}) and potassium chloride (100 kg K_2O ha^{-1}) were applied to all plots (including control).
B Digestate treatment – digestate as a source of 100 kg N ha^{-1}- rate: 45 m^3 ha^{-1}.
C Sewage sludge treatment – sewage sludge as a source of 100 kg N/ha - rate: 18.5 Mg ha^{-1}
D 1000 seeds weight were 34.57 g and 27.26 g for Sucrosorgo 506 and Rona 1, respectively.
E Chemical weeding was the same for all treatments - terbuthylazine 187.5 g dm^{-3} + mesotrione 37.5 g dm^{-3}+ s-metolachlor 312.5 g dm^{-3}
***

Table 4
Sorghum biomass yield, biogas, methane, ethanol production and yield.

Feedstock production methods		TS[1] yield (Mg ha^{-1})	Production per Mg of total solids[3]			Yield per hectare[3]		
Hybrids	Fertilization treatment		Biogas (Nm^3 Mg^{-1} TS)	Methane (Nm^3 Mg^{-1} TS)	Ethanol (L Mg^{-1} TS)	Biogas (Nm^3 ha^{-1})	Methane (Nm^3 ha^{-1})	Ethanol (L ha^{-1})
Sucrosorgo 506	control	7.03^d ± 0.12[2]	520.58 ± 15.73	303.58 ± 7.51	151.42^d ± 8.41	3661.12^e ± 110.62	2135.00^d ± 52.85	1065.17^g ± 59.13
	urea	16.89^a ± 2.22	539.90 ± 3.58	296.07 ± 34.17	196.23^c ± 3.58	9117.64^a ± 60.47	4999.88^a ± 576.98	3313.84^a ± 60.42
	sewage sludge	12.57^{ab} ± 2.32	550.21 ± 20.85	318.95 ± 14.29	166.24^d ± 18.82	6913.42^b ± 262.94	4007.60^b ± 179.56	2089.23^d ± 21.54
	digestate	10.45^{bc} ± 1.29	551.92 ± 15.72	327.57 ± 9.81	212.45^c ± 6,54	5769.85^c ± 164.31	3424.50^{bc} ± 102.53	2221.04^d ± 68.33
Rona 1	control	6.35^d ± 0.85	561.59 ± 23.46	340.20 ± 0.89	261.32^{ab} ± 5.95	3567.61^e ± 149.03	2161.21^d ± 5.63	1660.12^f ± 37.84
	urea	10.90^{bc} ± 2.18	535.33 ± 10.90	329.31 ± 4.53	255.69^b ± 3.03	5833.07^c ± 118.78	3588.16^{bc} ± 49.40	2786.75^c ± 33.18
	sewage sludge	9.85^{bc} ± 0.97	550.85 ± 23.20	336.16 ± 15.41	207.52^c ± 9.51	4877.25^d ± 205.42	2976.39^c ± 136.47	1837.43^e ± 84.20
	digestate	11.07^b ± 1.14	562.48 ± 19.70	342.36 ± 11.86	274.17^a ± 1.54	6227.74^c ± 218.11	3790.58^b ± 131.32	3035.59^b ± 10.31
Hybrids	Sucrosorgo 506	11.74	539.60	311.49^b	181.59^b	6149.86	3527.65	2171.32
	Rona 1	9.29	552.72	337.08^a	249.68^a	5149.07	3142.97	2329.97
Fertilization treatment	control	6.69^b	541.09	321.89	206.37	3641.37^b	2148.10^b	1362.6^c
	urea	13.89^a	537.16	316.01	225.96	6044.59^a	4152.85^a	3050.30^a
	sewage sludge	10.71^a	550.47	325.83	186.88	6098.95^a	3595.11^a	1963.33^b
	digestate	10.76^a	558.26	336.44	243.31	7146.90^a	3644.15^a	2618.32^b

a b c Means followed by similar letter within each column and columns that do not contain letters indicate no significance differences between treatments (p > 0.05) based on results of ANOVA followed by Tukey multiple range tests.
* TS total solids; results are mean of four replicates.
* ± standard deviation.
* Results are mean of triplicate.

Table 5
Sorghum biomass chemical composition.

Feedstock production methods		pH	TS[1] (%)	VS[2] % TS	Ash	N	P	Mg	Ca
Hybrids	Fertilization treatment								
Sucrosorgo 506	control	5.53 ± 0.05	23.95 ± 1.25[3]	96.51^{ab} ± 0.29	4.24^c ± 0.02	0.87^e ± 0.00	0.14 ± 0.00	0.23^b ± 0.00	0.47 ± 0.02
	urea	5.47 ± 0.03	25.82 ± 0.79	96.28^b ± 0.29	4.79^b ± 0.02	0.98^{cd} ± 0.01	0.15 ± 0.00	0.27^{ab} ± 0.01	0.48 ± 0.01
	sewage sludge	5.46 ± 0.01	23.34 ± 0.28	96.35^b ± 0.11	4.82^b ± 0.00	1.31^a ± 0.02	0.21 ± 0.00	0.34^a ± 0.02	0.56 ± 0.02
	digestate	5.51 ± 0.04	25.48 ± 0.74	95.92^b ± 0.08	5.61^a ± 0.01	1.00^c ± 0.00	0.17 ± 0.01	0.34^a ± 0.01	0.44 ± 0.08
Rona 1	control	5.24 ± 0.04	27.96 ± 0.38	97.16^a ± 0.03	3.59^e ± 0.01	0.93^{de} ± 0.01	0.12 ± 0.01	0.25^b ± 0.03	0.37 ± 0.02
	urea	5.36 ± 0.06	26.28 ± 1.24	96.36^b ± 0.30	4.19^c ± 0.00	1.18^b ± 0.01	0.14 ± 0.00	0.28^{ab} ± 0.02	0.35 ± 0.05
	sewage sludge	5.34 ± 0.10	25.70 ± 0.07	96.28^b ± 0.13	4.21^c ± 0.00	1.29^a ± 0.03	0.19 ± 0.00	0.27^{ab} ± 0.02	0.53 ± 0.02
	digestate	5.40 ± 0.02	27.72 ± 1.16	96.67^{ab} ± 0.18	3.91^d ± 0.02	0.87^e ± 0.00	0.15 ± 0.01	0.24^b ± 0.02	0.37 ± 0.02
Average for factors									
Hybrids	Sucrosorgo 506	5.50^b	24.65^b	96.26	4.88^a	1.04	0.17	0.29	0.49
	Rona 1	5.31^a	26.92^a	96.61	3.97^b	1.07	0.15	0.26	0.41
	control	5.38	25.96	96.83	3.91	0.90^c	0.13^c	0.24	0.42^b
Fertilizer	urea	5.43	26.05	96.32	4.49	1.08^b	0.14^{bc}	0.27	0.41^b
	sewage sludge	5.41	24.52	96.31	4.51	1.30^a	0.20^a	0.30	0.55^a
	digestate	5.47	26.60	96.29	4.76	0.94^{bc}	0.16^b	0.29	0.40^b

Data are reported as mean of three replicates and standard deviation.
**b c Different superscript letters indicate significant differences at p < 0.05. Columns that do not contain letters indicate no significance differences between treatments (p > 0.05) based on results of ANOVA followed by Tukey multiple range tests.
TS total solids.
VS volatile solids.
± standard deviation.

3. Discussion and results

3.1. Biomass yield

The average feedstock yields for two sorghum hybrids from the different fertilization treatments and the non-fertilized reference are given in Table 4. The ANOVA analysis showed that the dry matter production was significantly affected by interaction between the ex-perimental factors under study. The average dry matter yields ranged from 6.35 (for non-fertilized Rona 1) to 16.89 Mg ha^{-1} (for Sucrosorgo 506 fertilized with urea). The dry matter yield of Sucrosorgo 506 fer-tilized with urea achieved in the present study was similar to those recorded in a previous study in the same research site – 14.80 Mg ha^{-1} [43] – and in northeastern Poland: 15.77 Mg ha^{-1} [10]. In experi-mental trials carried out under similar environmental conditions, Głąb and Sowiński [15] recorded a slightly higher yield for Rona 1 fertilized with the same rate of N and form – urea, 15.0 Mg ha^{-1} – as compared to the value achieved in this study – 10.90 Mg ha^{-1}.
Both sewage sludge and digestate application significantly increased the yields across the hybrids (10.71 and 10.76 Mg ha^{-1}, respectively)

which were on average 38% higher than that for the control (6.69Mgha−1). Irrespective of the hybrid used, application of waste products provided a yield statistically comparable to that for urea. These results are consistent with the findings of Kołodziej et al. [31], who found that application of municipal sewage sludge significantly enhanced yields of Rona 1 and Sucrosorgo 506. Similarly, a study conducted by Formowitz and Fritz [44] showed that biogas digestate could be an adequate substitute for mineral fertilizer in sweet sorghum cultivation in southwestern Germany.

3.2. Chemical composition

The chemical composition of crops used for energy production is crucial in the assessment of the suitability of a feedstock for ethanol and methane production [12]. Both total solids and volatile solids contents are key parameters providing optimal conditions in a biogas digester. Results of chemical analyses of sorghum biomass are given in Table 5. Total solids content was not affected by interaction between the experimental factors. The highest volatile solids content of an average 97.16% TS was observed for Sucrosorgo 506 without nitrogen fertilization. Among all the treatments, the highest ash content was observed in biomass of Sucrosorgo 506 after application of digestate. Significant interaction between the tested factors was recorded for nitrogen content, with the highest nitrogen value being noted for biomass fertilized with sewage sludge – for both hybrids. Application of sewage sludge and digestate significantly increased magnesium content in Sucrosorgo 506 in comparison to the reference treatment without fertilizer. This trend was not observed for biomass from Rona 1, which contains magnesium at the same level across all fertilization treatments. Irrespective of fertilization treatment, biomass from Rona 1 had a significantly lower pH value. This hybrid had a significantly higher TS content averaged across fertilization treatments. Data on cellulose, hemicellulose and lignin contents in sorghum biomass are given in Table 6. Significant interaction between the tested factors was recorded for cellulose, hemicellulose and lignin content. The highest cellulose content was observed in biomass of Sucrosorgo 506 after application of digestate. Rona 1 was characterized by significant lower cellulose and hemicellulose contents, by 4.94 and 2.69% points (p.p.), compared with Sucrosorgo 506. Lignin content was at similar level (0.31 p. p.) in the biomass of both tested hybrids. Irrespective of hybrids, there was no effect of fertilization treatment on cellulose and hemicellulose contents. However, the lignin content was lowest, when biomass was fertilized with sewage sludge. Biomass from sorghum hybrids did not significantly differ in terms of the following macronutrient contents: nitrogen, phosphorus and magnesium. Contrary observations were noted by Kołodziej et al. [31], who recorded a significantly greater accumulation of Mg and P by Sucrosorgo 506 compared to Rona 1, while biomass of Rona 1 was characterized by a higher content of N. The content of macronutrients especially nitrogen affects ethanol production [45]. Results averaged across the hybrids showed that application of sewage sludge caused significant increases in nitrogen, phosphorus and calcium contents in sorghum biomass compared to the other fertilization treatments. Similar results were also reported by Kołodziej et al. [31], suggesting an important role of sewage sludge as a fertilizer. The structure of lignocelullosic complex and contents of fiber fractions was shown to have a significant effect on ethanol and methane fermentation [46]. In the present study, content of hemicellulose in sorghum biomass was quite high. This compound is degradably and provides a good source of carbon for microorganism, during fermentation process [47]. In study of Mahmood [48], the content of NDF was similar to that in this study and varied between sorghum hybrids, from 46% TS for Agrogas to 60% TS for Cerberus. The high content of lignin in substrate for biofuels production have negative effect on methane fermentation process [48]. It is as a physical barrier to microbial enzymes, because of its polymer structure.

Table 6
Fiber fractions contents in sorghum biomass.

Feedstock production methods		Cellulose	Hemicellulose	NDF[(1)]	ADF[(2)]	Lignin
Hybrids	Fertilization treatment	% TS[(3)]				
Sucrosorgo 506	control	25.53^{c}	19.62^{h}	57.20^{c}	34.31^{c}	4.53^{cd}
		± 0.02[(4)]	± 0.04	± 0.06	± 0.09	± 0.03
	urea	27.01^{b}	20.13^{g}	58.19^{b}	37.01^{b}	5.20^{a}
		± 0.01	± 0.02	± 0.12	± 0.05	± 0.02
	sewage sludge	23.83^{d}	21.19^{f}	55.52^{d}	32.38^{d}	3.72^{f}
		± 0.03	± 0.01	± 0.05	± 0.07	± 0.04
	digestate	28.21^{a}	21.61^{e}	65.21^{a}	38.66^{a}	4.87^{b}
		± 0.03	± 0.01	± 0.08	± 0.12	± 0.03
Rona 1	control	22.52^{e}	21.65^{d}	52.28^{e}	30.69^{f}	4.59^{c}
		± 0.01	± 0.02	± 0.05	± 0.13	± 0.01
	urea	22.19^{f}	22.90^{c}	50.95^{f}	30.81^{e}	4.42^{d}
		± 0.01	± 0.01	± 0.04	± 0.07	± 0.04
	sewage sludge	19.93^{h}	23.14^{b}	49.88^{g}	28.23^{g}	4.07^{e}
		± 0.05	± 0.03	± 0.06	± 0.05	± 0.02
	digestate	20.17^{g}	26.54^{a}	47.65^{h}	28.04^{h}	3.98^{e}
		± 0.02	± 0.03	± 0.07	± 0.09	± 0.03
Average for factors						
Hybrids	Sucrosorgo 506	26.14^{a}	23.44^{a}	59.03^{a}	35.59^{a}	4.58
	Rona 1	21.20^{b}	20.75^{b}	50.19^{b}	29.44^{b}	4.27
Fertilizer	control	24.02	22.25	54.74	32.49	4.56^{ab}
	urea	24.60	20.66	54.57	33.91	4.81^{a}
	sewage sludge	21.88 m	22.39	52.70	30.30	3.90^{b}
	digestate	24.19	23.08	56.43	33.36	4.43^{ab}

Data are reported as mean of three replicates and standard deviation.

*** b c Different superscript letters indicate significant differences at $p < 0.05$. Columns that do not contain letters indicate no significance differences between treatments ($p > 0.05$) based on results of ANOVA followed by Tukey multiple range tests.

NDF neutral detergent fiber.

ADF acidic detergent fiber.

TS total solids.

± standard deviation.

Table 7
Structure of energy inputs for sorghum production based on mineral fertilizer and waste biomass.

Hybrids	Fertilization treatment	Tractors and machines	Fuel	Labor	Materials			Total input	EI(1)
					Seeds	Fertilizers	Herbicides		
		(MJ ha^{-1})							(MJ Mg^{-1} TS(2))
Sucrosorgo 506	Control	1174	3896	626	415	2050	323	8484	1206
	Urea	1196	3965	646	415	7700	323	14244	843
	sewage sludge	1329	4271	683	415	2050	323	9070	722
	digestate	1252	4204	655	415	2050	323	8898	851
Rona 1	control	1174	3896	626	307	2050	323	8376	1318
	urea	1196	3965	646	307	7700	323	14136	1297
	sewage sludge	1329	4271	683	307	2050	323	8963	1012
	digestate	1252	4204	665	307	2050	323	8800	795

**** EI energy input per unit of production.
**** TS total solids.

3.3. Energy input in biomass production

It must be highlighted that in this study the evaluation of energy input included only feedstock production and transport. Table 7 shows the amount of energy required for crop management at field scale. The total energy input in biofuel feedstock production ranged from 8.4 GJ (Rona 1 without nitrogen fertilization) to 14.2 GJ (Sucrosorgo 506 fertilized with urea). A study conducted by Jankowski et al. [10] showed that production of sweet sorghum as biogas feedstock was more energy intensive (22.04 GJ) compared with the results of the present study. This difference can be attributed to the higher fertilizer rates (160/80/160 N/P_2O_5/K_2O kg ha^{-1}) used in field trials. Garofalo et al. [6] reported that the amount of energy required for sorghum cultiva-tion ranged from 10.0 GJ (no tillage system without N fertilization) to 19.1 (conventional tillage system with 150 kg N ha^{-1}). Results of a study conducted by Gissén et al. [12] in southern Sweden showed that energy input in the cultivation of maize as biogas feedstock was 16 GJ, and half of this value was associated with mineral fertilization.

Among the different energy sources, fertilization had the greatest impact on the energy flux. When urea was applied, energy input asso-ciated with mineral fertilization accounted for 54.1 and 54.5% of the total energy used in the production of Sucrosorgo 506 and Rona 1, respectively. These results are in accordance with another study carried out by Jankowski et al. [10], who reported nitrogen fertilizer as the most significant factor in the energy consumption, accounting for 68.6% of the total energy used for sorghum production.
In this study, when sewage sludge and digestate replaced urea, the energy inputs for machines and tractors increased by 11.1 and 4.7%, respectively (Table 7), and led to an increase in energy input for fuel (7.7 and 6.0%, respectively) and labor (5.7 and 1.4%, respectively). Gissén et al. [12] also noted that partly replacing urea with digestate increased energy inputs for machinery and diesel due to the transport and application of a waste product characterized by a low nutrient concentration.

When sewage sludge or digestate was applied the total required energy inputs decreased 36.3 and 37.5% for Sucrosorgo 506 and 37.1 and 37.7% for Rona 1 as compared with the application of urea. These results are in line with findings of Gissén et al. [12], who stated that, when digestate partly replaced mineral fertilizer, the required energy input decreased from 16 GJ to 9 GJ.

3.4. Methane and ethanol production

Rona 1, as a medium-maturing hybrid, forms seeds early under the moderate climatic conditions of Central Europe and reaches dough ri-peness or even fully ripeness when it is harvested. Sucrosorgo 506 is a late-maturing hybrid and thus it is harvested in the flowering stage or at the beginning of grain development. Ren et al. [5] reported that sorghum grain had a six-fold higher energetic value than its vegetative parts. In the present study, Rona 1 produced more biofuel. The more favorable and balanced chemical composition of Rona 1 likely con-tributed to an increase in the TS-based production of biogas and ethanol by 8.2 and 37.5%, respectively, as compared with Sucrosorgo 506 (Table 4). These results are in accordance with Klimiuk et al. [49], who suggested that a lower fiber content resulted in higher methane yields.
Methane production per Mg TS was not affected by fertilizer treat-ments. In the present study, biogas and methane production was eval-uated on total solids basis (Table 4). The TS-based methane production ranged from 296 m^3 Mg^{-1} TS (308 m^3/Mg VS) for Sucrosorgo 506 fer-tilized with urea to 342 m^3 Mg^{-1} TS (354 m^3 Mg^{-1} VS) for Rona 1 fertilized with digestate. The total production of methane from the in-oculum (blank) was much lower than that for the other treatments, demonstrating high sorghum methane production capacity. These findings are in accordance with several previous studies. Herrmann et al. [50] noted that methane production based on VS of sorghum hybrid (Sorghum bicolor x sudanense) was 317.1 Nm^3 Mg^{-1}. Results of a study conducted by Mahmood and Honermeier [51] reveal that me-thane production was similar to that achieved in the present study. However, these authors reported higher VS-based methane production for Rona 1–387 Nm^3 Mg^{-1} compared to the results of present study –

349 Nm^3 Mg^{-1} VS (337 Nm^3 Mg^{-1} TS). In the Czech Republic, Pazderu et al. [52] noted that methane production was significantly affected by sorghum hybrids and varied between 207 and 246 Nm^3 Mg^{-1} TS. In another study conducted in Germany, sorghum hybrids produced a significantly different amount of biogas [48]. Hybrid selection and the choice of an appropriate harvesting date are the most important factors determining methane production from sorghum [53].
The courses of biogas formation determined in batch anaerobic di-gestion tests are shown in Fig. 1. A plateau was reached after ap-proximately thirty days, which indicates that the biomass has been exhausted. Irrespective of the hybrid, the biogas formation during 30 days of the anaerobic digestion process was stable and very similar. The cumulative methane production increased most intensively during the initial phase of digestion up to five days after the beginning of the experiment. These findings are in line with results obtained by Herr-mann et al. [50], who reported rapid methane formation during the first days without inhibition or time lag for tested crops: sorghum, maize, rye and triticale. This intensive biogas production in the first few days is a result of digestion of readily biodegradable organic material [46].

The biogas composition produced during digestion processes in the days on which the measurements of biogas volume and composition were done is shown in Figs. 2 and 3. The first two days were char-acterized by the highest share of other volatile compounds (balance): 52–84% during first day and 10–45% during second day. In the fol-lowing days, the percentage of these compounds showed a decreasing tendency. Biogas produced from Sucrosorgo 506 fertilized with urea

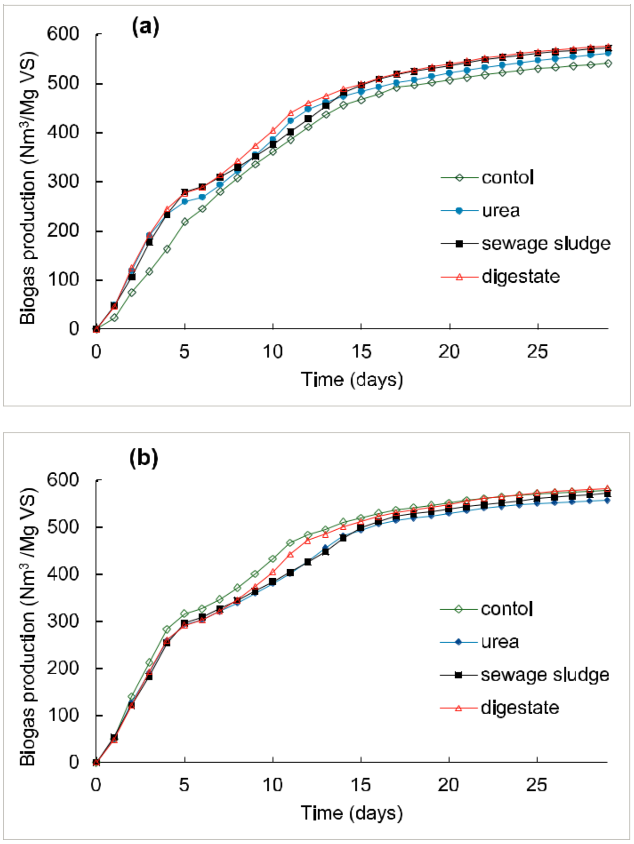

Fig. 1. Cumulative biogas production determined in batch anaerobic tests of (a) Sucrosorgo 506 and (b) Rona 1 with and without fertilization.

was characterized by the highest content balance (5–10%) during the whole process compared to other treatments (Fig. 2b). The contents of ammonia and hydrogen sulfide were highly dynamic. At the beginning of the digestion process on the second and third days, an increase in the concentration of these gases was observed. Their contents decreased on the fourth and fifth days and returned to initial values in 7–10 days. In the following days, the percentages of NH_3 and H_2S in biogas compo-sition were stable, with a decreasing tendency. Sucrosorgo 506 ferti-lized with sewage sludge showed a significantly different tendency. During the initial phase of digestion, the content of H_2S reached 1200 ppm, 7-10-fold higher than in the other treatments (Fig. 2c). On the third day after the beginning of the biomethanation, content of H_2S decreased to a value similar to that in other treatments – 200 ppm – and remained at this level until the end of the process. In terms of the composition of biogas produced from Rona 1, irrespective of the ferti-lization, the contents of NH_3 and H_2S were similar.

Hydrogen sulfide has a corrosive effect on installations and shows toxic inhibitory activity on microbial communities, causing protein denaturation of methanogens and sulfate reducers [54]. Therefore, this gas should be removed or its concentration should be decreased to an optimal level during the biogas production process. Based on the DVGW Code of Practice 260 [55], the H_2S concentration should be below 5 mg Nm^{-3} biogas. Hydrogen sulfide concentration depends on the sub-strates and ranges from a few ppm to several thousand ppm, e.g. during the fermentation of biological waste and slurry [56]. Quinn et al. [57] reported that in vitro ruminal fluid hydrogen sulfide production is correlated with sulfur concentration in the substrates.

Ethanol production per 1 Mg TS ranged from 151.41 dm^3 (Sucrosorgo 506 without nitrogen fertilization) to 274.21 dm^3 (Rona 1 fertilized with digestate) (Table 4). Regardless of the fertilization treatment, Rona 1 (249.71 dm^3 Mg^{-1} TS) produced significantly higher TS-based amounts of ethanol than Sucrosorgo 506 (181.61 dm^3 Mg^{-1} TS). Fertilization treatments did not affect ethanol production. Anioł et al. [58] reported that the TS-based ethanol pro-duction from sweet sorghum ranged from 157.2 to 225.2 dm^3 Mg^{-1} TS. In another study, the highest TS-based ethanol production (138–161 dm^3 Mg^{-1} TS) was observed at the end of September and at the beginning of October, whereas ethanol production from biomass harvested both earlier and later was lower due to the less appropriate chemical composition of the biomass [4]. In the present study, research did not included method of improving ethanol production from lig-nocellulosic biomass of sorghum. It should be mentioned, that prob-ably, at the pre-treatment stage, inhibitors had the negative impact on enzymes activities, particularly cellulases and as a result affected ethanol efficiency production [59–61]. The use of acid (e.g., H_2SO_4) mainly causes breakdown of hemicellulose into monosaccharides, but simultaneously compounds such as phenols, furfural or 5-hydro-xymethylfurfural are formed, which can significantly inhibit the en-zymes and microorganisms [62]. Testing another methods of pre-treatments, which allow to enhance process efficiency should be a scope of future experiments in this area. Physical (e.g. membrane detox-ification), chemical (ion exchange resins), biological (enzymatic methods using e.g. laccase, peroxidases) methods as well as micro-biological in-situ detoxification should be tested as pre-treatment of sorghum biomass, which will be alternative to this one used in this study [63].

3.5. Methane and ethanol yield per hectare

In the present study, interaction between the tested factors and biofuel yield per hectare was noted (Table 4). Both hybrids had the lowest biogas (3700 Nm^3 ha^{-1} for Sucrosorgo 506 and 3600 Nm^3 ha^{-1} for Rona 1) and methane (2100 Nm^3 ha^{-1} for Sucrosorgo 506 and 2200 Nm^3 ha^{-1} for Rona 1) yields when cultivated without nitrogen fertili-zation. The biogas and methane yields of both hybrids were sig-nificantly higher when sorghum was fertilized with urea or waste products. Application of urea for Sucrosorgo 506 provided the highest biogas (9100 Nm^3 ha^{-1}) and methane (5000 Nm^3 ha^{-1}) yields of all the treatments. Both tested hybrids in the present study produced si-milar amounts of biogas and methane per hectare. Mahmood et al. [48] evaluated the utility of 14 sorghum hybrids for biogas production. Some of the tested sweet sorghum genotypes, such as Maja, Lussi, Branko and Supersile 20, gave biogas and methane yields per hectare at levels comparable to that of maize [48]. Under the climatic conditions of southern Austria, methane yield produced from sweet sorghum biomass ranged from 3700 to 6500 Nm^3 and was highly dependent on chemical composition, in particular the content of lignocellulosic frac-tions [53]. Those hybrids with lower lignin and higher non-structural carbohydrate contents have the potential to generate a higher ethanol yield per hectare. Mahmood et al. [48] stated that hybrids with a higher methane production per Mg as well as those characterized by higher biomass yield should be chosen to maximize methane yield per hectare.

The lowest ethanol yield per hectare (1065 dm^3 ha^{-1}) was obtained from Sucrosorgo 506 biomass not fertilized with nitrogen. When urea was applied in the cultivation of this hybrid, the ethanol yield was 3-fold higher (3313 dm^3 ha^{-1}). Regardless of the hybrid, the highest ethanol amount per hectare (3050 dm^3 ha^{-1}) was received after urea application. A study conducted by Capecchi et al. [64] showed that the average ethanol yield from Sucrosorgo 506 was 2575 dm^3 ha^{-1} and was dependent on soil moisture and date of harvest. The delaying of harvest to the dough-ripe stage led to a 107% increase in ethanol yield as compared to harvest at the shoot formation stage [64]. Chmielewska et al. [4] demonstrated a significant relationship between neutral de-tergent fiber (NDF) and acid detergent fiber (ADF) contents in sorghum biomass and ethanol yields per hectare.

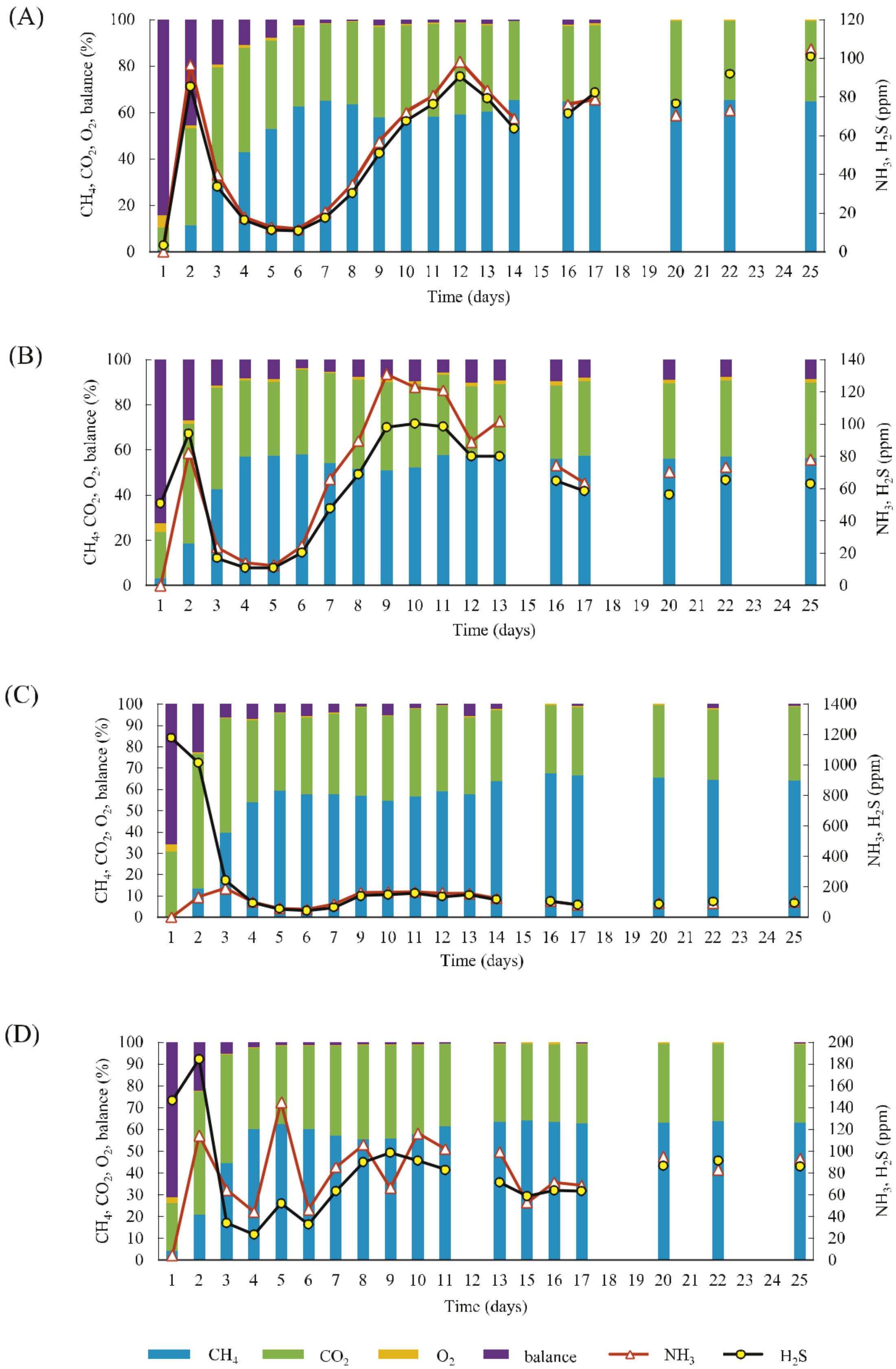

Fig. 2. Biogas composition from Sucrosorgo 506 (A) control, (B) urea, (C) sewage sludge, (D) digestate.

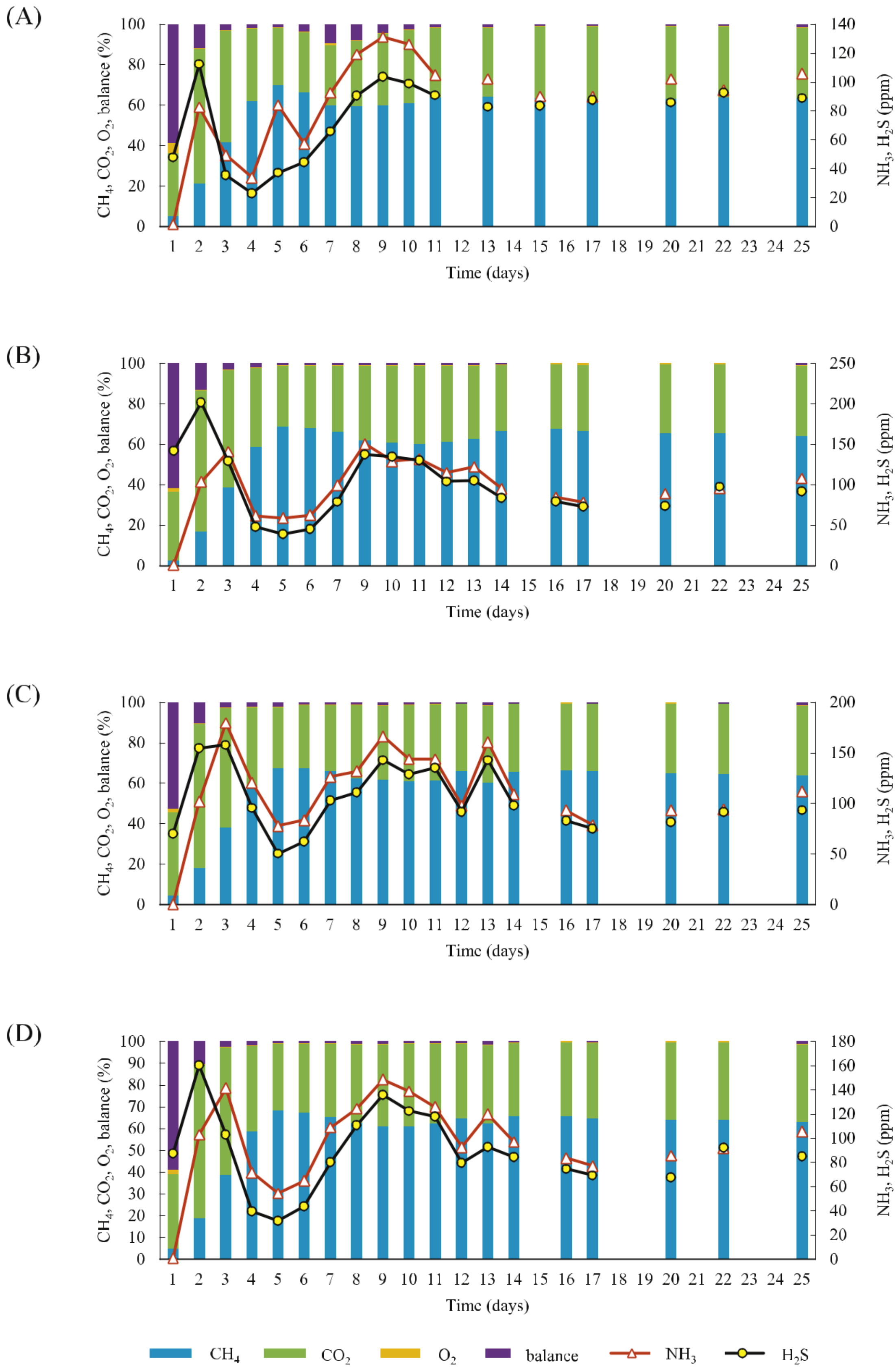

Fig. 3. Biogas composition from Rona 1 (A) control, (B) urea, (C) sewage sludge, (D) digestate.

Table 8
Lower heating value (LHV), net energy output and energy efficiency ratio of methane and ethanol produced from sorghum.

Feedstock production methods		LHV[(1)] (MJ Mg^{-1} TS[(2)])			Net energy output (GJ ha^{-1})		Energy efficiency ratio	
Hybrids	Fertilization treatment	Methane		Ethanol	Methane	Ethanol	Methane	Ethanol
Sucrosorgo 506	control	10895.48	± 269.69[(3)]	3219.26^{d} ± 178.70	76.64^{d} ± 1.90	22.64^{g} ± 1.26	9.0^{d} ± 0.22	2.7^{e} ± 0.15
	urea	10625.95	± 1226.23	4171.92^{c} ± 76.10	179.45^{a} ± 20.71	70.45^{a} ± 1.29	12.6^{c} ± 0.45	4.9^{c} ± 0.09
	sewage sludge	11447.11	± 512.90	3534.19^{d} ± 36.46	143.86^{b} ± 6.45	44.42^{d} ± 0.46	15.8^{a} ± 0.71	4.9^{c} ± 0.05
	digestate	11756.54	± 352.01	4516.76^{c} ± 138.96	122.91^{bc} ± 3.68	47.22^{d} ± 1.45	13.8^{bc} ± 0.41	5.3^{b} ± 0.16
Rona 1	control	12209.90	± 31.81	5555.73^{ab} ± 126.57	77.57^{d} ± 0.20	35.29^{f} ± 0.80	9.2^{d} ± 0.02	4.2^{d} ± 0.10
	urea	11818.82	± 162.73	5436.04^{b} ± 64.31	128.80^{bc} ± 1.77	59.24^{c} ± 0.70	9.1^{d} ± 0.13	4.2^{d} ± 0.05
	sewage sludge	12064.76	± 553.18	4411.95^{c} ± 202.20	106.82^{c} ± 4.90	39.06^{e} ± 1.79	11.9^{c} ± 0.55	4.4^{d} ± 0.20
	digestate	12287.28	± 425.67	5828.85^{a} ± 25.06	136.04^{b} ± 4.71	64.54^{b} ± 0.33	15.5^{ab} ± 0.54	7.3^{a} ± 0.02
		Average for factors						
Hybrids	Sucrosorgo 506	11179.28^{b}		3860.53^{b}	126.62	46.18	12.8	4.5
	Rona 1	12097.96^{a}		5308.14^{a}	112.81	49.53	11.4	5.0
Fertilizer	control	11552.69		4387.50	77.10^{b}	28.97^{c}	9.1^{b}	3.4^{b}
	urea	11341.67		4803.98	149.06^{a}	64.85^{a}	10.5^{b}	4.6^{b}
	sewage sludge	11694.17		3973.07	129.05^{a}	41.74^{b}	14.3^{a}	4.6^{b}
	digestate	12074.98		5172.81	130.79^{a}	55.88^{a}	14.8^{a}	6.3^{a}

a b c Means followed by similar letter within each column and columns that do not contain letters indicate no significance differences between treatments ($p > 0.05$) based on results of ANOVA followed by Tukey multiple range tests.
(1) LHV lower heating value.
(2) TS total solids.
3 ± standard deviation.

3.6. Methane and ethanol energy output

Energy output from biogas produced from Mg TS was 2.1–3.4-fold higher than from ethanol (Table 8). Rona 1 produced a significantly higher energy yield per Mg TS: 12.1 and 5.3 GJ for biogas and ethanol yields, respectively. The fertilization treatments did not affect energy output per 1 Mg TS. The land use efficiency expressed as gross biofuel energy output per land unit (GJ ha^{-1}) was several times higher when the sorghum biomass was subjected to anaerobic digestion. Conversion of fresh sorghum biomass into methane generated 76.6–179.5 GJ per hectare, while the gross energy output from ethanol was only 22.6–70.5 GJ per hectare. Interaction between the tested factors in re-gard to the energy output of both biofuels was reported. The fertiliza-tion treatments had a significant effect on land use efficiency. In the present study, energy input included only feedstock production and transport to the place of biomass conversion to biofuels. The energy efficiency ratio of ethanol production ranged from 2.7 (non-fertilized Sucrosorgo 506) to 7.3 (Rona 1 fertilized with digestate). A several times higher energy efficiency was noted for methane production. Ap-plication of digestate provided the highest energy efficiency ratio in terms of the production of methane for Rona 1 and in ethanol pro-duction for both hybrids. The highest energy efficiency from Sucrosorgo 506 was received after sewage sludge application. In the study by Jankowski et al. [10], of the six tested crops the highest energy effi-ciencies were obtained in the cultivation of miscanthus (Miscanthus x giganteus L.) (21.5), maize (18.6) and sweet sorghum (11.4). Crop species characterized by high energy yield and high energy efficiency ratio expressed as a relationship between energy outputs and inputs represent a reliable feedstock for biofuel production. Sweet sorghum, due to the high content of both non-structural and structural carbohy-drates in its stalks, represents a very promising alternative feedstock for ethanol production. However, based on the results obtained in this study, it can be concluded that, when the presented technology in production of biofuels is used, production of methane is a significantly more energy efficient method of sorghum biomass conversion com-pared to ethanol production. Cost-competitive ethanol production is still a highly challenging endeavor because of the bioconversion of all carbohydrates from sugar and lignocellulose fractions into ethanol [11].

4. Conclusions

Varied energy efficiency levels were obtained from conversion to biogas and ethanol of biomass from sorghum cultivated under the same climatic and soil conditions and subjected to different technological processes. Sweet sorghum should be used as a biogas feedstock in the temperate climate of Central Europe. The fertilization potential of di-gestate and sewage was comparable to that of urea. The use of these waste products in the cultivation of sorghum contributed to an im-provement in energy efficiency and, provided its suitable management, this is consistent with the European circular economy strategy.

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ROZDZIAŁ 6

Potencjał allelopatyczny sorga (*Sorghum bicolor* (L.) Moench) w aspekcie kontroli zachwaszczenia: obszerny przegląd literatury

CHAPTER TWO

Allelopathic Potential of Sorghum (Sorghum bicolor (L.) Moench) in Weed Control: A Comprehensive Review

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Abstract

Weeds constitute the largest biotic threat affecting the yield of cultivated plants. While conventional agriculture relies principally on chemicals for weed control, alter-native biological methods may be important tools to reduce weed pressure in

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agroecosystems. Furthermore, as the problem of excessive residue of plant protection agents in agroecosystems and the growing number of herbicide resistant weed bio-types continue to increase, new solutions that have smaller impacts on the environment are becoming increasingly desirable. One promising such method is the use of crops that exert a negative phytotoxic influence on weeds. This natural phenomenon describ-ing the ability of certain plant species to produce compounds that affect the growth of other plants in their surroundings is called allelopathy. Managing weed infestations in cultivated fields by planting allelopathic crops is a sustainable, economic, and environ-mentally friendly approach that has been strongly articulated in the international arena. Among cultivated crops, sorghum (*Sorghum bicolor* (L.) Moench) has been intensively studied because of demonstrated allelopathic potential.

This report provides a comprehensive literature review of the applications of sorghum allelopathy in agriculture. A critical analysis of the allelopathic properties of sorghum identified the following areas contributing to its ability to reduce weed infestation in agroecosystems:

1. a large number of compounds produced by sorghum have allelopathic properties,
2. allelopathic compounds can be applied in the form of mixed plant extracts or in combination with herbicides,
3. sorghum extracts have a broad spectrum of activity,
4. sorghum may be used to produce bioherbicides.

1. INTRODUCTION

Reducing weeds in crop cultivation leads to an increase in production costs. Weeds have a negative effect on the qualitative and quantitative aspects of yield as they compete for light, water, nutrients, and space with crops (Farooq et al., 2013). In comparison to other biotic factors, weeds cause the highest yield losses. According to estimates, 34% of yield losses are caused by the presence of weeds, while disease (18%) or pests (16%) have a lesser impact (Jabran et al., 2015; Oerke, 2006, Oerke et al., 1999).

Agriculture faces current challenges from various origins. One challenge is the penetration of plant-protection agent residues into soil, groundwater, and the food chain (Beckie and McKercher, 1990). The improper use of herbicides, such as application in unsuitable weather conditions or at the incorrect developmental crop phase, could lead to serious environmental consequences, such as the leaching of active compounds into groundwater or accumulation in the soil (Kruidhof et al., 2008; Walker et al., 2013). The strong selection pressure exerted by herbicides also increases the evo-lutionary pace of herbicide resistance in weeds (e.g., common windgrass (*Apera spica-venti* (L.), P. Beauv.), cornflower (*Centaurea cyanus* L.), lambsquarter (Chenopodium album L.), redroot pigweed (Amaranthus retroflexus L.), rigid ryegrass (*Lolium rigidum* Gaud.), wild oat (*Avena fatua* L.)). There are currently at least 250 herbicide-resistant weed species in the world (International Survey of Herbicide Resistant Weeds, 2016). These species have evolved resistance to 23 of the 26 known herbicide sites of action and to 160 different herbicide active ingredients. Another problem is public lack of acceptance of the use of crop protection agents in agriculture (Dayan et al., 2009a). Indeed, social awareness of environmental threats and the increasing number of customers looking for high-quality agricultural produce are prompting new, safer, and sustainable approaches.

Environmental pollution and the threat to human and animal health caused by incorrect or excessive application of plant-protection agents have driven new searches for alternative methods of weed control (Scarabel et al., 2015; Shaner, 2014; Singh et al., 2003; Sowinski, 2014). Decisions aiming to optimize plant production are made while at the same time respecting the principles of sustainable management. The adoption of safer plant protection methods associated with lower environmental risks, such as biological methods, is gaining popularity. This trend is representative of greener technologies being developed in numerous fields of human activity, such as the development of allelopathic crops in agriculture (Gealy and Yan, 2012, Gealy et al., 2013). The phytotoxic effect of allelochemicals, i.e., biologically active secondary metabolites exuded by higher plants, fungi, or microorganisms, may become a useful way to reduce weed infestation in crop cultivation (Farooq et al., 2011). This report provides a comprehensive literature review of the applications of the allelopathic potential of Sorghum bicolor (L.) Moench in agriculture.

2. ALLELOPATHY PHENOMENON

According to Rice (1984), allelopathy is a natural phenomenon involving either direct or indirect effects of one plant (including microorganisms) on another plant through the release of chemical compounds into the environment. The term allelopathy is derived from two Greek words: “allelon,” meaning “of each other,” and “pathos,” meaning “to suffer” (Rizvi et al., 1992). Classical researchers were aware of this concept in the Greek and Roman era (Wills, 2007). Interference between plants was mentioned in the literature for over 2000 years and was formally recognized in 1937 when Austrian plant physiologist, Hans Molisch, named it allelopathy. Consequently, Molisch is considered the father of allelopathy (Li et al., 2010 quot. Molisch, 1937). A plant with allelopathic potential is called the "donor plant," while the plant affected by allelopathic com-pounds from the donor plant is called the "acceptor plant" (Muller, 1969).

Allelochemicals can be produced and/or accumulate in nearly all plant parts and tissues, such as leaves, roots, stems, rhizomes, flowers, fruits, and seeds. These bioactive metabolites are released from plants in a number of ways, such as volatilization, foliar leaching, root exudation, or decomposition of residues and leaf litter (Ben-Hammouda et al., 2001; Bonanomi et al., 2006; Kumar et al., 2009; Rice, 1984). When released into the soil, these natural chemicals cause allelopathic effects, which are typically detri-mental (inhibitory) or sometimes beneficial (stimulatory) to target organisms (Ghafarbi et al., 2012; Rice, 1984). Effects of allelochemicals can be observed at all levels of organization of a living organism, from physiological responses, through cellular and molecular levels (Rice, 1984). For example, certain allelochemicals can affect germination of surrounding species seed by inhibiting cell division and preventing hydrolysis of nutrient reserves (Balke, 1985; Irshad and Cheemas, 2004). Others inhibit electron transport in pho-tosynthesis and the respiratory chain by altering enzyme activity (Hejl and Koster, 2004; Meazza et al., 2002; Silva et al., 1996). However, the molecular target site of most allelochemicals is poorly understood (Bertin et al., 2007, 2009, Kato-Noguchi and Peters, 2013, Romagni et al., 2000, Toyomasu et al., 2014).

Li et al. (2010) proposed a following classification of allelochemicals according to their different structures and properties: (1) water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones; (2) simple lactones; (3) long-chain fatty acids and polyacetylenes; (4) quinones (benzoquinones, anthraquinones, and complex quinones); (5) phenolics; (6) cinnamic acid and its derivatives; (7) coumarins; (8) flavonoids; (9) tannins;

× steroids; and terpenoids (sesquiterpene lactones, diterpenes, and triterpenoids).

Numerous crops have been reported to show allelopathic effects on associated weeds. Examples include sunflower (Anjum and Bajwa, 2007; Batlang and Shushu, 2007; Khaliq et al., 2012; Mahmood et al., 2010), rice (Jabran et al., 2008; Kayode and Ayeni, 2009; Rehman et al., 2010), brassica (Awan et al., 2012; Khan et al., 2012b; Mahmood et al., 2015a), rapeseed (Mushtaq et al., 2010a), barley (Bertholdsson, 2003; Overland, 1966), wheat (Bertholdsson et al., 2012), and sorghum (Breazeale, 1924; Hozayn et al., 2011; Khan et al., 2015; Khandro et al., 2014; Lehle and Putman, 1983).

3. SORGHUM SPECIES

Sorghum is an annual grass from the *Panicoideae* subfamily most likely descending from the wild species *Sorghum arundinaceum* (Desv.) Stapf (Owuama, 1997). Sorghum originates from Ethiopia, serving as a dietary sta-ple to endogenous populations with the first records of cultivation dating back to 4000 BC. This tropical cereal later migrated from East Africa to other continents (Owuama, 1997; Se`ne et al., 2001). According to FAOSTAT (2014), sorghum was recently cultivated on 45 million hectares that produced 68.9 million metric tons of grain, making it the fifth most cultivated crop in the global cereal area structure. The United States is the global leader in sorghum production, accounting for more than 22% of world production with an export revenue that exceeds 1.5 billion US dollars.

The Sorghum genus includes approximately 25 species that are widely cultivated throughout the world (Hodnett et al., 2005). Over the years, more than 10,000 varieties and genotypes have been cultivated, yet the positions of many of these taxa in taxonomy are ambiguous (Liu et al., 2014). The following subtypes are particularly important among the functional types of Sorghum bicolor ssp. bicolor: (1) high-stem sweet sorghum, (2) low-stem varieties cultivated for grain, (3) broomcorn (Sorghum vulgare var. technicum) for technical applications, (4) sudangrass (Sorghum sudanense (Piper) Stapf.), and (5) sudex (sorghum:sudangrass hybrid), harvested several times a year (Dahlberg et al., 2011). Sorghum is a species with a wide spectrum of applications, ranging from consumption purposes through the pro-duction of animal fodder to technological and construction applications (Sowinski and Szydełko-Rabska, 2013). The importance of this species is increasing worldwide because of the ability to adapt well to changing habitat conditions, particularly increased drought, and a high functional value (Berenji and Dahlberg, 2004; Sowinski, 2009; Sowinski and Liszka-Podkowa, 2008). Another sorghum species, Johnsongrass (*S. halepense* (L.) Pers.), is currently recognized as one of the worst weeds in the world and classified as an invasive species in the United States by the Department of Agriculture (NISIC, 2016).

Within the four past decades, research has documented the allelopathic potential of sorghum and evaluated its extent depending on the part of the plant, age, environmental factors, and species of acceptor plants. This weed suppressive potential is determined by the presence of hydrophilic

Fig. 1 Sorghum allelopathy phenomenon. Allelochemicals are released into the environment from above- and belowground sorghum plant parts.

compounds, phenolic acids, and their aldehyde derivatives, as well as hydrophobic substances, such as sorgoleone and its analogues (Czarnota et al., 2003a; Lehle and Putman, 1983) (Fig. 1).

4. ALLELOCHEMICALS IN SORGHUM

4.1 Phenolic Compounds

Phenolic compounds are important plant products and include several phytotoxins. The basic chemical backbone of phenolic compounds consists of a hydroxy group (–OH) bonded directly to an aromatic ring. They are ubiquitous to the plant kingdom and are released in the soil as plant decomposition products (Li et al., 2010). Phenolic acids and their aldehyde derivatives can also leach from aboveground parts or be exuded from the root system (Funnell-Harris et al., 2008). Sorghum produces many primary phenolic acids that have phytotoxic activity (Al-Tavaha and Odat, 2010; Cheema, 1988; Cheema et al., 2007a) (Fig. 2); however, relative amounts differ between cultivars. For example, Cheema et al. (2007a) reported that p-hydroxybenzoic, gallic, syringic, and protocatechuic acids were more abundant than other phytotoxins and were present in all cultivars used in the study. The levels of vanillic, benzoic, p-coumaric, and benzoic acids were relatively lower than those mentioned previously and were not present in all cultivars. There was greater phenolic compound diversity between

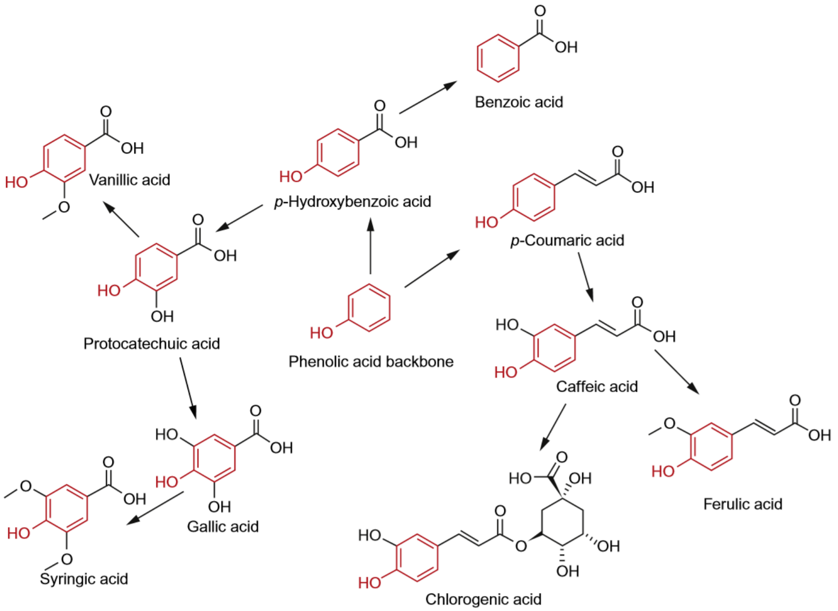

Fig. 2 Phenolic acid backbone (red) and its presence in common molecules produced by sorghum

sorghum hybrids (Cheema et al., 2007a). The JS-263 cultivar had the highest content of total isolated phenolic compounds (904.6 µg g $^{-1}$), while extracts of the cultivar Sibbi contained the lowest phenolic content (51.35 µg g $^{-1}$). Giza 15 and Giza 115 hybrids accumulated five times more p-hydroxybenzoic acid than the Rabeh hybrid (Alsaadawi et al., 2007).

The total content of phenolic acids of sweet sorghum ranges from 2.0% to 2.2% in aboveground dry mass, and 1.1% to 1.6% in roots (Se`ne et al., 2001). However, phenolic acid levels change during plant development and tend to decrease as plants age (Marchi et al., 2008; Won et al., 2013). These dynamic changes in phenolics may account for some of the variation in overall phytotoxicity of sorghum extracts (Weston et al., 1989).

The literature is replete with such reports on the phenolic contents of sorghum cultivars and hybrids, but their role in allelopathy is overestimated (Cheema et al., 2009; Nicollier et al., 1983; Weston et al., 1989; Won et al., 2013). Indeed, common phenolic acids derived from the shikimate pathway are often cited as potential allelochemicals. However, these compounds are weakly phytotoxic molecules that are ubiquitous to higher plants, making them unlikely to play a role in allelopathy (see review Dayan and Duke, 2009 for more explanations).

4.2 Sorgoleone - The Main Sorghum Allelochemical

4.2.1 Chemical Structure of Sorgoleone and Its Analogues

Sorgoleone, 2-hydroxy-5-methoxy-3-[(Z,Z)-8',11',14'-pentadecatriene]-p-benzoquinone, is a lipophilic secondary metabolite consisting of a quinone ring and aliphatic chain (Netzly and Butler, 1986) (Fig. 3). Yet, in literature, this term has also been used to refer to the oily root exudate containing the parent molecule and its congeners (Soltys et al., 2010). Sorgoleone analogues have aliphatic side chains of varying lengths and different degrees of saturation (one to three double bonds). Other analogues may also have an additional methoxy group at the third and fifth atom in the ring (Dayan et al., 2003; Kagan et al., 2003).

Sorgoleone and its 1,4-dihydroxy form (resorcinol) account for 90% of compounds that are present in the root exudates of sorghum (Czarnota et al., 2003a; Fate and Lynn, 1996; Kagan et al., 2003; Rimando et al., 1998). The remaining 10% of root exudate components include sorgoleone analogues originating from the same path of biosynthesis, e.g., 5-ethoxy-sorgoleone (Rimando et al., 1998, 2003), small amounts of protein, and anthocyanins (Dayan and Duke, 2009; Rasmussen et al., 1992). Netzly et al. (1988) identified three other minor p-benzoquinones, similar in chemical structure to sorgoleone. Czarnota et al. (2003b) also noted another minor compound in sorghum root exudates, 2,5-dimethoxysorgoleone, which is closely related to sorgoleone.

4.2.2 Biosynthesis

Sorgoleone is produced exclusively by species in the Sorghum genus, upheld by testing of 17 other species from the Poaceae family, including closely related *Panicoideae* subfamily members (Baerson et al., 2008). Sorgoleone and its analogues are synthesized specifically in root hair cells (Dayan et al., 2007a), making these specialized cells natural herbicide factories

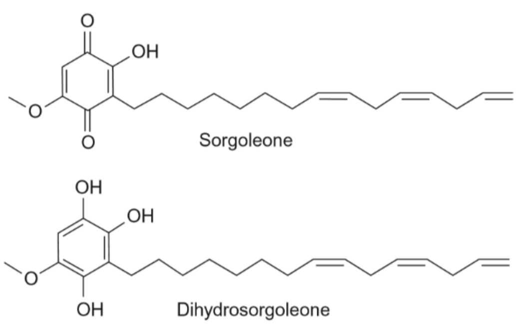

Fig. 3 Structures of sorgoleone and its reduced analogue dihydrosorgoleone.

(Dayan and Duke, 2003). In agreement with this observation, sorghum seedlings grown under conditions where root hairs do not develop have little to no sorgoleone (Yang et al., 2004a). Our group has investigated the biosynthesis of sorgoleone in great detail, starting with a retrobiosynthetic NMR analysis approach (Dayan et al., 2003) that follows the incorporation of ^{13}C-labeled substrates into the carbon backbone of sorgoleone (Fig. 4). This preliminary study identified key enzymes involved in the biosynthesis of sorgoleone, namely, a specialized fatty acid desaturase that introduces three double bonds in the aliphatic tail, a polyketide synthase that forms the ring, an O-methyltransferase that methylates one or more of the hydroxy groups of the resorcinol intermediate, and a P450 monooxygenase that completes synthesis. The biosynthesis of sorgoleone that takes place with the participation of the endoplasmic reticulum and the Golgi apparatus is constitutive and proportional to the biomass of roots (Czarnota et al., 2003a; Dayan, 2006). A functional genomic approach analyzing an EST library confirmed that the genes encoding enzymes involved in the biosyn-thesis of sorgoleone were enriched in root hair cells (Baerson et al., 2006, 2008). Yang et al. (2004a) identified a fatty acid desaturase gene (SOR1) that

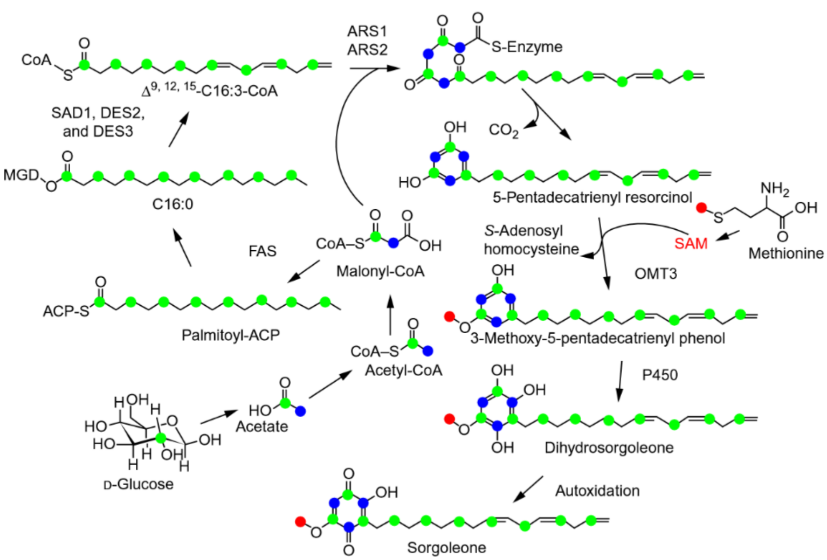

Fig. 4 Sorgoleone biosynthesis as determined from a retrobiosynthetic NMR analysis using ^{13}C-labeled substrates. Green = incorporation of 2-^{13}C-glucose, blue = incorporation of 2-^{13}C-acetate, and red = incorporation of methyl-^{13}C-methionine (Dayan et al., 2003).

was putatively involved in the introduction of some of the double bonds in the aliphatic tail of sorgoleone, but the function of this gene, now called DES1 (Fig. 4), was not confirmed until a later study (Pan et al., 2007). Another study on temporal expression of this gene in several sorghum cultivars found that highest expression occurred within the first 5 days after emergence and subsequently decreased as the plants aged (Dos Santos et al., 2014).

The genes involved in sorgoleone biosynthesis and their functions in isolated Sorghum bicolor root hair cells are now fully characterized (Baerson et al., 2008; Cook et al., 2010; Pan et al., 2007) (Fig. 4).

Expression levels of the DES2, DES3, ARS1, ARS2, and OMT3 genes involved in sorgoleone biosynthesis responded positively to the following auxin treatments: indole-3-acetic acid, indole-3-butyric acid, and 1-naphthaleneacetic acid (Uddin et al., 2011). The amount of sorgoleone was highly dependent on exposure time (3, 6, 12, 24, 48, and 72 h after auxin application) and auxin concentration. It was confirmed that, with increased auxin application duration, sorgoleone content also increased. In further studies, Uddin et al. (2013b) found that both methyl jasmonate and jasmonic acid significantly promoted secondary root development, root hair formation, root growth of sorghum, and, as consequence, sorgoleone accumulation. Transcript accumulation was apparent for all genes involved in sorgoleone biosynthesis. The highest increase in expression levels was observed for the O-methyltransferase 3 gene. The authors suggested that jasmonates be recognized as potent substances for promoting root hair formation, upregulating expression of genes involved in sorgoleone synthesis, resulting in sorgoleone accumulation in sorghum.

4.2.3 Exudation

The biosynthesis of lipid benzoquinones and resorcinol is a dynamic pro-cess. The system that regulates the dynamics of sorgoleone generation and exudation is based on the feedback loop principle (Dayan et al., 2009b). Sorgoleone, deposited in the space between the cell membrane and cell wall, is transported by bulk transport to the top part of root hairs, from which it is then exuded as oily droplets (Czarnota et al., 2003a; Field et al., 2006). The biosynthesis and exudation of sorgoleone can start when root hairs are developed and have reached their final size (Dayan, 2006). According to Dayan et al. (2009b) release of sorgoleone and an associated dimethylated resorcinol analogue is regulated by overall accumulation at root hair tips (Fig. 5).

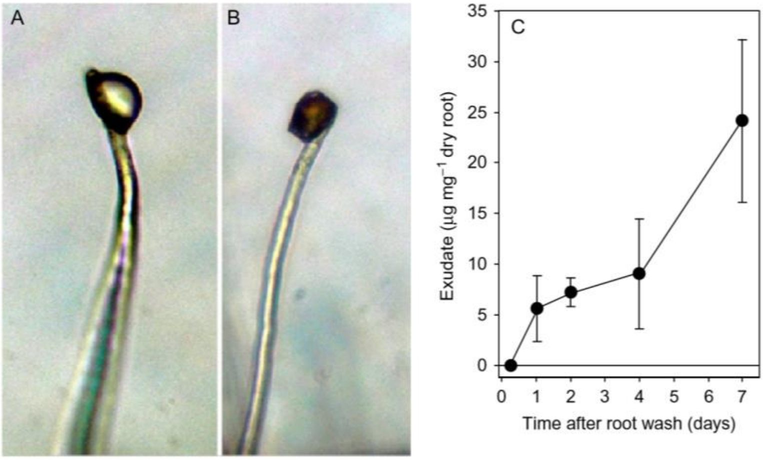

Fig. 5 Droplets of sorgoleone exuded by sorghum root hairs. (A) A droplet, consisting primarily of dihydrosorgoleone, is initially yellowish when roots are in a low oxygen envi-ronment. (B) When exposed to air, the droplet rapidly turns dark brown as its consistency shifts to predominately sorgoleone. (C) Synthesis and exudation of sorgoleone resumes when the oily droplet is removed.

The intensity of sorgoleone production is influenced by a series of environmental factors (Hess et al., 1992). An excessive amount of water impedes growth of root hairs and, as a result, reduces sorgoleone exudation (Dayan, 2006; Hess et al., 1992; Yang et al., 2004b). Temperatures below 25°C or above 35°C and an alkaline pH also have a limiting effect on the intensity of sorgoleone generation (Dayan, 2006). The greatest amount of sorgoleone is secreted at a pH between 4 and 5, suggesting that biosynthesis in acidic soil should be higher than that of alkaline soil. Illumination of sorghum seedlings with blue and red light inhibits sorgoleone synthesis by 50% and 23%, respectively (Dayan, 2006).

The amount of sorgoleone produced varies between and within sor-ghum species. Certain genotypes may accumulate up to 15 mg of sorgoleone per gram of fresh sorghum biomass (Weston and Czarnota, 2001). The Chalsusu hybrid accumulates 16.5 times more sorgoleone than the Hinsusu hybrid (Uddin et al., 2009). Bertin et al. (2003) found that sorghum, includ-ing grain and forage cultivars, typically produces quantities ranging from 1.3 to 1.9 mg of exudate per gram of fresh root biomass. Czarnota et al. (2003b) evaluated chemical composition of root exudates of seven genetically diverse sorghum species. It was demonstrated that Johnsongrass produced the greatest amount of exudate (14.75 mg per gram of fresh root biomass) compared to other species, which produced only 0.5–1.85 mg per gram of fresh root biomass, although sorgoleone content is generally lower in Johnsongrass than other sorghum biotypes. According to Nimbal et al. (1996a) sorgoleone content may vary from 0.67 to 17.8 mg per gram of fresh root biomass.

4.2.4 Mode of Action

Detailed studies on the phytotoxic activity of sorgoleone demonstrated that its mechanism of action targets the photosynthetic electron transport chain (Czarnota et al., 2001; Rasmussen et al., 1992). Structurally, sorgoleone is similar to plastoquinone (a lipid benzoquinone), resulting in competition with the natural electron acceptor at the plastoquinone binding site on the D1 PSII protein (Fig. 6).

By binding to the D1 protein, sorgoleone is able to block reoxidation of plastoquinone A (Q_A) by plastoquinone B (QB) (Gonzalez et al., 1997; Hejl and Koster, 2004). This operating mechanism is identical to that of

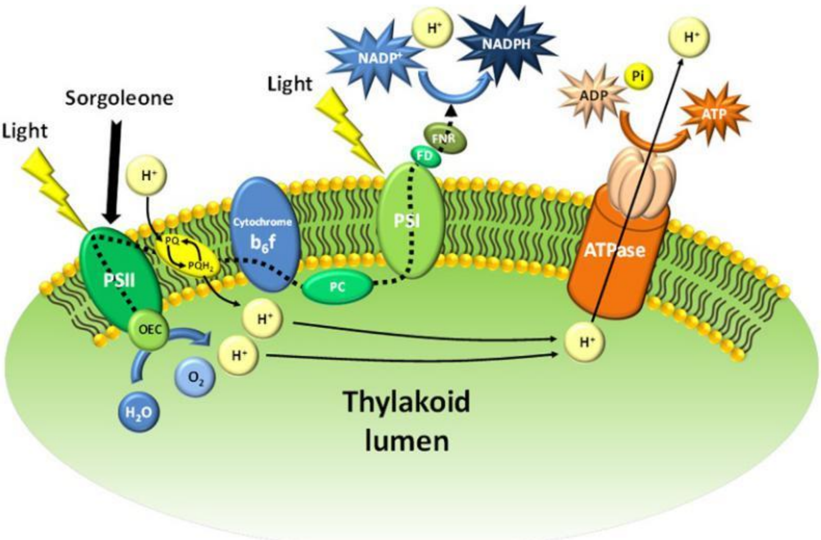

Fig. 6 Schematic of the light reaction of photosynthesis (Z-scheme or Hill reaction) and the location of the binding site of sorgoleone in the D1 protein of photosystem II (PSII). PC, plastocyanin; PQ, plastoquinone; PSI, photosystem I. Figure adapted from Dayan, F.E., Duke, S.O., 2014. Natural compounds as next-generation herbicides. Plant Physiol. 166 (3) 1090–1105. http://dx.doi.org/10.1104/pp.114.239061.

atrazine (6-chloro-N-ethyl-N8-(1-methylethyl)-1,3,5-triazine-2,4-diamine) (Gonzalez et al., 1997; Nimbal et al., 1996b; Streibig et al., 1999). As demonstrated in isolated spinach thylakoids (Nimbal et al., 1996b), sorgoleone is therefore a competitive inhibitor that competes with atra-zine for the plastoquinone-binding domain. The authors stated that in the case of atrazine-susceptible redroot pigweed and potato thylakoids, sorgoleone bound to the same Q_B niche of the D1 protein as diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] and metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazine-5(4H)-one]. Nimbal et al. (1996b) did not observe competition for bentazon [3-(methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide], another inhibitor of the Hill reaction, which was used as a control. Interestingly, the mutation in the D1 protein imparting resistance to typical triazine inhibitors does not affect the efficacy of sorgoleone on the target site (Dayan et al., 2009c).

An additional mechanism of sorghum phytotoxic activity (Meazza et al., 2002) is the reduction of carotenoid production through inhibition of p-hydroxyphenyl pyruvate dioxygenase, a key enzyme in carotenoid syn-thesis and a target for new herbicide classes. Carotenoid reduction leads to a decreased amount of chlorophyll and subsequent reduced photosyn-thetic capability. Additionally, sorgoleone lowers the membrane activity of H^+ ATPase, which, in turn, leads to disturbances in water uptake (Hejl and Koster, 2004).

4.2.5 Autotoxicity

In plants that produce phytotoxins, an autotoxicity avoidance mechanism is necessary to prevent internal translocation of potentially toxic root exudates to sensitive parts of the plant (Bertin et al., 2003). Avoidance mechanisms include sequestration, secretion, resistance at the target site, and metabolic inactivation (Dayan and Duke, 2003; Duke et al., 2001). For sorgoleone, rapid, efficient transport and deposition to the outside of root hair cells most likely prevents further transport into more sensitive aboveground plant tis-sues (Bertin et al., 2003). Furthermore, the high lipophilicity (logP) of sorgoleone impedes acropetal translocation via the transpiration stream (Dayan, 2002; Dayan et al., 2009b). Phenolic compounds also necessitate autotoxicity avoidance. To prevent autotoxicity, phenolics are stored in plant cells as inactive esters or glycosides that may be later activated by plant hydrolases (Ben-Hammouda et al., 1995).

5. SCREENING METHODS TO EVALUATE SORGHUM ALLELOPATHIC POTENTIAL

The importance of allelopathy is extremely difficult to test in real eco-systems, causing some authors to question its existence (Hiradate, 2006; Hiradate et al., 2010). Studies on allelopathic properties of plants are potentially complicated by interactions including additive, antagonistic, or synergistic relationships with other compounds in the soil. Many other factors may also modulate allelopathic potential of a plant, such as growth stage and physiological status, species composition of rhizosphere microorganisms, or environmental factors, including moisture, temperature, and pH (Duke, 2015; Inderjit, 2005; Romeo and Weidenhamer, 1999; Wang et al., 2012).

Allelopathy research can be conducted both in field and within con-trolled environments, including laboratory or greenhouse conditions (Falquet et al., 2014). A laboratory bioassay is the first step used to demonstrate the possible existence of allelopathy (Foy, 1999). Preliminary studies should aim to support or refute that a suspected species has allelopathic potential with a rapid, easy to operate, and inexpensive assay (Wu et al., 2001). After preliminary experiments, genotypes with stronger allelopathic potential can be selected for further greenhouse and field studies. This procedure reduces the time, labor, and space needed for an otherwise large screening project.

Another problem in allelopathy research is the extremely challenging task of differentiating the effect of allelopathy from competition for resources (Falquet et al., 2014; He et al., 2012; Inderjit and Del Moral, 1997; Nilsson, 1994; Weidenhamer, 1996). Falquet et al. (2014) instituted a simple, inexpensive allelopathy screening method that provided a means of separating competition for light, nutrients, and water from allelopathic root interactions between a sudex hybrid (donor species) and redroot pigweed (receiver plant). In this method, donor and receiver plants were grown in pots where either roots made contact or were separated by impenetrable barriers. The authors also evaluated the effect of shading by the presence or absence of vertical nets between sorghum and redroot pigweed seedlings. With this simple methodology, it was possible to assess the effects of sorghum root exudates on acceptor plants regardless of competition. Furthermore, activated carbon can be added to the soil to evaluate the contribution of allelochemicals to overall competition between donor and receiver plants.

Activated carbon traps the allelopathic compounds released by donor plants, although it may introduce confounding factors through effects on nutrient availability and plant growth. It is important to consider that the reversal of plant growth inhibition may be a plant response to changes in soil nutrient availability (Lau et al., 2008). Nonetheless, the size of redroot pigweed seedlings grown in the presence of sorghum was dramatically larger when planted in soils amended with activated charcoal than in unamended soils (Fig. 7).

From an ecological standpoint, it is important to select plants species grown in the same ecosystem as the allelopathic plants tested. When con-ducting a study to evaluate a crop with allelopathic potential for weed control, it is necessary to use relevant weed species as receiver plants (Wu et al., 2000).

5.1 Water Extract From Leaves—Sorgaab

Many studies on sorghum allelopathy have utilized sorgaab water extracts. The term "sorgaab" is the combination of two words: "sorg," derived from "sorghum," and "aab," which means "water" in Urdu (Cheema et al., 2007b). Sorgaab is made from green parts of mature sorghum plants. The extract preparation is easy, inexpensive, and does not require the use of

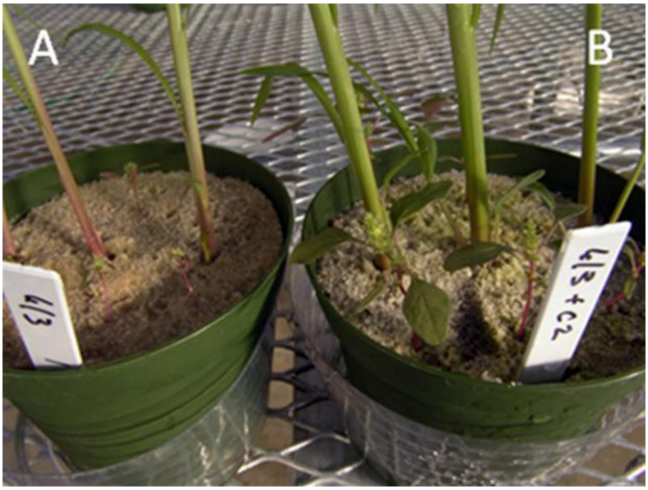

Fig. 7 Effect of activated charcoal on the allelopathic effect of sorghum roots. Notice the difference in size between redroot pigweed seedlings grown in the presence of sorghum (A) in sandy soil and (B) in sandy soils amended with activated carbon.

specialized laboratory equipment. Plant material is first cut into several centimeter-long pieces that are soaked with a specific amount of distilled water for 24 h. After this period, the extract is filtered and then concentrated through boiling. Al-Tavaha and Odat (2010) included a centrifugation step after the first filtration of sorgaab, but this is rarely used in practice.

It should be noted that sorgaab is not likely to contain any sorgoleone since this allelochemical is produced exclusively in roots. This compound is very lipophilic (logP = 3.56) and does not translocate (Dayan et al., 2009b). Therefore, it is unlikely that sorgoleone is present in sorghum leaves and the toxic effect of sorgaab may be associated with the presence of other bioactive secondary metabolites.

The effect of sorgaab on weed species can be assessed under both con-trolled and field conditions. In the laboratory, the effect of sorghum extract on germination and early phases of weed development can be tested in Petri dishes (Al-Tavaha and Odat, 2010; Randhawa et al., 2002; Yarnia et al., 2009). The experiments can also be carried out in phytotrons or green-houses, where seed of the receiver plant weeds species is germinated in pots and watered with solutions containing sorgaab (Al-Bedairy et al., 2013; Cheema et al., 2007a). Biometric parameters such as germination, shoot, root and seedling length, and dry mass are measured and compared to a control (i.e., seedlings exposed to water). One of the limitations of this method is the influence of the osmotic potential of sorgaab, since a concentrated extract promotes plant water loss rather than uptake. Seed density and size may also affect the potency of natural phytotoxins (Weidenhamer et al., 1987).

The efficiency of foliar applications of sorgaab to manage weeds in field crops (e.g., corn or wheat) can be evaluated by measuring weed density, fresh and dry mass, and quantitative aspects of crop yield. These results are compared to the level of weed infestation reduction obtained by mechanical weed control or herbicide application. Economic analysis of such studies provides an indication of the potential profitability of sorgaab use.

In another type of study, sorghum residues rather than sorgaab are incorporated into the soil or used as a mulch to evaluate allelopathic effects on weed and crop growth (Correia et al., 2005b; Lahmod and Alsaadawi, 2014). In container experiments, sorghum residues can be mixed into soil in a powder form or as chopped pieces (Ayeni and Kayode, 2013a,b; Khaliq et al., 2011a,b). In field trials, sorghum residues are incorporated into the soil by tilling twice with a disc plow (Alsaadawi et al., 2013). Several authors have also studied the allelopathic potential of sorghum to control weeds by intercropping sorghum with a main crop (Kandhro et al., 2014; Khalil et al., 2010; Mahmood et al., 2013a).

5.2 Analytical Approaches to Study Allelopathy

As mentioned above, it is difficult to quantify the allelopathic potential of a plant. To validate the role of allelopathy, it is necessary to isolate metabolites from plants tissues and purify the extracts until the structure of a putative allelochemical can be determined. According to Duke (2015), there are two fundamental approaches to detecting allelochemicals: (1) identifying known compounds in crude plant extracts or (2) using bioassay-guided isolation of unknown phytotoxins. The second strategy is scientifically more appropriate. With this method, the tissues or exudates of the potentially allelopathic plant must first be extracted to obtain a crude extract with the highest level of phytotoxic activity. These extracts are then fractionated with solvents of varying polarity. In the case of sorghum, sorgoleone may be extracted with organic solvents such as methanol, chloroform, ethanol (Dayan et al., 2009b; Uddin et al., 2010a) or methylene chloride and 1% acetic acid (Netzly and Butler, 1986). According to Uddin et al. (2010a), methanol provides the greatest sorgoleone recovery. After extraction, the allelopathic potential of separated fractions is evaluated with the use of a rapid bioassay, and finally, active fractions can be further fractionated by additional chromatographic methods (Cheema et al., 2007a; Czarnota et al., 2001; Duke, 2015) and structures can be determined by high-resolution mass spectrometry, proton and carbon NMR, and other advanced methods (Lang et al., 2008; Reid and Sarker, 2012).

Studies at the biochemical level are useful tools for determining the effect of allelochemicals on crucial processes in cells of acceptor plants (Dayan et al., 2000). Such experiments can be conducted using isolated mitochondria and chloroplasts from the etiolated seedlings or leaf discs of receiver plants (Einhellig et al., 1993; Rasmussen et al., 1992; Uddin et al., 2012). A very dynamic, highly promising approach for evaluating allelochemical effects is the combination of molecular and genomic studies, which enables metabolic pathway description through the identification and characterization of all the enzyme-encoding genes involved in biosynthesis (Ju et al., 2014). This approach has successfully been used to study the genes and enzymes involved in the biosynthesis of sorgoleone (Cook et al., 2010).

Detailed cultivar characterization and variability at the molecular level for allelochemical synthesis could contribute to the selection of top, allelopathic lines in breeding programs that may be useful in weed control (Dos Santos et al., 2014; Wu et al., 2001). Before breeding programs can develop sorghum cultivars with increased allelopathic potential, more screening work needs to be accomplished (Wu et al., 2001). Promising results are apparent in rice, where germplasm with higher allelopathic potential than current commercial lines has been developed (Gealy and Yan, 2012; Gealy et al., 2013).

6 APPLICATION OF THE ALLELOPATHIC PROPERTIES OF SORGHUM IN AGRICULTURE

The allelopathic potential of sorghum toward weed species has been extensively studied (Table 1). Although early studies were carried out in laboratory or greenhouse environments, more recent research has been conducted in-field. In-field experiments are particularly valuable, as they shed light on practical allelopathic interactions and their consequences in agroecosystems. Weed repression potential of sorghum has been investigated through (1) foliar application of sorgaab (sorghum water extract), (2) crop rotation systems including sorghum, (3) cultivation of sorghum as cover or intercrops, (4) incorporation of sorghum residues into soil, and (5) development of sorghum-derived alleloherbicides (Alsaadawi and Dayan, 2009; Farooq et al., 2013).

6.1 Sorghum Water Extract

6.1.1 Laboratory Tests of Sorgaab Effectiveness

Allelopathic potential of sorghum extract, sorgaab, varies between developmental stages of both sorghum and weed plants. Typically, sorgaab has the greatest impact on early plant growth stages. For example, extract derived from young, vegetative sorghum plants exhibited greatest growth inhibition on seedlings of redroot pigweed and wild barley (Hordeum spontaneum K. Koch) rather than full-grown plants (Al-Tavaha and Odat, 2010; Yarnia et al., 2009). Sorgaab application (1 g/20 mL ^{1} of distilled water) in another study indicated a 15%–20% germination reduction of desert horse purslane (*Trianthema portulacastrum* L.), a persistent weed that infests cotton (Gossypium L.), corn (*Zea mays* L.), and sugar cane (*Saccharum officinarum* L.) in tropical and subtropical regions (Randhawa et al., 2002). Investigations using similar concentrations of sorgaab also revealed shoot length and dry

Table 1 Summary of Weed Species With Growth Inhibition in the Presence of Sorghum or Sorghum-Derived Products

Weed Species	Common Name	Weed Bayer Code	Literature
Abutilon theophrasti Medik.	Velvetleaf	ABUTH	Czarnota et al. (2001), Hoffman et al. (1996)
Aeschynomene indica L.	Indian jointvetch	AESIN	Uddin et al. (2012, 2013a)
Amaranthus hybridus L.	Smooth pigweed	AMACH	Correia et al. (2005b), Hoffman et al. (1996)
Amaranthus palmeri S.Wats.	Palmer amaranth	AMAPA	Burgos and Talbert (1996)
Amaranthus retroflexus L.	Redroot pigweed	AMARE	Alsaadawi et al. (1986), Correia et al. (2005b), Czarnota et al. (2001), Einhellig and Rasmussen (1989), Falquet et al. (2014), Marchi et al. (2008), Uddin et al. (2012), Won et al. (2013), Yarnia et al. (2009)
Amaranthus spinosus L.	Spiny amaranth	AMASP	Correia et al. (2005b), Erasmo et al. (2004)
Ammi majus L.	Bishop's weed	AMIMA	Alsaadawi et al. (2013)
Anagallis arvensis L.	Scarlet pimpernel	ANGAR	Ahmad et al. (1991), Cheema et al. (2004), Ashraf and Akhlaq (2007)
Alternanthera tenella			Correia et al. (2005b)
Avena fatua L.	Wild oat	AVEFA	Alsaadawi et al. (2013), Jamil et al. (2005b, 2009), Mahmood et al. (2015a), Mushtaq et al. (2010b), Sharif et al. (2005)
Beta maritima L.	Sea beet		Alsaadawi et al. (2007)
Beta vulgaris L.	Beet		Alsaadawi et al. (2013)
Bidens pilosa L.	Hairy beggarticks	BIDPI	Trezzi and Vidal (2004)

Continued

Table 1 Summary of Weed Species With Growth Inhibition in the Presence of Sorghum or Sorghum-Derived Products—cont'd

Weed Species	Common Name	Weed Bayer Code	Literature
Brachiaria plantaginea (Link) Hitchc.	Alexander grass		Trezzi and Vidal (2004)
Carthamus oxyacantha Bieb.	Wild safflower	CAUOX	Alsaadawi et al. (2007, 2013)
Cenchrus echinatus L.	Southern sandbur	CCHEC	Dos Santos et al. (2014)
Cercis canadensis L.	Eastern redbud		Geneve and Weston (1988)
Chenopodium album L.	Lambsquarter	CHEAL	Ahmad et al. (1991), Alsaadawi et al. (2007), Cheema et al. (1997, 2004), Czarnota et al. (2001), Hussain et al. (2014), Jabran et al. (2008, 2010a), Mahmood et al. (2015a), Shah et al. (2016), Sharif et al. (2005)
Chromolaena odoratum (L.) King & H.E. Robins.	Siam weed		Ayeni and Kayode (2011)
Commelina benghalensis L.	Benghal dayflower	COMBE	Correia et al. (2005b)
Convolvulus arvensis L.	Field bindweed	CONAR	Ahmad et al. (1991), Awan et al. (2012), Cheema et al. (2000a), Hussain et al. (2014), Khalil et al. (2010), Mahmood et al. (2015b)
Coronopus didymus (L.) Sm.	Lesser swine-cress	COPDI	Ahmad et al. (1991), Bhatti et al. (2000), Cheema et al. (1997, 2004), Jabran et al. (2008, 2010a), Mahmood et al. (2015a), Razzaq et al. (2010, 2012), Shah et al., 2016
Cynodon dactylon (L.) Pers.	Bermudagrass	CYNDA	Dos Santos et al. (2014); Khaliq et al. (1999), Mahmood et al. (2015b)

Table 1 Summary of Weed Species With Growth Inhibition in the Presence of Sorghum or Sorghum-Derived Products—cont'd

Weed Species	Common Name	Weed Bayer Code	Literature
Cyperus eragrostis Lam.	Tall flatsedge	CYER[a]	Rehman et al. (2010)
Cyperus iria L.	Rice flatsedge	CYPIR	Burgos and Talbert (1996), Khaliq et al. (2013a), Rehman et al. (2013)
Cyperus rotundus L.	Purple nutsedge	CYPRO	Ahmad et al. (1991, 1995), Bhatti et al. (2000), Cheema et al. (1997, 2000a, 2005b, 2009), Ihsan et al. (2015), Iqbal et al. (2009), Iqbal and Cheema (2007, 2008), Jabran et al. (2008, 2010a), Khalil et al. (2010), Khaliq et al. (2013a), Mahmood and Cheema, 2004, Mahmood et al. (,2013a, 2015b), Sharif et al. (2005)
Dactyloctenium aegyptium L.	Egyptian crowfootgrass	DTTAE	Mahmood et al. (2015b), Mubeen et al. (2012), Khaliq et al. (1999), Rehman et al. (2010)
Daucus carota L.	Wild carrot	DAUCA	Alsaadawi et al. (2013)
Digitaria sanguinalis L.	Large crabgrass	DIGSA	Nimbal et al. (1996a), Uddin et al. (2012)
Echinochloa colona L.	Junglerice	ECHCO	Cheema et al. (2005b, 2010), Khaliq et al. (2011a, 2013a), Kim et al. (1993), Mahmood et al. (2015b)
Echinochloa crus-galli (L.) P. Beauv.	Barnyardgrass	ECHCG	Cheema et al. (2005b, 2010), Dilipkumar and Chuah (2013), Irshad and Cheema (2005), Khaliq et al. (2013a), Rehman et al. (2010, 2013), Uddin et al. (2012), Weston et al. (1989), Won et al. (2013)

Continued

Table 1 Summary of Weed Species With Growth Inhibition in the Presence of Sorghum or Sorghum-Derived Products—cont'd

Weed Species	Common Name	Weed Bayer Code	Literature
Eclipta alba L.	False daisy	ECLAL	Uddin et al. (2012)
Eleusine indica (L.) Gaertn.	Goosegrass	ELEIN	Mahmood et al. (2015b), Mubeen et al. (2012)
Eriochloa acuminata (J. Presl) Kunth.	Southwestern cupgrass	ERBGR	Burgos and Talbert (1996)
Euphorbia dracunculoides Lam	Dragon spurge		Khaliq et al. (2012)
Euphorbia heterophylla L.	Wild poinsettia	EPHHL	Ayeni and Kayode (2013a, b), De Almeida Barbosa et al. (2001)
Fumaria indica L.	Lambsquarter fumitory		Ashraf and Akhlaq (2007), Awan et al. (2012), Hussain et al. (2014), Sharif et al. (2005)
Fumaria parviflora Lam.	Fineleaf fumitory	FUPA[a]	Cheema et al. (1997)
Galium aparine L.	Catchweed bedstraw	GALAP	Cheema et al. (2003a)
Galium spurium L.	False cleavers	GALSP	Uddin et al. (2012, 2013a)
Hordeum spontaneum K. Koch	Wild barley		Al-Tavaha and Odat (2010)
Hyptis lophanta Mart. ex Benth			Erasmo et al. (2004)
Ipomoea grandifolia (Dammer) O'Donell	Pink convolvulus		Correia et al. (2005b)
Leonotis nepetifolia (L.) R.Br.	Klip dagga	LEONE	Correia et al. (2005b)
Lolium rigidum Gaud.	Rigid ryegrass	LOLRI	Alsaadawi et al. (2007)
Lolium temulentum L.	Poison ryegrass	LOLTE	Alsaadawi et al. (2007, 2013)
Malva parviflora L.	Little mallow	MALPA	Alsaadawi et al. (2007)
Melilotus indicus (L.) All.	Indian sweet clover	MEUIN	Alsaadawi et al. (2007)

Table 1 Summary of Weed Species With Growth Inhibition in the Presence of Sorghum or Sorghum-Derived Products—cont'd

Weed Species	Common Name	Weed Bayer Code	Literature
Nicandra physalodes (L.) Gaertn.	Apple-of-Peru	NICPH	Correia et al. (2005b)
Parthenium hysterophorus L.	Congress grass	PTNHY	Javaid et al. (2006)
Phalaris minor Retz.	Littleseed canarygrass	PHAMI	Ahmad et al. (1991), Alsaadawi et al. (2013), Awan et al. (2012), Cheema et al. (1997, 2004), Jamil et al. (2005b, 2009), Mahmood et al. (2015a), Mushtaq et al. (2010b), Razzaq et al. (2010, 2012)
Plantago asiatica L.	Chinese plantain		Uddin et al. (2012, 2014)
Plantago ovata Forssk.	Blond plantain		Alsaadawi et al. (2007)
Polygonum bellardii All.	Narrowleaf knotweed		Cheema et al. (2004)
Polypogon monspeliensis (L.) Desf.	Rabbitfoot polypogon	POHMO	Alsaadawi et al. (2007)
Portulaca oleracea L.	Common purslane	POROL	Cheema et al. (2000a)
Rumex dentatus L.	Toothed dock	RUDE3[a]	Ahmad et al. (1991), Bhatti et al. (2000), Cheema et al. (1997, 2004), Mahmood et al. (2015b)
Rumex japonicus Houtt.			Uddin et al. (2012, 2013a, 2014)
Senecio vulgaris L.	Common groundsel	SENVU	Nimbal et al. (1996a)
Setaria italica (L.) Beauv.	Foxtail millet	SETIT	Weston et al. (1989)
Setaria viridis (L.) P. Beauv.	Green foxtail	SETVI	Bhatti et al. (2000), Hoffman et al. (1996)
Sida rhombifolia L.	Arrowleaf sida	SIDRH	Trezzi and Vidal (2004)

Continued

Table 1 Summary of Weed Species With Growth Inhibition in the Presence of Sorghum or Sorghum-Derived Products—cont'd

Weed Species	Common Name	Weed Bayer Code	Literature
Silybum marianum (L.) Gaertn.	Blessed milkthistle	SLYMA	Alsaadawi et al. (2007)
Sinapis arvensis L.	Wild mustard	SINAR	Urbano et al. (2006)
Solanum nigrum L.	Black nightshade	SOLNI	Czarnota et al. (2001), Nimbal et al. (1996a)
Trianthema portulacastrum L.	Horse purslane	TRTPO	Cheema et al. (2002a, 2003a, 2007a, 2010), Ihsan et al. (2015), Jabran et al. (2008, 2010a), Khalil et al. (2010), Khaliq et al. (2011b, 2013a), Khan et al. (2012a), Mahmood et al. (2010, 2015b), Mubeen et al. (2012), Mushtaq et al. (2010a), Randhawa et al. (2002)
Trifolium repens L.	White clover	TRFRE	Alsaadawi et al. (2007)

[a]US code is used when Bayer Weed Code is not available.

weight suppression of purple nutsedge (Cyperus rotundus L.) by 75% over a control (Cheema et al., 2009), as well as congress grass (Parthenium hysterophorus L.) and junglerice (Echinochloa colona L.) (Javaid et al., 2006; Kim et al., 1993).

In addition to developmental stage, sorgaab allelopathic potential may differ between sorghum cultivars. A survey of water extract allelopathic potential from nine grain sorghum cultivars on desert horse purslane growth reported that J2001 and Kashmor cultivars completely inhibited weed germination, while other cultivars partially inhibited germination (Cheema et al., 2007a). The survey authors speculated that extracts from the J-263 cultivar had the greatest effect on desert horse purslane seedling dry weight (99% reduction over control) due to a higher content of p-hydroxybenzoic and p-coumaric acids compared to other tested cultivars. Another study implicated that extracts from Jabbal, Hegari, and Sindhar cultivars were most potent to horse purslane. Nonetheless, correlations between sorgaab activity and the presence of p-hydroxybenzoic and p-coumaric acids have been demonstrated (Chung et al., 2002).

6.1.2 Field Tests of Sorgaab Effectiveness

Field trials substantiate sorgaab allelopathy manifested in controlled environments. In several trials, two sequential sprays of 10% sorgaab solutions reduced weed populations and biomass while resulting in 21% wheat (*Triticum* L.) yield increases (Anwar et al., 2003; Cheema and Khaliq, 2000; Cheema et al., 2000b). The allelopathic effect of sorgaab was tested on eight plant species: eucalyptus (*Eucalyptus camaldulensis* Dehnh), acacia (*Acacia nilotica* L. Willd. ex Delile), poplar (*Populus deltoides* W. Bartram ex H. Marshall), sheesham (*Dalbergia sissoo* Roxb.), sunflower, tobacco (*Nicotiana tobacu*m L.) congress grass, and wheat (Khan et al., 2015). Weed suppression expressed as a reduction of dry biomass (62%) through repeated application of sorgaab. Ashraf and Akhlaq (2007) also highlighted the herbicidal ability of sorgaab in wheat via a decrease in weed density, fresh weight, and dry weight by 29%, 31%, and 27% compared to a control, respectively. Furthermore, double spraying of both sorghum stem and sorghum stem plus leaf extracts led to wheat yield increases by 8% and 19%, yet caused substantial growth inhibition of the following weeds species: pimpernel (*Anagallis arvensis* L.), lambsquarter fumitory (*Fumaria indica* L.), and bur clover (*Medicago polymorpha* L.). In corn, sorgaab foliar sprays reduced weed infestation in one instance by 18%–50% and increased corn yield by 11%– 44%, where the most economically effective weed control method was a tri-ple spray with sorgaab (Cheema et al., 2004). Furthermore, a triple spray with sorgaab (at 1:10 volume concentration) applied to rice (*Oryza sativa* L.) reduced weed biomass by 45%–85%, while the application of solely pendimethalin reduced weed biomass by 45% relative to a control (Cheema et al., 2004). Sorgaab application was also reported to increase rice yields (Bhatti et al., 2000). The efficiency of sorgaab to manage weeds in lentil (Lens culinaris Medikus) was revealed in another study because of reduced weed dry biomass of 66% and increased lentil yields of 61% (Hozayn et al., 2011).

6.1.3 Effectiveness of Mixtures Containing Extracts From Sorghum and Other Allelopathic Plants

Duke et al. (2000) suggested that mixing two or more water extracts enhances weed control efficacy due to an increased number of allelochemicals. This concept was verified several times with sorgaab mixes

(Khaliq et al., 2012; Mahmood et al., 2010; Mubeen et al., 2012; Mushtaq et al., 2010a). In a corn study, a blend of sorghum and moringa (*Moringa oleifera* Lam.) extracts resulted in a 35% increase in yield associated with greater control of the weed population compared to sorgaab alone (Kamran et al., 2016; Khan et al., 2012b). Similarly, combined application of sunflower and sorghum extract sprayed at 6 L ha^{1} had the greatest negative impact on wild oat and littleseed canarygrass in wheat field trials (Jamil et al., 2009). Such benefits were also observed in other trials with combinations of sorghum, brassica, and sunflower extracts, where double applications of concentrated extracts of all three species at 45 and 75 days after sowing provided optimum weed control and the greatest wheat yield (Awan et al., 2012). When compared to conventional herbicide treatments (i.e., iodosulfuron plus mesosulfuron), double foliar sprays of sorghum, sun-flower, and brassica extract combinations at 18 L ha^{1} provided economical herbicide alternatives that resulted in 48%–58% weed reduction in a wheat production system (Mahmood et al., 2015a).

6.1.4 Sorghum Extracts Combined With Reduced Doses of Herbicides for Weed Control in Cereal Crops

An interesting potential application of allelopathy is the use of allelopathic plant extracts in combination with reduced doses of conventional herbicides to achieve similar levels of weed control equivalent to full rates of conventional herbicides. This application may enable production cost reductions, yield increases, and agricultural sustainability improvements. Reduced herbicide doses with allelopathic rice species extracts or sorgaab have demonstrated weed control success in cereal crop trials (Gealy and Yan, 2012; Gealy et al., 2013). In one study, the efficacy of a combination of sorgaab (20 L ha^{1} each) and a 50% field rate of iodosulfuron plus mesosulfuron was assessed using conventional, reduced, and zero tillage soil management practices for wheat (Khaliq et al., 2013b). The combination weed management approach was very effective, especially in conjunction with zero tillage, pro-viding up to 90% weed suppression and between 52% and 63% yield gains. Similar results were obtained in another study by integrating sorgaab (in ratio 2:10 w/w) with half the recommended dose of cereal herbicides (e.g., bromoxinil + MCPA, fenoxaprop-p-ethyl, and carfentrazone-ethyl ester) (Shahid et al., 2007). Further yet, an evaluation of sorghum, sunflower, and mulberry leaf extracts (18 L ha^{-1} each) in combination with half the recommended field rate of iodosulfuron plus mesosulfuron provided an 86% reduction in weed populations and an 88% reduction in weed dry mass

(Mahmood et al., 2013b), comparable to results of sorghum and sunflower extracts combined with 75% of the recommended field rate of iodosulfuron plus mesosulfuron in another trial (Hussain et al., 2014). There are many more reports in the literature documenting the promising use of allelopathic extracts in combination with reduced rates of conventional herbicides in cereal crops, and readers are encouraged to read the following articles: Cheema et al. (2003a, 2005b, 2010), Elahi et al. (2011), Irshad and Cheema (2005), Jamil et al. (2005a), Mahmood et al. (2015b), Mushtaq et al. (2010b), Razzaq et al. (2010), Rehman et al. (2010, 2013), and Sharif et al. (2005).

6.1.5 Sorghum Extracts Combined With Reduced Doses of Herbicides for Weed Control in Noncereal Crops

Trials applying the same principles as in Section 6.1.4 have been performed in noncereal crops. For rapeseed, sorgaab mixed with pendimethalin (400 and 600 g ha^{-1}), sunflower, rapeseed, or rice extract provided better weed control compared to 1200 g ha^{-1} herbicide alone in one field study, particularly for purple nutsedge control (Jabran et al., 2008). Sorgaab combinations have also demonstrated effectiveness for weed control in several cotton studies. Triple spray mixtures of concentrated sorgaab (12 L ha^{-1}) with pendimethalin, S-metolachlor, or trifluralin provided excellent weed control and netted the highest profit in one example (Cheema et al., 2002b). Other cotton studies have shown that the rate of pendimethalin can be reduced by half (625 g ha^{1}) to maintain weed control if combined with sorgaab (12 L ha^{-1}) (Cheema et al., 2003b, 2005a), and that increasing sorgaab dosage from 12 to 15 L ha^{-1} enables reduction of S-metolachlor application to one-third of the recommended rate while still providing control of weeds such as purple nutsedge (Iqbal and Cheema, 2008). Further research in cotton has also indicated effective, economic weed control using sorgaab in combination with sunflower or rapeseed extract (15 and 18 L ha^{-1}) and glyphosate, as well as conventional herbicide reduction of up to 75% to decrease production costs while maintaining satisfactory weed control and crop yield (Iqbal et al., 2009). In sunflower production, three foliar applications of sorgaab (15 L ha^{-1}) combined with one-third the recommended dose of S-metolachlor (1.6 L ha^{-1}) in one trial imparted 93.7% weed suppression and superior sunflower yield compared to extract or herbicide alone (Shah et al., 2016). Research of sorgaab and herbicide mixtures in noncereal crops suggests that allelopathic extracts are able to reduce conventional herbicide load by two-thirds while maintaining effective weed control.

6.2 Sorghum in Crop Rotation Systems

Allelopathic plants can be used directly in various cropping systems, including intercropping, cover cropping, crop rotation, and minimum to no tillage systems. These approaches were suggested decades ago (Hussain and Gadoon, 1981) and supported by early allelopathy research that revealed agroecosystem benefits (Leather, 1983). The interaction between crop plant density and allelopathic potential has also been studied and documented by several investigators (Al-Bedairy et al., 2013; Chunjie et al., 2010; Seal et al., 2004). Sorghum cultivated as a forecrop, follow crop, or intercrop should be considered a highly beneficial, allelopathic element of a crop rotation system. In early research, an accumulation of sorghum allelochemicals in soil following sorghum production appeared to provide residual activity that suppressed weed development (Geneve and Weston, 1988). Crop rotation systems that consisted of sorghum, corn, and soybean (*Glycine max* (L.) Merr.) exhibited reduced weed infestation, whereas soybean and corn rotations excluding sorghum had lower weed suppression (Einhellig and Leather, 1988). Sudex, a sorghum-sudangrass hybrid, has been shown to inhibit the growth of Palmer amaranth (*Amaranthus palmeri* S. Wats.), rice flatsegde, and southwestern cupgrass (*Eriochloa acuminata* (J. Presl) Kunth.) in no-till alfalfa (*Medicago sativa* L.) (Forney et al., 1985) and southern pea studies (Burgos and Talbert, 1996), during which increased sorghum plant density was positively correlated to root exudate concentration and weed suppression. Root exudate potency may vary by cultivar.

6.3 Intercropping With Sorghum

Intercropping is a common practice used by farmers in developing countries since it boosts crop yield while reducing soil erosion (Altieri et al., 1983). Another benefit of this system is the suppression of weeds (Liebman and Dyck, 1993) to achieve integrated weed management (Baumann et al., 2000; Schoofs and Entz, 2000). Sorghum is commonly used in intercropping systems due to allelopathic characteristics (Kondap et al., 1990; Sistachs et al., 1991). When intercropped with corn in one trial, sorghum provided suitable management of purple nutsedge (Mahmood et al., 2013a). In fact, one examination of intercropping systems revealed that sorghum had better weed control performance for purple nutsedge, field bindweed, and desert horse purslane than other systems, including other allelopathic crops, such as sunflower and mung bean (Khalil et al., 2010). Sorghum control of purple nutsedge was also confirmed in an intercropping system with cotton, resulting in 87%–95% reduction in weed density and 88%–96% reduction in dry biomass (Iqbal and Cheema, 2007). However, sorghum has also been cited to cause a 22% reduction in cottonseed yield, though greater total economic returns were achieved than those of untreated controls. According to Kandhro et al. (2014), intercropping sunflower and sorghum in cotton is an economic, efficient, and environmentally friendly method of weed control that resulted in greater profits than conventional chemical weed control with 2.5 L ha ^{1} metolachlor.

6.4 Sorghum as a Cover Crop

The incorporation of cover crops and green manures to field crop cultivation has an overall positive effect in the agroecosystem by reducing soil erosion, enriching soils with organic matter, improving soil moisture retention, and smothering weeds (Altieri et al., 2011; Hartwig, 1988; Hartwig and Ammon, 2002). Consequently, the allelopathic potential of sorghum makes it an effective cover crop. Putnam and DeFrank (1983) identified a negative influence of sweet sorghum, sudangrass, and sudex on the growth of weeds in cherry and apple tree orchards with 40% reduced weed biomass from sorghum planted in fall and 85%–90% reduced weed biomass from spring plantings (Putnam and DeFrank, 1983). A covercropping survey of 10 sorghum genotypes for impacts on lambsquarter, white clover *(Trifolium repens* L.), sea beet (*Beta maritima* L.), and little mallow (*Malva parviflora* L.) determined that 3 genotypes (Giza 15, Giza 115, Enkath) enabled weed biomass reduction and density by 58%–66% and 59%–67%, respectively (Alsaadawi et al., 2007). Sorghum and sudangrass are excellent cover crops for weed management in barley as evidenced by their significant inhibitory effect on weed density and biomass, particularly for wild mustard (*Sinapis arvensis* L.), while sustaining high barley grain yield (Urbano et al., 2006). Similar suppression of Canada thistle (*Cirsium arvense* (L.) Scop.) by sudangrass has also been reported (Bicksler and Masiunas, 2009). Green manure of sorghum hybrid BR304 has been shown to reduce dry biomass of spiny amaranth (*A. spinosus* L.) and *Hyptis lophanta* Mart. ex Benth, an invasive weed common in Central Brazil, as well (Erasmo et al., 2004).

Unfortunately, weed suppression of allelopathic plants may have some drawbacks, specifically crop injury on certain incompatible species. For example, one field study concluded that sudex used as a cover crop negatively affected cabbage (*Brassica oleracea* L. var. *capitata* L.) production (Finney et al., 2009). Nonetheless, under proper conditions and with compatible species, the incorporation of allelopathic crop mulches or residues into soil may enhance agricultural sustainability by suppressing weed growth and thereby reducing herbicide use (Nagabhushana et al., 2001).

6.5 Sorghum Crop Residues

A portion of allelopathy phenomenon may be the result of plant residue decomposition that gradually releases phytotoxic substances to the environment. In early allelopathy research, Putnam and DeFrank (1983) noted the high efficiency of sorghum mulch in reducing weed infestation. Sorghum residue provides selective weed management through physical presence on the soil surface as well as phytotoxin release (Inderjit and Keating, 1999). As a consequence of 1.3 t ha^{1} sorghum straw application, Trezzi and Vidal (2004) observed 50% reduction of Alexander grass (*Brachiaria plantaginea* (Link) Hitchc.) and arrowleaf sida (*Sida rhombifolia* L.) density. Application of 4 t ha^{1} of sorghum straw reduced Alexander grass, arrowleaf sida, and hairy beggarticks (*Bidens pilosa* L.) infestations by 91%, 96%, and 59%, respectively. In another study, sorghum residues incorporated into soil or used as mulch strongly inhibited purple nutsedge growth, although res-idue incorporated into soil was most active within the first 20 days and mulch was most active 40 days after mulching (DAM). By 40 DAM, equal application rates of sorghum residue or mulch (15 t ha^{1}) reduced purple nutsedge density and dry mass by 40% and 50% or 45% and 53%, respectively (Mahmood and Cheema, 2004). A study examining the early growth phases of wild poinsettia (*Euphorbia heterophylla* L.) also implicated growth inhibi-tion through powdered sorghum stem residue (Ayeni and Kayode, 2013a,b).

Multiple wheat field trials corroborate sorghum residue weed inhibition studies. In an irrigated wheat crop system, sorghum residues inhibited development of lambsquarter, littleseed canarygrass, purple nutsedge, lesser swine-cress, toothed dock (*Rumex dentatus* L.), pimpernel (*Anagallis arvensis* L.), and field bindweed (Ahmad et al., 1991). Similarly, incorporation of 2–6 t ha^{1} mature sorghum plant residue enabled weed infestation reductions of 40%–50% and wheat yield increases of up to 15% in one wheat production system (Cheema and Khaliq, 2000), as well as complete purple nutsedge growth inhibition in another (Cheema et al., 2009). Field trials of crops other than wheat with sorghum residue applications have also been con-ducted. In a mung bean trial, utilization of 10 and 15 t ha^{1} of sorghum mulch reduced weed infestation by 25% and 27%, respectively, with simultaneous crop yield increases of 19.7% and 13% in comparison to a control group (Cheema and Khaliq, 2000). Soil incorporation of the same application of chopped mature, sorghum plants reduced weed infestation by 26% and 37% and increased yields by 36% and 40% in a comparable corn field trial (Cheema et al., 2004).

As mentioned in Section 6.1 with sorgaab, combining sorghum residue with other allelopathic crops or reduced doses of conventional herbicides may achieve weed control similar to or better than full rates of conventional herbicides. Khaliq et al. (2011a) indicated growth suppression of junglerice by sorghum residues in a pot study through delayed germination plus decreased root and shoot dry biomass, which was enhanced with combinations of sorghum, sunflower, and brassica allelopathic residues in equal amounts. Similar combination trials were carried out to evaluate the impact of allelopathic residues on germination dynamics and early growth stages of horse purslane seedlings, demonstrating that combined allelopathic crops residues (6 g kg ^{1} of soil; 12 t ha ^{1}) increased horse purslane seedling suppression (Khaliq et al., 2011b). In field bean (*Vicia faba* L.) cultivation, Alsaadawi et al. (2013) found that the incorporation of 7.6 t ha ^{1} of crop residue into soil and the application of half a typical field dose of trifluralin resulted in greater reduction of weed dry mass than a full dose. A subsequent study of the same amount of sorghum residue with a half dose of iodosulfuron and mesosulfuron resulted in phytotoxicity and decreased wheat yield; however, reducing sorghum residue to 3.5 t ha $^{-1}$ combined with the same herbicides and application rates provided the greatest wheat yield (Lahmod and Alsaadawi, 2014). The authors suggested that blended methods of weed control (allelopathic crop residue and reduced rates of conventional herbicides) improved both physiochemical and biological properties of soil. Weed population densities of klip dagga (*Leonotis nepetifolia* (L.) R.Br.), *Alternanthera tenella*, pink convolvulus (*Ipomoea grandifolia* (Dammer) O'Donell), Benghal dayflower (*Commelina benghalensis* L.), apple of Peru (*Nicandra physalodes* (L.) Gaertn.), smooth pigweed (*Amaranthus hybridus* L.), redroot pigweed, and spiny amaranth were also reduced by sorghum mulch in combination with reduced rates of imazamox in a soybean field trial (Correia et al., 2005b).

6.6 Sorgoleone as the Precursor for the Development of Alleloherbicide

6.6.1 Efficiency of Sorgoleone in Laboratory Settings

Weeds that produce small seeds tend to be more sensitive to the phytotoxic influence of sorgoleone (De Souza et al., 1999; Einhellig and de Souza, 1992; Peterson et al., 2001). Contrastingly, larger seeded weeds tend to grow faster during early development, enabling their root systems to grow beyond the sorghum rhizosphere and consequently facilitate lower susceptibility to sorghum allelochemicals (Bertin et al., 2003; Leishman et al., 2000). Resistance may also result from lower levels of sorgoleone absorbtion and translocation or faster metabolic degradation (De Almeida Barbosa et al., 2001; Rimando et al., 1998). With ^{14}C-labeled sorgoleone in velvetleaf (*Abutilon theophrasti* Medik.), sorgoleone photosynthesis inhibition was shown to be limited to developing plants in a laboratory environment (Dayan et al., 2009b). Sorgoleone deposition period, decomposition rate, and biotransformation in soil may be applied to evaluate the environmental risks connected with using sorgoleone as an herbicide. In another experiment with ^{14}C-labeled sorgoleone, evaluation of the rate of sorgoleone mineralization in four soil types, including two originating from the United States and two from Denmark, demonstrated that the methoxy group (Fig. 3) decomposed the fastest at a rate of up to 26% during the first 48 h after soil incorporation (Gimsing et al., 2009); other parts of sorgoleone, including the ring and lipophilic tail, degraded slower. The varied rate of functional group decomposition in sorgoleone molecules is recognized as one of the crucial properties for its herbicidal potential. The mineralization of the methoxy group may be associated with reduced allelopathic activity, whereas degradation of the ring or tail renders the molecule inactive. Increased intensity of the mineralization process in soil samples from the United States resulted from the presence of microorganisms that used sorgoleone as a source of energy.

Einhellig and Rasmussen (1989) indicated a higher sensitivity of broad-leaf compared to grass weed species to the allelopathic effect of grain sorghum introduced in a crop rotation system. Hydroponic tests suggested that sorgoleone is phytotoxic at concentrations lower than 10 μM, and that grasses are typically more tolerant to this secondary metabolite than broad-leaf species (Einhellig and de Souza, 1992; Nimbal et al., 1996a). Further studies indicate that broadleaf seedling growth may be inhibited by as much as 70%–80% (Uddin et al., 2009). These conclusions are supported by more recent greenhouse research examining the application of 150 μg/ mL sorgoleone combined with 7.5 mg/mL of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) hairy root extract, where broadleaf weeds exhibited greater growth inhibition than grass weeds for combined rather than individual extracts in particular (Uddin et al., 2013a). Specifically, the mix-ture of these two natural products inhibited the growth of false cleavers

(Galium spurium L.), Rumex japonicus Houtt., and Indian jointvetch (*Aeschynomene indica* L.) by 100%, 96%, and 90%, respectively, whereas application of sorgoleone alone led to growth suppression of these weeds species by 81%, 83%, and 75%, respectively.

In laboratory trials, sorgoleone blocked electron transport in mitochondria isolated from etiolated seedlings of corn and soybean (Rasmussen et al., 1992). The target appears to be the step between state III and state IV respiration in both soybean and corn. Sorgoleone is most active as an inhibitor of oxygen evolution in soybean leaf disks and isolated chloroplasts from many species including weeds resistant to conventional PSII inhibitors (Dayan et al., 2009c; Einhellig et al., 1993). Similar investigation have been carried out by Uddin et al. (2012), who studied inhibition of chlorophyll fluorescence and growth by sorgoleone in several weed species under in vivo conditions, including false cleavers, Indian jointvetch, Rumex japonicus, Chinese plantain (Plantago asiatica L.), redroot pigweed, false Daisy (Eclipta alba L.), barnyardgrass, and hairy crabgrass. Significant growth reduction was observed in plants exposed to sorgoleone at 200 μg mL, with the broadleaf weeds (*R. japonicus*, false cleavers, and Indian jointvetch) being most susceptible. Maximum quantum efficiency of PSII (Fv/Fm) was inhibited by 200 μg sorgoleone mL^{-1} 6 h after application.

6.6.2 Development of Alleloherbicide

A promising use of allelopathic plants is to develop the active component as structural scaffolds to develop new herbicide classes, as has been done successfully with the triketone herbicides (Beaudegnies et al., 2009; Dayan et al., 2007b; Gray et al., 1980). The discovery of chemistry with novel modes of action is greatly needed to overcome rising problems associated with evolution of herbicide-resistant weed biotypes to current herbicides (Albuquerque et al., 2011). It should be mentioned that allelochemicals must meet several criteria to become registered herbicides: proved phytotoxic properties between 10^{5} and 10^{7} M, described chemical structure, identified mode of action in plants, known time of residence in soil, possible toxic activity on human health, and viability of production on an industrial scale (Soltys et al., 2013 quot. Bhowmik and Inderjit, 2003).

Many studies conducted under laboratory conditions with purified sorgoleone have shown its high efficiency as a broad-spectrum inhibitor of agronomically important weed species (Bertin et al., 2003; Czarnota et al., 2001; Nimbal et al., 1996a; Uddin et al., 2009, 2010b). The phyto-toxic activity of sorgoleone combined with its multiple target sites and relatively long soil half-life are characteristics that could lead to the development of a natural herbicide. Sorgoleone could be developed as a preemergence herbicide, inhibiting photosynthesis in very young weed seedlings (Dayan et al., 2009a). The hydrophobic properties of sorgoleone (Dayan, 2002; Trezzi et al., 2006) enable it to adsorb strongly to soil, especially to organic matter and other hydrophobic molecules. This property is necessary from the point of view of the practical use of the sorgoleone as an herbicide. The herbicide must have defined persistence in the weed (target species) seed germination zone, which implies effective biological activity (Trezzi et al., 2006).

Synthetic analogues of sorgoleone with saturated site chains (hydrogenated sorgoleone and 2-acetoxy-5-methoxy-3-(pent-1-yl)-1,4-benzoquinone) retained their activity against the development roots of cucumber (Cucumis sativus L.), lettuce (Lactuca sativa L.), Desmodium tortuosum (Sw.) DC, pignut (Hyptis suaveolens (L.) Poit), and Mexican fireplant (De Almeida Barbosa et al., 2001). The synthetic quinone obtained in this experiment was as active as the natural product. These data are in agreement with another study that demonstrated that the level of unsaturation of the aliphatic tail had no effect on the ability of the benzoquinone to inhibit photosynthesis (Kagan et al., 2003). Therefore, the synthesis of natural products like quinones described by De Almeida Barbosa et al. (2001) can be used to prepare novel quinone herbicides, assuming that these molecules have appropriate toxicologic pro-files. Pure sorgoleone remains stable for up to 21 days at 20 °C (Franco et al., 2011). Similarly, storing roots in a freezer did not alter the quality of sorghum root extracts.

Commercial herbicides are most often formulated with adjuvants to improve their water solubility, increase spreading on leaves and absorp-tion in plants, or prolong their soil stability (Amali et al., 2014). Uddin et al. (2014) developed a wettable powder (WP) formulation of sorgoleone by combining methanol sorgoleone extract with silicon diox-ide, kaolinite, calcium carbonate, and polyethylene ether. In Petri dishes, 0.2 g L^{-1} of active ingredient completely inhibited the germination and development of broad-leaved weeds. In greenhouse experiments, the growth of sorrel (*Rumex japonicus* Houtt.) and Chinese plantain (*Plantago asiatica* L.) was also completely inhibited after the application of 0.4 g L^{-1} of the active substance. It was noted that improved weed reduction results may be obtained if the developed bioherbicide is applied after germination.

7. EFFECT OF SORGHUM ON OTHER CROP SPECIES

The deleterious effect of sorghum on the growth of other crop species has been known for a long time (Breazeale, 1924). In laboratory bioassays, aqueous extracts prepared from leaves, stem, and roots of five sorghum hybrids have been shown to inhibit soybean radicle development (Correia et al., 2005a). Sorgoleone alone has also demonstrated phytotoxicity to lettuce, cucumber, and rice (De Almeida Barbosa et al., 2001; Khaliq et al., 2011a; Uddin et al., 2010b). Marchi et al. (2008) observed significant seed germination inhibition as well as reduced radicle and shoot growth of lettuce and tomato treated by a sudex water extract, especially for seedlings after 10 days. Phytotoxicity symptoms, including stunting, leaf necrosis, and color change in tomato, lettuce, and broccoli (Brassica oleracea var. italica) following sudex extract application, are indicative of sorghum allelopathic potential, particularly as a cover plant (Summers et al., 2009). To reduce the risk of injury to similarly sensitive crops, it has been suggested that planting be delayed by 6–8 weeks and that crops are not directly planted into sudex residue. According to Roth et al. (2000), the effect of sorghum residue on wheat is highly dependent on the degree of decomposition of the straw before planting wheat. Additionally, tilled sorghum residues do not affect wheat grain yield, though delays in wheat development have been observed. No-till sorghum straw reduces wheat grain yield, perhaps due to slower leaching and degradation of allelochemicals in the soil. Although sorghum has been implicated in a negative effect on yield for peanuts (Arachis hypogea) cultivated in tropical areas (Se`ne et al., 2000 quot. Delafond and Burgos-Leon, 1978), effects may be mitigated by planting peanuts between rows of a previous sorghum crop (Se`ne et al., 2000). Suppression of germination and early growth in cotton seedlings has been observed after soil application of sorghum powder and aqueous extracts (Kandhro et al., 2016). Mung beans are also sensitive to sorghum aqueous extracts (Moosavi et al., 2011). Consequently, rotations between sorghum and mung bean are not recommended.

In contrast to formerly described observations and studies, one research group has reported that sorgoleone did not have any influence on rice, bar-ley, wheat, corn, tomato (*Solanum lycopercisum* L.), soybean, or Chinese cabbage *(Brassica rapa* L. subsp. *chinensis* (L.) Hanelt) (Uddin et al., 2010b). Other research groups have moreover reported positive, stimulatory effects of sorghum on major crops. For example, an intercropping of sorghum and cotton was shown to exhibit positive effects such as increased cotton canopy and root mass by 22% and 41%, respectively (Dos Santos et al., 2014). Cheema et al. (2003c) also pointed out a stimulatory effect of foliar applied sorgaab on wheat yield, which is supported by studies conducted by Afzal and Iqbal (2015) that showed increased wheat yield after application of soghum water extracts. Techniques that capitalize on the benefits of sorghum allelopathy while minimizing negative effects on other crop plants are critical for the implementation of sorghum as a weed control tool (Kandhro et al., 2016). One such technique may be delaying planting following sor-ghum residue incorporation for at least 1–2 weeks, which was successful in rice (Khaliq et al., 2011a).

8. CONCLUSION

Knowledge of the allelopathic properties of plants is important not only from an academic or scientific point of view but also for its potential impact in agricultural practice (Vyvyan, 2002). Ongoing research in allelopathy should focus on donor plants that produce chemical compounds capable of accumulating to bioactive concentrations (i.e., phytotoxic) that persist within soil long enough to influence the growth of nearby receiver plant species (Chou, 1999; Hiradate, 2006; Hiradate et al., 2010).

The effectiveness of allelopathy under field conditions is often questioned (Cheema et al., 2009), and difficulty in differentiation of allelopathy from plant competition has hindered the development of methods that capitalize on the weed repression potential of allelopathic crops. The pro-duction and release of allelochemicals is highly dependent on many external factors including mineral deficiency, light, temperature, and water stress (Kobayashi, 2004). Complex, interlinked physical, chemical, and biological processes occur in the soil and may lead to the modification of properties of exuded allelopathic substances (Tharayil et al., 2008).

The combination of water extracts from sorghum and other plants with lower doses of herbicides may help reduce the overall amount of herbicide introduced into the environment (Einhellig, 1996). Many years of studies on sorgaab demonstrate that the aqueous extract enhances weed control by conventional herbicides, making it an economically viable biological plant protection method (Cheema et al., 2000b; Irshad and Cheema, 2005).

Allelopathy is becoming an increasingly popular alternative for the application of synthetic plant protection agents. It has been projected that biopreparations will account for approximately 20% of environmentally friendly plant protection agents in the upcoming decades (Li et al., 2003; Turnera et al., 2007). In a broader perspective, new possibilities associated with the use of biotechnology to enhance allelopathy and the synthesis of bioherbicides by crops offer exciting future novel weed management tools. Attempts at genetic manipulation aimed at allelopathy enhancement in donor plants are currently underway. Specifically, overexpression of key enzymes in the sorgoleone biosynthetic pathway may increase the allelopathic potential of sorghum, resulting in greater opportunities for practical applications (Gniazdowska, 2007). Holistic approaches using multi-disciplinary programs will be necessary to carry out the research required for implementation of allelopathy as a useful weed control tool. Molecular research on the genetic control of the synthesis and exudation of allelochemicals as well as the functional characterization of allelochemicals and their fate in soil is necessary.

One should bear in mind that the cost of bioherbicides must be competitive in order to become realistic alternatives to conventional herbicides. Thus, new plant protection methods should be developed in a way that minimizes the cost of crop production. The search and implementation of innovative biological methods of weed control that are competitive with synthetic herbicides and translate into actual economic profits remains challenging in modern agriculture.

It should be noted that, in the last 20 years, agriculture has witnessed a noticeable trend toward the search for new methods to reduce plant production costs, especially with respect to plant protection agents and expenses. The application of allelopathy in agriculture may meet economic needs by reducing costs while at the same time exhibiting a more desirable environmental profile.

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ROZDZIAŁ 7

Wnioski

Przeprowadzone badania wskazują, że:

1. Zastosowanie mocznika w polimerowej otoczce w niedzielonej dawce 90 kg ha^{-1} pozwala na uzyskanie biomasy sorga i wytłoków zawierających bezpieczny dla przeżuwaczy poziom azotanów. Taki sposób nawożenia spełnia zasady zrównoważonej produkcji sorga cukrowego na cele paszowe.
2. Nawożenie saletrą amonową sorga przeznaczonego na paszę w dawkach 90 kg ha^{-1} i większych nie jest wskazane ze względu na zwiększone ryzyko wystąpienia, zwłaszcza w lata o deficycie opadów toksycznych ilości azotanów w biomasie i w wytłokach.
3. Określenie indeksu zieloności liści (SPAD) w okresie kwitnienia roślin sorga może stanowić prostą i nieinwazyjną metodę przewidywania zawartości azotanów w biomasie sorga.
4. Zastosowanie osadu ściekowego i pofermentu w nawożeniu sorga cukrowego zapewnia uzyskanie plonu biomasy na zbliżonym poziomie w porównaniu do plonu otrzymanego po nawożeniu mocznikiem. W badaniach wykazano znaczący potencjał nawozowy badanych produktów odpadowych.
5. Wykorzystanie produktów odpadowych takich jak osad ściekowych pozwala na recyrkulację makro- i mikroskładników i dzięki temu wpisuje się w gospodarkę obiegu zamkniętego.
6. Zastosowanie osadu ściekowego i pofermentu jako zamiennik konwencjonalnego nawozu azotowego pozwala na znaczące zmniejszenie śladu węglowego w produkcji sorga cukrowego przeznaczonego na cele energetyczne. Zastosowanie tych produktów odpadowych powinno być rekomendowane w zrównoważonej produkcji sorga uwzględniającej zmniejszoną emisję gazów cieplarnianych i zmniejszenie wpływu na globalne zmiany klimatu.
7. Produkcja metanu z biomasy sorga cukrowego odznacza się kilkakrotnie wyższym współczynnikiem efektywności energetycznej niż produkcja etanolu. Dlatego w warunkach klimatu umiarkowanego sorgo powinno być wykorzystywane jako substrat do produkcji biogazu.
8. Stosowanie pofermentu zapewnia najwyższy współczynnik efektywności energetycznej u odmiany Rona 1 (w przypadku produkcji metanu). Zastosowanie do nawożenia osadu ściekowego pozwala na uzyskanie najwyższej efektywności energetycznej produkcji biopaliw z odmiany Sucrosorgo 506. Zastosowanie produktów odpadowych zmniejsza nakłady energetyczne w porównaniu do aplikacji mocznika.

9. Właściwości allelopatyczne sorga pozwalają na wykorzystywane substacji występujących w tej roślinie jako substytut konwencjonalnych, chemicznych metod kontroli zachwaszczenia. W oparciu o zebrane publikacje stwierdzono, że bioherbicydy wytwarzane na bazie właściwości allelopatycznych sorga muszą być cenowo konkurencyjne w stosunku do chemicznej kontroli zachwaszczenia.
10. Koniecznością są badania molekularne mające na celu genetyczne kontrolowanie syntezy i wydzielania allelochemikalii.

ROZDZIAŁ 8

Streszczenie

W obszarze występowania klimatu umiarkowanego sorgo (*Sorghum bicolor* (L.) Moench) to gatunek relatywnie nowy i brakuje doniesień naukowych o wielu istotnych aspektach jego uprawy.

Mając na uwadze powyższe, w niniejszej rozprawie w sposób transdyscyplinarny ujęto 2 tematy badawcze, które zostały uznane za ważne z ogólnoświatowego punktu widzenia, czego wyrazem jest przyjęcie opracowań w ramach tych tematów do renomowanych czasopism naukowych. Po pierwsze ukazanie wielokierunkowości uprawy sorga a po drugie skupienie się na wieloaspektowym jej wpływie na środowisko. Na wielokierunkowe wykorzystanie składa się przydatność biomasy z sorga do celów paszowych, produkcji biopaliw (bioetanol i biogaz) oraz specjalne (do ograniczania zachwaszczenia). W zakresie oddziaływania na środowisko przeanalizowano emisję CO_2 do atmosfery przy różnych sposobach uprawy i nawożenia sorga oraz efektywność energetyczną produkcji metanu i bioetanolu. Podjęcie tematu nawozowego wykorzystania produktów odpadowych (pofermentu czy osadów ściekowych) to ważny element gospodarki obiegu zamkniętego.

Mieszańce sorga i spokrewniona trawa sudańska oraz mieszańce między tymi gatunkami są przede wszystkim ważnymi roślinami paszowymi, szczególnie w ciepłych i suchych regionach świata. Jednak ich biomasa może powodować zatrucia azotanami u zwierząt gospodarskich. Doświadczenie polowe zostało przeprowadzone w celu oceny wpływu nawożenia azotem na wielkość plonu sorga paszowego i nagromadzenie azotanów w biomasie oraz wytłokach. Zastosowano 2 dawki nawozu – 90 i 180 kg N ha^{-1}. Nawóz był aplikowany pojedynczo lub w dawce dzielonej. Użyto mocznika o kontrolowanym uwalnianiu azotu poprzez zastosowanie otoczki polimerowej z poliolefinu oraz nawozów o charakterze konwencjonalnym – saletry amonowej i mocznika. Zastosowanie azotu w postaci mocznika otoczkowanego było podyktowane tym, że wiele doniesień naukowych dowodzi, że nawozy tego typu zwiększają efektywność wykorzystania azotu i plon roślin oraz zmniejszają straty azotu.

Przeprowadzone badania wskazują, że zastosowanie nawozu o kontrolowanym uwalnianiu azotu w dawce 90 kg N ha^{-1} zapewniło bezpieczny poziom azotanów w biomasie sorga i może być rekomendowane w zrównoważonej produkcji sorga na cele paszowe. Dodatkowo, w badaniach wykazano przydatność pomiaru zieloności liści (SPAD – *Soil Plant Analysis Development*) wykonywanego w czasie sezonu wegetacyjnego do przewidywania zawartości azotanów w biomasie podczas zbioru. Badania pokazały, że ta prosta i nieinwazyjna metoda może zapewnić przydatną informację o potencjalnym zagrożeniu zatruciem zwierząt.

W kolejnym aspektach (agronomicznym jak i wpływie na środowisko) podjęto tematy nawozowego potencjału produktów odpadowych oraz emisji gazów cieplarnianych przy zróżnicowanych sposobach nawożenia. Zgodnie z wynikami badań zastosowanie osadu ściekowego i pofermentu można uznać za alternatywne źródło składników pokarmowych bez zmniejszenia plonu sorga w stosunku do konwencjonalnego nawożenia mocznikiem.

Obliczenia śladu węglowego wykonano zgodnie z normą ISO TS 14067 przy użyciu tzw. kalkulatora BioGrace Excel GHG bezpłatnie dostępnego w źródłach internetowych. Z bazy kalkulatora przyjęto standardowe wartości i wskaźniki konwersji. Obliczenia są zgodne z wytycznymi IPCC (*Internationl Panel of Climate Change*). Wydzielone zostały 2 zakresy emisji – (1) zewnętrzne wynikające z procesów produkcyjnych pestycydów, nawozów, nasion oraz (2) wewnętrzne w obrębie gospodarstwa. Stwierdzono, że zastosowanie azotu w formie mocznika miało najwyższy wpływ na zewnętrzną emisję gazów cieplarnianych (*Greenhouse Gases*- GHG. Zastosowanie osadu ściekowego i pofermentu zmniejszało ogólną emisję gazów cieplarnianych. Co sprawia, że użycie produktów odpadowych w celach nawozowych może stanowić perspektywiczną strategię zapewniająca produkcję sorga na cele energetyczne zmniejszającą emisje GHG.

Sorgo cukrowe oprócz wykorzystania paszowego, które dominuje w klimacie umiarkowanym stanowi również surowiec do produkcji biopaliw drugiej generacji ze względu na zawartość w biomasie cukrów rozpuszczalnych w wodzie i kompleks ligninocelulozowy. Jednym z kluczowych czynników zrównoważonej uprawy roślin energetycznych jest poprawa efektywności energetycznej. Przeprowadzone badania wskazują, że zapotrzebowanie na produkcję i aplikację mineralnego nawozu azotowego stanowi najwyższy udział w ogólnym zużyciu energii w przygotowaniu surowca. Oceniono efekt zastąpienia mocznika produktami odpadowymi – osadem ściekowym i pofermentem na produkcję energii z biopaliw z dwóch odmian sorga cukrowego (Rona 1 i Sucrosorgo 506). W badaniach oceniono nakłady energetyczne obejmujące etapy od przygotowania surowca - czyli uprawy sorga i transportu surowca. Po zastosowaniu zarówno osadu ściekowego jak i pofermentu dla obu odmian całkowity nakład energetyczny obniżył się średnio o 30% w porównaniu do aplikacji mocznika. Konwersja świeżej biomasy sorga do metanu pozwoliła na uzyskanie znacznie większej ilości energii niż produkcja etanolu. W przypadku zastosowania pofermentu otrzymano najwyższy współczynnik energii dla produkcji etanolu z obu odmian oraz dla produkcji metanu z odmiany Rona 1. Na podstawie uzyskanych wyników stwierdzono, że sorgo w warunkach klimatu umiarkowanego powinno stanowić surowiec do produkcji biogazu. Zastosowanie produktów odpadowych zwiększało współczynnik efektywności energetycznej.

Jak wspomniano sorgo charakteryzuje wielokierunkowość użytkowania. Oprócz wykorzystania paszowego i energetycznego, związki w nim występujące mogą zostać użyte w kontroli zachwaszczenia. Dokonano obszernego przeglądu literatury w oparciu o zebranie wielu doniesień naukowych o możliwości praktycznego wykorzystania allelopatycznych właściwości sorga. Krytyczna analiza pozwoliła na zidentyfikowanie kilku płaszczyzn wykorzystania właściwości allelopatycznych w ograniczeniu zachwaszczenia w agrocenozach. Scharakteryzowane związki o charakterze allelochemikalii mogą być zastosowane w kombinacji z ekstraktami innych roślin lub herbicydami. Ponadto mogą zostać wykorzystane do produkcji bioherbicydów.

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Summary

Sorghum (*Sorghum bicolor* (L.) Moench) is relatively new species in temperate climate and some aspects of sorghum production are still unknow. Therefore, this dissertation shows two research area in transdisciplinary approaches. On the one hand it is focusing on large sorghum uses and the other hand indicating effects of sorghum production on environment. This species can be use as forage, biofuels (bioethanol and biogas) feedstock and allechochemicals donor to weeds control. Environmental aspects of this paper included GHG emission in terms of different feedstock management technologies and energy efficiency of bioethanol and biogas production. Moreover, another issue of this dissertation is possibility of application waste products (sewage sludge and digestate) as fertilizer of circular economy management.

Same sorghum hybrids, the related Sudangrass and hybrids between these two species are important forage crops, particularly in warm, dry regions. However, feeding with sorghum or Sudangrass can pose the threat of inadvertent NO^{3-} poisoning. A field experiment was conducted to test the impact of N fertilization management on sweet sorghum yield and NO_3^- accumulation in sorghum biomass and bagasse. Sorghum was grown under two levels of N dosses – 90 and 180 kg N ha^{-1}, once or split applied as enhanced-efficiency N fertilizer – polyolefin polymer coated urea and as common N sources – ammonium nitrate and urea. Polymer coated urea was used because some research indicated that this fertilizer improves nitrogen efficiency and crop yield and decreases N losses.

This study demonstrates that polymer coated urea at the rate of 90 kg N ha^{-1} provides biomass with a safe level of NO_3^- and can be recommended in sustainable sweet sorghum production for forage. In addition, in this paper an indirect strategy based on Soil Plant Analysis Development (SPAD) readings measured during growing season was proposed to predict NO_3^- level in biomass at harvest. Results showed that this non-invasive method could provide valuable information on potential NO_3^- accumulation and animal poisoning risk.

The next issues of this paper are assessing the fertilizer potential of biowaste products and evaluation the emission greenhouse gas (GHG) from sweet sorghum cultivation as a bioenergy crop. Three years experiment was carried out in the field condition. According to obtained results, sewage sludge and digestate could be recognized as a nutrient substitute without sorghum yield losses.

Calculations of (GHG) were performed based on Intergovernmental Panel on Climate Change methodology. The quantification of GHG emissions was made according to ISO TS

14067 norm. The freely available BioGrace Excel GHG calculation tool was used to estimate the C footprint of sorghum production. Standard values containing conversion factors and LHV (lower heating values) from the database developed by IPCC were used for computing GHG emissions. Greenhouse gas emissions were divided into external and on-farm emissions. These emissions area result of production processes and application of agricultural inputs, such as pesticides, fertilizers, seeds, and combustion of diesel oil during farm operation. Nitrogen application had the greatest impact on the external GHG emissions. CO_{2eq} emissions decreased when sewage sludge and digestate were applied. This fertilization practice represents a promising strategy for low emission C agriculture and could be recommended to provide sustainable sorghum production as a bioenergy crop to mitigate GHG emissions.

One of the crucial factors in the sustainable production of energy crops is improvement of the energy balance and efficiency. Nitrogen demands contribute to the highest proportions of total energy consumption of all energy inputs for feedstock management. The effect of bio-based waste products – sewage sludge and digestate replacing urea – on the energy output of biofuels produced from two different hybrids of sweet sorghum (Rona 1 and Sucrosorgo 506) was evaluated.

In this study the evaluation of energy input included only feedstock production and transport. When sewage sludge or digestate was applied the total required energy inputs decreased by 1/3 in case of both varieties as compared with the application of urea. Conversion of fresh sorghum biomass into methane provided significantly more the gross energy output as compared ethanol production. Application of digestate allowed the highest energy efficiency ratio to be obtained in terms of ethanol production for both tested hybrids and in terms of methane for Rona 1. Sweet sorghum should be used as biogas feedstock in the temperate climate. The application of waste – sewage sludge and digestate – in feedstock management increased the energy efficiency of biofuel production.

As mentioned above, sorghum is multifunctional crop. Because of chemical composition it can be use in weed control. This report provides a comprehensive literature review of the applications of sorghum allelopathy in agriculture. A critical analysis of the allelopathic properties of sorghum identified the following areas contributing to its ability to reduce weed infestation in agroecosystems:

1. a large number of compounds produced by sorghum have allelopathic properties,

2. allelopathic compounds can be applied in the form of mixed plant extracts or in combination with herbicides,
3. sorghum extracts have a broad spectrum of activity,
4. sorghum may be used to produce bioherbicides.

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Załączniki

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