

ORIGINAL RESEARCH

The bioenergy crop *Sorghum bicolor* is a relevant pollen source for honey bees (*Apis mellifera*)

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Abstract

Abundance and diversity of pollinating insects are decreasing. Intensification of agricultural bioenergy production is presumed to accelerate the decline of pollinators. Sorghum (*Sorghum bicolor* L. Moench) is a promising bioenergy crop. Enhanced dual-purpose type cultivars have been developed and tested for suitability for bioenergy cropping in Germany. Sorghum is assumed to be a nutritional resource for pollen-collecting insects. To evaluate this assumption, we studied the foraging strategy of *A. mellifera* colonies, which were migrated to sorghum fields in Germany. The bee hives were equipped with bottom fixed pollen traps. The pollen loads of the colonies contained variable shares of sorghum pollen ranging between approx. 10% and 50% (weight/weight). Sorghum pollen occurred frequently in more than 50% of all pollen samples. Experimental mini colonies were placed in plots which were grown with two varieties of sorghum, phacelia (*Phacelia tanacetifolia*), maize (*Zea mays*) and a control plot without any vegetation. All plots were encased with flight tents. Significant effects of the crop were found for the productivity parameters brood rearing and pollen collection. The sorghum and maize variants performed significantly better than the controls but significantly poorer than phacelia ($p < 0.05$). The parameters number of dead bees and colony sizes were not affected by the crops ($p < 0.05$). Pollen of sorghum is a valuable food for bees which supports nursing of bee brood, but its availability proved to be inferior to phacelia as the pollen shedding of sorghum lasted a considerably shorter time. Pollen collection by honeybees did not negatively affect seed yield of sorghum in any case. Under unfavourable weather conditions, flower visiting bees enhanced seed yield of sorghum.

KEYWORDS

food resource, insect-friendly bioenergy cropping, pollinator conservation, *Sorghum bicolor*

1 | INTRODUCTION

Global warming has been accelerating during the last decades (IPCC, 2015). Atmospheric concentrations of greenhouse gases

have reached historic highs (UNEP, 2020). Dramatic negative consequences are expected for agriculture, for the functionality of ecosystems and for human societies. Counteractivities are the subject of intense political debate. Scientists advise a

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decarbonization of the energy sector (Rockström et al., 2017). One component of the strategy against global warming is the use of renewable energies, especially energy crops, which can be converted to electrical power and thermal energy in biogas plants (Dhillon & Wuehlisch, 2013). In Germany, there are presently approx. 9500 biogas plants in operation. The substrate of biogas plants consists of at least 50% of bioenergy crops (Scarlat et al., 2018). Maize (*Zea mays*) is the most prominent bioenergy crop due to high biomass yields, excellent fermentation properties and high methane yields. The predominance of maize raises controversial debates (Theuerl et al., 2019). Monocropping of maize and growing maize in self-succession can be accompanied by severe phyto-sanitary problems (e.g. pandemic occurrence of *Diabrotica virgifera*), soil degradation and is believed to affect biodiversity. Since maize does not provide nectar and its pollen has a rather low nutritive value, pollinators in regions with high corn cultivation intensity are at risk of facing food shortages, which raises concerns as the functionality of the ecosystem relies on the service of pollinating insects (Steffan-Dewenter et al., 2005). The debate is held against the backdrop of a massive decline of the entomofauna (Forister et al., 2019; Hallmann et al., 2017; Seibold et al., 2019), for which intensification of the agricultural land use has been identified among the most important drivers (Sánchez-Bayo & Wyckhuys, 2019; Vanbergen, 2013). In particular, intensive agricultural bioenergy crop production can exacerbate this trend by converting non-crop land into crop land, as has been documented for the Great Plains in the United States, for instance (Otto et al., 2016). Global cultivation of biofuel crops affects biodiversity primarily through land-use change (Immerzeel et al., 2014). In Central Europe, bioenergy crop cultivation led to a partial conversion of previously extensively managed perennial grassland into intensively managed maize fields, which is assumed to be associated with a deterioration of pollinator diversity (BfN, 2009). European policy makers counteracted in 2014 by issuing regulations to prevent the plowing up of grassland.

The agro-ecological value of landscapes for pollinating insects is mainly defined by the availability of abundant food resources (Tscharntke et al., 2005) and their botanical diversity (Goulson et al., 2015). Pollen diets are monotonous in intensively farmed landscapes. The lack of botanical diversity is claimed to affect the fitness of honey bee colonies (Di Pasquale et al., 2013). From a social perspective maize dominated farming reduces the common acceptance of bioenergy systems (Herbes et al., 2014). Hence, alternative bioenergy crops are sought to complementing the existing maize crop rotations. Sorghum (*Sorghum bicolor* L. Moench) is considered a promising alternative to maize also for Central Europe, due to its drought tolerance, low input requirements and *diabrotica* resistance (Oyediran et al., 2004). Even though further enhancements in cold tolerance are necessary to improve its competitiveness (Schaffasz, Windpassinger, Friedt, et al., 2019; Windpassinger et al., 2017), it has already a satisfying

yield potential. Its potential growing regions in temperate Europe are expected to expand due to both climate change and breeding progress. Moreover, it can be cultivated with the existing machinery (Mathur et al., 2017), and being an annual crop facilitates its integration into existing crop rotations (in contrast to perennial bioenergy crops as *miscanthus*). Presently, dual-purpose type varieties with enhanced stress tolerance and energy density are being developed. Compared to tall and late-maturing biomass sorghum types, which have been the predominating sorghum variety types for biogas generation in Germany so far, these dual-purpose type varieties are shorter (approx. 180–250 cm plant height), of an earlier maturity and have larger and more compact panicles enabling a higher pollen shedding. Hence, the proportion of grains on total biomass and starch content can be as high as in maize, facilitating comparable energy densities (Windpassinger et al., 2015).

Pollen-collecting honey bees are frequently observed in sorghum inflorescences. It was therefore hypothesized that sorghum may be an important source of pollen. This study was conducted to verify the assumptions that (a) foragers of *A. mellifera* collect pollen of sorghum under the cool and humid climatic conditions of Central Europe, (b) sorghum pollen has a nutritive value for honeybees and (c) bee foraging activity has an impact on the seed yield of sorghum. The strategic goal of the current study is to amplify the diversity of insect-friendly bioenergy crops.

2 | MATERIAL AND METHODS

2.1 | Study sites

The experiments were conducted at the field stations of the Justus-Liebig-University Giessen in Gross-Gerau (GG, 49°55'N, 8°29'E), Germany, and in Rauschholzhausen (RHH, 50°46'N, 8°53'E), Germany. Land use within a foraging area around the bee hives of 3 km radius was estimated from the CORINE land cover data, which were analysed with the program QGIS (CLC, Deutschland, 25 ha square grid; accessed on 14.04.2021). RHH is dominated by arable land (60%), pastures (14%) and broad-leaved forests (19%). The GG area includes mainly forests (54%), arable land (26%) and settlement and industrial areas (17%, details see Table S2). At both sites there were sorghum nursery and variety testing plots (approx. 2–3 ha). In the surroundings of both field stations additional sorghum was grown on small acreages. The proportion of crops grown on the arable land within the foraging areas was obtained for the respective years from the corresponding agricultural funding agency (WiBank, Frankfurt, Germany, InVeKos data). RHH is characterized by predominant cropping of cereals (60% of the arable land) as wheat (*Triticum* spp. approx. 40%) and barley (*Hordeum* spp. approx. 18%). Moreover, silage maize (*Zea mays*, 14%)

and winter oilseed rape (*Brassica napus*, above 10%; for details see Table S2) are other important crops. In contrast, in the area of GG mainly special crops are cultivated: vegetables (around 10% of the arable land), asparagus (*Asparagus* spp. approx. 20%) and strawberries (*Fragaria* spp. 2%). Important field crops are barley (*Hordeum* spp., >10%), wheat (*Triticum* spp. >10%) and potatoes (*Solanum* spp., approx. 9%). Also regarding their climate, GG and RHH represent contrasting environments. While GG is characterized by warm and sunny summers, providing favourable growth conditions for sorghum, temperatures in RHH are 1.6°C cooler on average, implying usually suboptimal conditions for sorghum (see Table 1 and S1). The climatic conditions relevant for sorghum can be well described with the growing degree days approach (GDD 10/40, see McMaster et al., 2016 with a lower limit of 10°C and an upper limit of 40°C). The meteorological data required for this purpose were obtained from the two nearby weather stations Gross-Gerau and Kirchhain (Data available <https://llh.hessen.de/pflanze/wetter/>).

2.2 | Pollen foraging of free-flying colonies

From 13 August 2015 to 26 August 2015 and from 25 July 2017 to 1 September 2017 four colonies were placed at GG. In the nursery of RHH one colony was set up from 25 August 2016 to 23 September 2016. In 2017, there were four colonies from 30 August 2017 to 22 September 2017. Pollen traps were installed in the bottom boards of each colony. The pollen yield was collected regularly at least once a week and stored at -20°C until analysis.

2.3 | Determination of the botanical origin of the pollen collected by free-flying colonies

The air-dried mass of each sample was determined. From samples whose mass exceeded 30 g a subsample of exact 30 g was used for the analysis. The pollen pellets were sorted manually by colour. Each fraction was weighted. A randomly selected pollen pellet of each fraction was prepared for microscopic specification according to the standard

procedures (Louveaux et al., 1978). In brief, the pellet was suspended in 250 µl H₂O, soaked for 24 h and dropped in gelatine on the microscopic slide and determined microscopically by an experienced person. When the pollen pellet consisted of pollen of different species, approximately 200 pollen grains were determined and the percentage of each species was estimated. Using these counts the total mass of each pollen species per sample was calculated. Based on the weights of the colour fractions the share in per cent (w/w) of the most prominent species was calculated.

2.4 | Flight tent experiments

2.4.1 | Experiments to evaluate the nutritional value of sorghum pollen for honey bees

Flight tent experiments to evaluate the value of sorghum and other crops on honey bees were conducted at the agricultural experimental station of Justus-Liebig-University Giessen in Gross-Gerau, Germany, in 2017 and 2018. A total of 16 randomized plots (four per plant species) of 100 m² each were sown with *Phacelia tanacetifolia* (PH), maize (ZM), a *S. bicolor* F1 hybrid (SBH) and a *S. bicolor* inbred line (SBL). The cultivars were for PH 'Amerigo' (Petersen Saatzucht Lundsgaard GmbH, Grundhof, Germany), for ZM 'LG 30222' (Limagrain Edemissen, Germany) and for SBH and SBL dual-purpose type experimental material, originating from a joint breeding program of Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Holtsee, Germany, Deutsche Saatveredelung AG, Lippstadt, Germany and Justus-Liebig-University Giessen. These different plant species were sown at different dates (first sowing sorghum: mid-May; first sowing maize: early June; first sowing Phacelia: mid-June) to facilitate overlap of flowering periods. Furthermore, each plot was split into two subplots, with the first subplot sown 14 days earlier than the second one, in order to extend the duration of flowering. While the sowing dates for sorghum were in the recommended time period for commercial agriculture in that area, those for maize were somewhat delayed, corresponding rather to typical sowing dates for maize as a second or catch crop, which is still a common practice in bioenergy cropping systems. Phacelia is usually planted as a summer catch crop with high flexibility in sowing time. To

TABLE 1 Climatic characteristics of the experimental sites GG and RHH

Site	Coordinates (UTM zone 32 N)	Elevation m asl.	Temperature ¹	Precipitation ¹	CWB ²	GDD ³
GG	464294 5532095	92 m	10.6°C	620 mm/a	-10 mm/a	1283.4
RHH	490707 5623389	248 m	9°C	700 mm/a	110 mm/a	1131.7

¹ Deutsche Wetter Dienst (DWD) climate map, 1 × 1 km long-term medium 1981–2010.

² CWB: Mean Annual Climatic Water Balance.

³ Mean of the years 2016, 2017 and 2019; calculated for the period from 15 May to the 15 October.

avoid drought stress affecting pollen or nectar production, all plots were irrigated several times. By this measure, also possible added stress on maize due to the later sowing was excluded.

Tunnel tents were set up with aluminum tubes (outer diameter 34 mm, wall strength 2 mm) and clamp fittings of iron (Globosign BV Rotterdam). The constructions were covered with radish gaze (Hartmann-Brockhaus, Pfaffenhofen-Wagenhofen, Germany) with a mesh size of 1.35 mm. The tents were 4 m wide (W), 3 m high (H) and 25 m long (L), and were set up before the crops reached BBCH 60 (start of anthesis). The hives were set up in the middle of the tents. Entrances were orientated to the south. In 2018 a zero check variant (C) was included. For that purpose, four additional tents of 4 m (L) * 4 m (W) * 3 m (H) were set up on plots which were cleared of vegetation so that the bees could not forage on any pollen source at all.

Colony management

From healthy colonies bees of all ages were brushed off the combs and stored in shook swarm boxes. Small polystyrol hive boxes (internal dimensions 23 cm * 23 cm * 16 cm; Mini Plus®, Holtermann, Germany) were provided with six frames (16 cm * 22 cm) which had been equipped with foundations. In each box 0.500 kg bees were weighed in and fitted with a young queen less than 3 months old. All queens were sister queens originating from the Carnica breeding program of the Bee Institute and were mated on the isolated Carnica mating yard Gehlberg, Germany. Each experimental colony was fed 0.5 L of sugar syrup (ApiInvert®, Südzucker, Germany) at the beginning of the trials. Later, additional rations of sugar syrup followed as needed.

Determination of the parameters

The colonies were assessed 10 times in 2017 and 9 times in 2018 at 7 days intervals. The adult bees were carefully moistened with a sprayer, brushed off the frames in a funnel in such a way that they slipped in a box on a balance (Kern, Germany). The weight of the adult bees was recorded. Each side of all frames was photographed with the help of a device similar to the description of (Jeker et al., 2012). Images were loaded in the software tool ImageJ for counting the number of cells containing eggs, larvae, capped brood or bee bread (Delaplane et al., 2013). Dead bees were collected every 7 days from 1 m² large sheets in front of the entrances. The plant developmental stages were scored using the BBCH scale (Meier, 1997). Bee visits per flower were counted once a week ante meridiem by a surveyor walking through each plot along a pre-defined walking path for 5 minutes. Assessment of BBCH and flower visitation was conducted in the earlier sown subplot initially, and shifted to the second, later sown subplot when full bloom was reached there. Concurrently with the counting of the bee visits per flower, a second surveyor observed the entrances for 5 minutes and counted the number of pollen foragers returning home as well as the returning bees without

pollen pellets and the number of outgoing bees. Pollen foragers were defined as bees carrying clearly observable pollen pellets in their baskets. The assessments started at the end of July and ended at the beginning of October.

2.4.2 | Experiments to analyse effects of bees on the seed yield of sorghum

Plots grown with sorghum (4 m × 4 m each) were integrated in a maize field to minimize wind pollination between different plots and variants. A honey bee nuc per cage was set up in 50% of the plots shortly before the onset of sorghum flowering till the end of bloom. The nucs occupied five Zander frames and were fed with sugar candy ad libitum. They raised brood and consisted of a mixture of brood caring and foraging bees. During the flowering period, the number of flower visits was regularly counted five times for 1 minute in each flight tent with a nuc. At the same time, the number of returning bees without pollen and those with pollen, as well as the number of departing bees, was determined at the hive entrances for 5 minutes. All sorghum plots were individually covered with a 3 m high flight cage before anthesis, regardless if they were equipped with nucs (bees) or not.

In 2017, a sorghum dual-purpose type hybrid (SBH, see above) was grown in one sowing time on eight plots at GG and RHH each. Hence, the experiment consisted of the factors site (GG and RHH) and bees (four plots at each site with and four plots at each site without bees).

In 2019 the experiment was conducted only at RHH. SBH (from here on for a better distinction referred to as type A) was grown on 16 plots and another dual-purpose hybrid (SBH type B) was cultivated on further 16 plots. Half of the plots of each hybrid were sown 14 days earlier than the second half. Thus, the factors for the 2019 experiment were sorghum hybrid (2), sowing time (2) and bees (with or without). Each variant was replicated four times.

During both years, in each plot 20 sorghum main shoots were marked randomly before bloom. In October (end of vegetation period) the marked plants were hand harvested, dried and used for the determination of seed yield per panicle and seed number per panicle.

2.5 | Statistical analysis

The serial data were evaluated by a classical summary measures approach. The data had a peaked shape. The time intervals between the measure points were equal. Therefore, the repeated observations of each colony were aggregated to arithmetic means (Matthews et al., 1990). Means of number of eggs, larvae, sealed brood and mass of the bees were calculated from 10 measurements per plot in 2017 and from

9 observations in 2018. The number of observations for the parameter dead bees, visits of flowers and flight activity differed slightly as specified in the chapter results. Data were checked for the assumption of normality with the Shapiro–Wilk test and for homogeneity of variances with Levene's test. If a deviation from normality and/or from the assumption of the homogeneity of variance was detected, a non-parametric analysis of the variance with the Kruskal–Wallis test (KWT) was performed. The procedure according to Campbell und Skillings as preset in the statistical software package SPSS was used for multiple comparisons. For the experiments on the impact of bees on seed yield the data of the 20 panicles were averaged per plot and tested for significant effects of the factors site (resp. for 2019 sowing time), sorghum hybrid and bees on the dependent variables seed yield and seed number with the procedure glm multivariate. Numbers of flower visitation and of returning foragers with pollen pellets were counted three times in 2017, aggregated and subjected to ANOVA. In 2019 these dependent variables were counted nine times and analysed with a linear mixed model with date of counting, sowing time and sorghum hybrids as fixed factors and plot identity as random factor. All statistical calculations were performed in SPSS version 19.

3 | RESULTS

3.1 | Foraging behaviour of free-flying colonies

All colonies collected pollen from sorghum, on both sites and in each year (Table 2). The overall prevalence summarized over

both sites, all sampling dates and all colonies was 56.7%. From the total of 136 pollen samples collected on both sites during the whole experiment 77 samples contained pollen of sorghum. In 2015, 20 of 40 samples in GG were positive for sorghum. The respective rates were 66.7% in 2016 and 68.8% in 2017 in RHH and 54.8% in 2017 in GG. The maximum mass share of *Sorghum* pollen collected by single colonies per week amounted to 89% in 2015, in GG, cw 35, with 30.736 g Sorghum pollen out of a total of 34.424 g pollen. In RHH in 2016 the maximum mass rate of *Sorghum* pollen was 59.3% [w/w; 16.92 g *Sorghum* pollen/27.466 g total pollen] and 88.2% [w/w; 8.395 g *Sorghum* pollen/9.518 g total pollen] (see Table 2).

Other important genus which were predominantly used by the foragers were *Liliaceae*, *Asteraceae*, *Brassicaceae* and *Fabaceae* and in particular in RHH also *Vitaceae* (see Figure 1). In GG, *Sorghum* pollen was collected during 3 weeks in 2015 [calendar week (cw) 33–35] and 6 weeks in 2017 (cw 30–35). The foraging time of *Sorghum* pollen in RHH was 4 weeks for both years (2016: cw 34–37; 2017: cw 35–38).

3.2 | Flight tent experiments to evaluate the nutritional value of sorghum for honey bees

3.2.1 | Development of the crops

The crops started flowering simultaneously in the first week after initiation of the experiment (see Figure 2). The main blossom of the *Poaceae* (maize and sorghum) occurred in the second and third week and terminated 4 weeks after initiation of the experiment (wai). Phacelia bloomed continuously during the whole test period whereas the flowering periods

TABLE 2 Characteristics of the Sorghum foraging behaviour of the freely foraging colonies in GG and RHH, derived from the collection of pollen traps

Year	2015	2017	2016	2017
Site	GG	GG	RHH	RHH
N colonies	4	4	1	4
N samples	40	62	18	16
Maximum S. b. pollen [% w/w] per colony and per sampling date	89	40.8	59.3	88.2
Mean [%, w/w] S. b. pollen over all colonies and dates	50.6	9	15.8	15.3
Duration of S. b. positive samples cw calendar week	33–35	30–35	34–37	35–38
First. most dominant species [mean %w/w]	<i>Poaceae</i> 50.6	<i>Liliaceae</i> 54.2	<i>Asteraceae</i> 30.9	<i>Vitaceae</i> 81.5
Second. most dominant species [mean %w/w]	<i>Liliaceae</i> 15.3	<i>Brassicaceae</i> 14.6	<i>Brassicaceae</i> 30.6	<i>Poaceae</i> 15.3
Third. most dominant species [mean %w/w]	<i>Asteraceae</i> 10.9	<i>Fabaceae</i> 14.6	<i>Poaceae</i> 15.8	<i>Fabaceae</i> 1.2

S. b.: *Sorghum bicolor*; Mean values were calculated by summing the pollen weights of each plant taxa over the entire period of pollen collection from all colonies per site, and then relating this sum to the total weight of pollen collected.

of the *Poacea* plots were restricted to approx. 4 weeks. Each plot consisted of two relay plots, which were sown with an interval of 14 days to extend the blooming period in the flight tents.

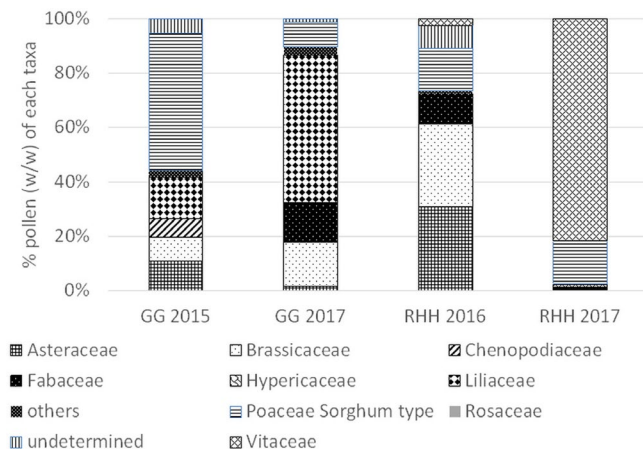


FIGURE 1 Mass percentage of pollen [w/w] of each taxa for the sites Gross-Gerau (GG) and Rauschholzhausen, (RHH)

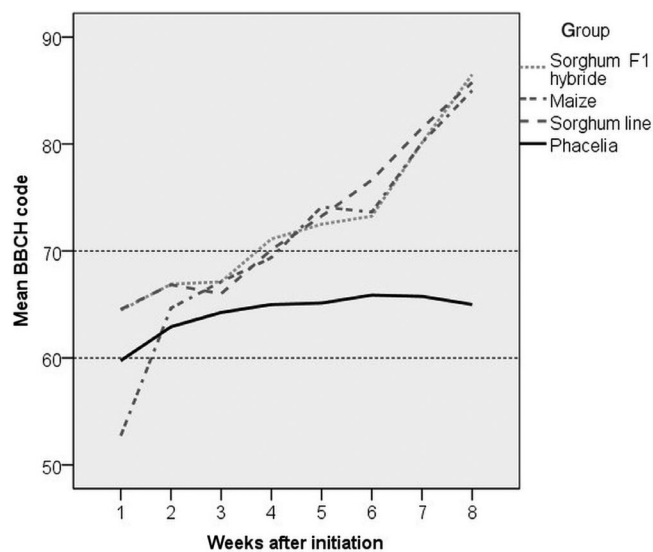


FIGURE 2 development of the crops in terms of the BBCH scale. Plants were flowering in the range between BBCH 60 and 70 marked by the dashed lines. wai = 1: calendar week (cw) = 30 in 2017 and cw = 31 in 2018

	N	# flower visits	# pollen foragers	# outgoing bees	# returners - pollen
<i>Z mays</i>	68	14.6a	4.46a	5.4a	3.1a
<i>S. bicolor</i> hybrid	64	45.5ab	9.48b	9.8ab	8.5b
<i>S. bicolor</i> line	68	41.7ab	10.09b	14.1bc	9.1b
<i>P. tanacetifolia</i>	79	124.3b	3.52a	22.3c	22.7c

N: number of observations of each parameter. 'returners - pollen': returning bees without pollen pellets, groups marked with the same letter are statistically not distinguishable ($p = 0.05$, Campbell and Skillings; # returning pollen foragers: Scheffe test).

3.3 | Flower visitation and pollen foraging in the flight tents

Pollen-collecting foragers were observed in the inflorescences of all four variants. PH blossoms were visited most frequently, followed by SBH and SBL. The number of flower visitations were lowest for ZM. The differences in the flower visitation rates were significant between PH and ZM (see Table 3, $p = 0.05$, Campbell and Skillings). The visitation of the *Poacea* flowers was strongly dependent on time: The highest rate was observed in wai 3 with a mean of 104 flower visits for SBH and 109 visitations for SBL. A significant difference was found for the number of returning pollen foragers. SBH and SBL had twice as much returners than PH and ZM (see Table 3). PH colonies had a significantly elevated flight activity whereas the ZM colonies were the less active ones (Table 3).

3.4 | Performance of the colonies

The factor year had significant effects on the parameters 'number of unsealed brood cells' (KWT, $p = 0.01$), 'number of sealed brood cells' (KWT, $p = 0.008$), 'number of cells with bee bread' (KWT, $p = 0.001$) and 'mass of adult bees' (KWT, $p < 0.001$). The three characteristics were in 2017 superior to 2018. The number of eggs did not show a significant difference between both years (KWT, $p = 0.143$). Significant effects of the factor 'foraging crop' occurred on all parameters ($p < 0.008$) except the parameter 'mass of adult bees' ($p = 0.478$). The queens of all variants produced eggs. The number of eggs did not differ significantly between the three *Poacea* variants ZM, SBH and SBL and the C colonies (Campbell and Skillings, $p = 0.05$, see Table 4), even though the queens of the control colonies laid the lowest amount of eggs. In contrast, queens of PH colonies were significantly more productive (Campbell and Skillings, $p = 0.05$) and had the highest number of eggs. With respect to the number of unsealed brood cells and the number of sealed brood cells, C was significantly less productive than ZM, SBH and SBL. However, PH produced significantly more unsealed and sealed brood than all other variants (see Figure 3 and Table 4, Campbell and Skillings, $p = 0.05$). Also for the number of

TABLE 3 Mean number of flower visits per 5 minutes and of the returning foragers with pollen pellets at the flight entrance during 5 minutes (pooled data from the flight tent experiments in Gross-Gerau 2017 and 2018)

TABLE 4 Performance of the test colonies assessed by the productivity parameters egg laying, brood rearing activity, mass of the adult bees and amount of stored pollen (pooled data from the experiments in Gross-Gerau 2017 and 2018)

	N	# eggs	# unsealed brood cells	# sealed brood cells	# bee bread cells	mass of adult bees (g)
<i>Z. mays</i>	76	387.1a	131.4a	201.1a	45.7a	200.9a
<i>S. bicolor</i> hybrid	76	382.1a	123.8a	245.7a	76.0ab	248.5a
<i>S. bicolor</i> line	76	391.5a	147.5a	272.0a	76.1ab	233.1a
<i>P. tanacetifolia</i>	76	613.4b	378.2b	761.0b	123.7b	261.6a
Control	36	317.1a	24.3c	16.9c	0.0c	159.5a
<i>p</i> (KWT)		0.008	<0.001	<0.001	0.001	0.478

N: number of observations of each parameter. Groups marked with the same letter are statistically not distinguishable.

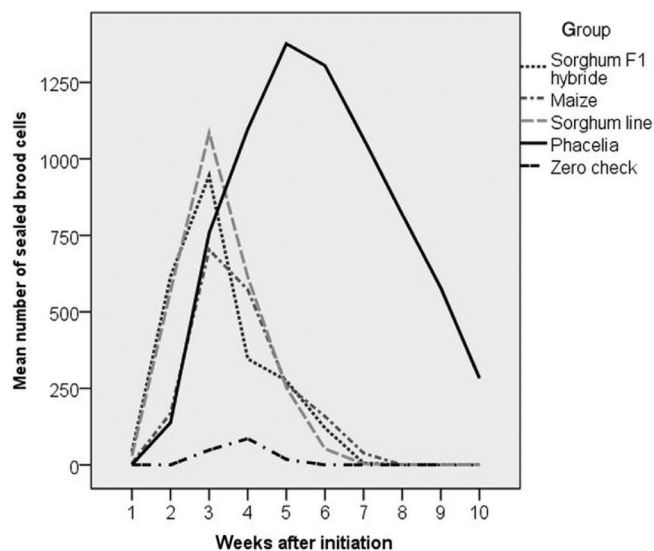


FIGURE 3 Number of capped brood cells in dependence of time and pollen sources. The curves of sorghum F1 hybrid, sorghum inbred line and maize are statistically not distinguishable ($p = 0.05$, Campbell and Skillings test). *P. tanacetifolia* and the zero checks are both statistically discriminable from all other variants. $N = 8$; $N_{\text{zero checks}} = 4$ replicates. Curves display means of both years

cells with bee bread C showed the lowest number, followed by ZM, SH, SL and PH. The *Poaceae* could not be discriminated statistically from each other. PH had the highest amount of bee bread cells, but the differences to SBH and SBL were not significant (see Table 4). Regarding the total bee mass of each colony, C colonies were the weakest, while PH colonies had the largest mass of bees. Colonies of ZM, SH and SL showed an intermediate mass, but none of these differences were statistically significant ($p = 0.478$, see Table 4).

3.5 | Dead bees

The mean number of dead bees was normally distributed for all variants except for the group *S. bicolor* hybrid (SBH) in 2017 as assessed by the Shapiro–Wilk test ($\alpha = 0.05$).

TABLE 5 Number of dead bees in the different flight tent variants, pooled data from 2017 and 2018

	N	# dead bees
<i>Z. mays</i>	53	17.96a
<i>S. bicolor</i> hybrid	52	26.31a
<i>S. bicolor</i> line	52	22.98a
<i>P. tanacetifolia</i>	61	45.64a
Control	31	28.67a

N: number of observations of each parameter. Groups marked with the same letter are statistically not distinguishable ($p = 0.775$, Kruskal–Wallis test).

Homogeneity of variances was asserted using Levene's test which showed that equal variances could not be assumed ($p = 0.037$). While the factor year was significant ($p < 0.001$, Mann–Whitney U test; mean # dead bees in 2017: 8.5; mean in 2018: 44.1) no significant differences among the variants (plant species) were found ($p = 0.775$ KWT, see Table 5).

3.5.1 | Impact of bees on sorghum seed yield

Foragers collected pollen from the sorghum inflorescences in this set of experiments as well. In 2017 significantly more returning foragers carrying pollen pellets were counted under favourable growth conditions for sorghum at GG (27.33 against 3.83 returners at RHH, $p < 0.001$, ANOVA). Also, the number of flower visitation with 117 flower contacts was higher at GG, but statistically not discriminable from RHH (54 flower contacts, $p = 0.076$, ANOVA).

Both factors of the 2017 experiments, 'site' and 'bee', showed significant effects on seed yield and seed number ($p < 0.001$ and $p = 0.01$, respectively, Pillai–Spur). Interaction between both factors was not significant (0.056). The activity of the bees increased seed yield and seed number by a factor of 2.5 at RHH. Figures are given in Table 6.

In 2019 the experiment was repeated at RHH with two different sorghum hybrids and two dates of sowing. The importance of

TABLE 6 Impact of bee foraging activity on the seed yield formation for the experiments of 2017 (a) and of 2019 (b); $N = 4$ replicates, three repeated measurements per plot in 2017 and nine repeated measurements in 2019

(a) Variety	Site	Bees	Seed yield per panicle (g)	Seed number per panicle
A	GG	Without	76.61c	2587c
A	GG	With	82.67c	2704c
A	RHH	Without	6.74a	712a
A	RHH	With	17.72b	1829b
		∅ without	41.68	967
		∅ with	50.19	1243
(b) Variety	Sowing date	Bees	Seed yield per panicle	Seed number per panicle
A	Early	Without	41.22bcd	1911abc
A	Early	With	36.80bcd	1835ab
A	Late	Without	16.62a	1453a
A	Late	With	18.27a	1559a
B	Early	Without	50.34d	2823d
B	Early	With	45.69cd	2511cd
B	Late	Without	29.42ab	2391bcd
B	Late	With	31.04abc	2421bcd
		∅ without	34.40	2145
		∅ with	32.95	2082

Weight in [g]; average value comparisons within a column, variants marked with different letters differ significantly from each other (Tukey Test, $p = 0.05$, $N = 4$ plots per variant, seed yield of 2017 $\log(10)$ transformed), A and B = experimental Sorghum F1-hybrides; ∅ = overall means.

bees as a significant yield-generating factor was not reproduced ($p = 0.853$, Pillai-Spur), whereas variety and date of sowing were significant factors (for both $p < 0.001$ Pillai-Spur). The earlier date of sowing was highly significant for a higher seed number ($p = 0.004$) and higher seed yield ($p < 0.001$). Inflorescences of SBH type B were significantly more attractive to bees than flowers of SBH type A (122.6 flower contacts vs. 60.2 contacts; $p = 0.002$, glm mixed). The number of returning foragers with pollen pellets of the higher yielding SBH type B doubled the number of the lower yielding variety A (12.85 vs. 6.1, $p = 0.001$, glm mixed). Earlier sowing was also a significant factor for more flower visits and more pollen returners ($p = 0.012$ and $p < 0.001$). Altogether, it was found for both years that bees did not reduce any seed yield trait in any case (see Table 6a,b).

Both years differed with regard to the weather. 2017 was the more humid and cooler year, especially at the site RHH. In 2017 the differences of GDD between RHH and GG amounted to 195 against 135 for 2019 (see Table S1).

4 | DISCUSSION

4.1 | Sorghum is a pollen crop for bees

Sorghum is an anemophilous plant lacking any insect attracting stimuli as colour or odour. Nevertheless, bees with large

pollen pellets are frequently observed in the inflorescences of sorghum. The crop has been described as a pollen source for honey bees in Western regions of France (Odoux et al., 2012), the United States, Africa and Asia (Bhusari et al., 2005; O'Neal and Waller, 1984; Saunders, 2018; Schmidt & Bothma, 2005; USDA, 2017). The experimental sites of the present study were located in Germany, which is characterized by a rather cool, temperate climate. Sorghum is sensitive to cool temperatures, which can hamper the shedding of pollen (Osuna-Ortega et al., 2003; Wood et al., 2006). While the summer of 2018 was unusually hot in Central Europe, providing optimal growth conditions for sorghum, during the summers of 2016 and 2017 several cool periods affected the pollen shedding potential of sorghum especially at RHH. Nevertheless, it was found that the colonies collected pollen of sorghum even under these suboptimal conditions of Central Europe. At the GG site, asparagus was grown on 85 ha in 2015 and 134 ha in 2017 (see Table S2). Asparagus is an attractive nectar and pollen plant for bees. Its flowering period overlaps with the flowering window of *S. bicolor*. Significant amounts of asparagus pollen were collected by bees in GG, but interestingly, bees also collected sorghum pollen near the asparagus fields. From a methodological point of view, the proportion of pollen plants could be biased. Pollen was sorted by colour. A single pellet of each colour fraction was used for microscopic determination of botanical origin. Pollen from

different species may have the same colour, so that this sampling technique has limited representativeness. However, this shortcoming is outweighed by the considerable number of 136 samples (see Table 2), so that it can be concluded that sorghum is an attractive pollen plant for bees.

4.2 | Sorghum pollen is an adequate bee forage

Having shown that bee colonies collect pollen from sorghum, its nutritive value had to be characterized. It is not clear how the pollen foraging behaviour of honey bees is linked to the pollen quality (Keller et al., 2005). There is a controversial debate whether bees can perceive the nutritional requirements of the colony, communicate it to the foragers and whether they can depict pollen quality in the field and adapt their foraging behaviour accordingly (Goulson et al., 2015). In choice test experiments foragers preferred high-quality pollen (Cook et al., 2003). However, there is evidence for divergent hypotheses as well. While some studies showed that foragers opt for a highly diverse pollen diet to compensate for nutrient deficiencies (Danner et al., 2017; Nürnberger et al., 2019; Requier et al., 2015), other studies concluded that they are rather attracted by the easiness and the efficiency of the collection process (Pernal & Currie, 2001), or that they use other cues as colour or an experienced odour (Pernal & Currie, 2001). Liolios et al. (2015) underlined that bees collect what they get. Also the results of the present study suggest that foragers target pollen plants which are available in the surroundings of their colonies. They do not visit high-quality plants selectively. As the rate of flower visitation does not reflect the quality of pollen, its nutritive value has to be verified experimentally. There are basically two approaches to evaluate the dietetic quality of pollen, the evaluation of the chemical contents and the capability of specific diets to sustain the performance of bee colonies (Keller et al., 2005). Biochemical properties of the pollen were described, for example, by Fuenmayor et al. (2014). Other authors performed both, the chemical analysis and the investigation of the biological activity of specific pollen diets (Herbert et al., 1978). Life expectancy, the capability of brood rearing or growth and size of the colony might be informative biological parameters (Haydak, 1970; Loper & Berdel, 1980; Saffari et al., 2010; Schmidt et al., 1987). Further approaches quantified the size of organs or other physiological traits of individual bees as indicator for the nutritive quality of a specific pollen diet (Di Pasquale et al., 2013; Frias et al., 2016; Omar et al., 2017). Here we adopted a biological research strategy with small but fully functional experimental colonies. The measurement of direct performance parameters on the colony level was judged to be the most relevant approach, as it is not fully understood how performance traits of the colony are linked to specific contents of the pollen or to traits of individual bees (Keller et al., 2005). Therefore,

small colonies were placed in the middle of single crop plots, which were covered by flight tents. These colonies had no possibility to use other crops than the single pollen source in the tent. An inbred line of *S. bicolor*, an F₁ hybrid of *S. bicolor*, *P. tanacetifolia*, and *Zea mays* were grown on the plots. Tents of the zero check were free of any vegetation, so that bees had no access to any pollen at all. The brood rearing capability and the growth of the colonies were used as indicator for the pollen quality. These experiments showed that a monofloral diet of sorghum pollen was apt to raise bee brood. Bees of the sorghum variants generated more brood than the colonies in the maize tents, but the differences were not significant. Colonies foraging on *P. tanacetifolia* were the most productive ones. The control generated nearly no brood, proving that brood rearing could not be explained by the use of the body reserves of the nurse bees. Brood rearing did depend on the support of external pollen. Differences between the *P. tanacetifolia* colonies and the *Poacea* colonies might be caused by differences in the quality of the pollen. However, pollen of sorghum has been reported to contain between 13% (Standifer, 1967) and approx. 26% raw protein (RP) (Shen, 1992) and thus corresponds to the RP content of the high grade rapeseed pollen. Other ingredients, such as vitamins, fatty acids and essential amino acids might be deficient though. Also, the digestibility of *Poacea* pollen could be inferior to that of known high grade pollen as, for example, oilseed rape. Pollen of some plants contains toxic or deterrent compounds which hamper the development of bee larvae (Rivest & Forrest, 2020). However, suspected toxicity of sorghum pollen should not be considered as a cause for the lower brood rearing performance. Sorghum pollen is not listed as a toxic pollen. Even if there were harmful substances, the early stages of bee brood would be protected as nurse bees filter out toxins from the food stream when supporting larvae with nursing jelly (Lucchetti et al., 2018). Adverse effects could have been seen on the later, pollen-consuming larval stages, but at least no visually apparent signs of direct larval damage were observed by the experimenters. Therefore, neither qualitative deficiencies nor toxic compounds, but rather quantitative effects can be assumed as the cause for the different brood productivity. *P. tanacetifolia* flowered during the whole period of the experiment. Foragers were observed in *P. tanacetifolia* flowers continuously the whole day long over the entire duration of the study. In contrast, *Poacea* flowered only from week 1 to week 4, and foragers visited their flowers exclusively in the morning time. This behaviour coincides with the pollen shedding pattern of maize and sorghum, which release fresh pollen mainly in the morning. It should be taken into account that for all crops in the tent experiments, the flowering period was purposefully extended by two different sowing times. If a single crop variety is planted in one sowing time on a field, as it is common practice in agriculture, the flowering period is much shorter. For dual-purpose or grain sorghum in monoculture, the duration of flowering can be estimated at 7–14 days, depending

on the weather conditions and amount of tillers. A single sorghum panicle usually flowers for 3–4 days, but the duration of bloom in a field is prolonged by tillering and the fact that even in a monoculture of a genetically uniform variety not all plants start flowering simultaneously. For a maize field, a shorter period of pollen shedding than for sorghum can be assumed, since modern maize varieties do not form tillers.

The confinement of colonies in tents is a standard technique for testing chemical substances (Medrzycki et al., 2013). As flight tunnels restrict the foraging radius of bee colonies to specific diets, tents are used for studying foraging behaviour of bees (Hendriksma & Shafir, 2016; Hendriksma et al., 2019). Bees cannot use other flowers than the target crop. The limitation of the tunnel tent approach is the restriction in space. The amount of pollen might be scarce. Alternatively, colonies could be transferred to regions which are predominately grown with the targeted crops. As there are so far no regions grown mainly with sorghum in Central Europe, that approach could not be adopted. Another experimental approach is the addition of pollen food in form of pollen supplements (Branchiccela et al., 2019). However, feeding pollen supplements, for example, prepared from manually collected pollen do not reflect the real situation, and sensitive contents of the pollen might be subject to deterioration. Moreover, practically it is hardly feasible. Maize inflorescences can be shaken to obtain sufficient amounts of pollen (Höcherl et al., 2012), but as our own experiments showed, manual collection of *Sorghum* pollen yielded low amounts. Therefore, this study is based on tent trials despite their limitation in space.

Colonies of the zero check did not produce sealed brood, but interestingly, the colony size did not differ significantly from the colony sizes of the brood rearing colonies of the variants with plants. The bees of the zero check had a higher life expectancy than the pollen collecting and brood rearing units: Caring for brood is a life time consuming business. Nurse bees are short lived (Amdam & Omholt, 2002; Omholt, 1988). The authors assume that the bees of the zero check were physiologically non-productive, but long-lived winter bees. (Mattila & Otis, 2007). The more productive *P. tanacetifolia* colonies showed more dead bees, more brood cells and had more bees. These PH colonies were much more active, which can also be seen on the flight data, and, in consequence, had the highest turnover. Activity is costly (Neukirch, 1982) and reduces the life expectancy of individual bees.

4.3 | Impact of foraging activity on sorghum seed yield

Prior to our experiments, it was unclear whether the foraging activity of honey bees has an impact on the seed yield of sorghum. It might be hypothesized that seed setting is reduced by bees. There are examples of impaired fruit setting by pollen consumption, described as ‘consumptive emasculation’ (Hargreaves et al., 2009), by damaging the reproductive

structures (Sáez et al., 2014) or in a broader sense by disrupting established mutual pollinator flower networks (Valido et al., 2019). The data of the present study do not support the idea that pollen foraging bees can negatively affect seed yield of sorghum. None of the variants without bees had a higher seed yield than the corresponding reference groups with bees.

In contrast, our results suggest that bees can improve sorghum seed yield under stressful conditions. Sorghum is an anemophilous plant. Fertilization depends on abundant pollen shed. However, pollen shedding and in consequence seed set of sorghum have been shown to be reduced by temperature stress (e.g. Osuna-Ortega et al., 2003; Schaffasz, Windpassinger, Snowdon, et al., 2019). Induction of pollen sterility is a quantitative process depending on low temperatures (Schaffasz, Windpassinger, Snowdon, et al., 2019). Therefore, GDD values were considered to be an appropriate parameter to describe a possible cause of low seed set in RHH in 2017. Under stress conditions, cross pollination has a much higher importance than under no-stress conditions (Osuna-Ortega et al., 2003). This finding is consistent with the results of the present study. In the year 2017, cool and cloudy weather during critical growth stages implied stress for sorghum, especially at the site RHH, where bees enhanced sorghum seed yield 2.5-fold. The bees could presumably at least partly compensate for the lack of fertile pollen by buzzing around the flowers. As a result, the scarce pollen was probably better transferred to the stigma, resulting in an enhanced fertilization and seed set. However, in the year 2019 no temperature stress occurred, so that the results of 2017 could not be reproduced. Nevertheless, bees (*Apoidea*) are likely to have the potential to improve the seed yield as similar observations are reported from maize (Fohouo et al., 2002).

4.4 | Sorghum upvalues bioenergy crop rotations for insects

Many pollen eating insects collect pollen of anemophilous plants and especially of *Poaceae* (Bertrand et al., 2019; Saunders, 2018). An article reviewing mainly Swiss studies claims that maize is among the five most frequently collected pollen resources (Keller et al., 2005). *Poaceae* produce large amounts of pollen (Prieto-Baena et al., 2003). *Hymenoptera* uses *Poaceae* as pollen source (D’Apolito et al., 2010; Pangestika et al., 2017; Rivernider et al., 2017; Simeão et al., 2015; Tchuenguem Fohouo et al., 2013). Hence, the authors of the present study assume that integrating sorghum in the biogas crop rotation system can enhance the nutrition of bee colonies. Sorghum can improve the agro-ecological value of bioenergy cropping systems as it satisfies the nutritional needs of pollen eating insects. Compared to maize, the presently predominant bioenergy crop in Central Europe, sorghum showed a better impact on several of the scored parameters on trend, but significant differences could hardly be

found. Although, the later flowering of sorghum (depending on region and variety, from end of July to early September), coinciding with a critical period for honeybees when major pollen sources are missing, and its longer duration of flowering due to tillering represent agro-ecological assets. It should be emphasized that the results and inferences of the present study only apply for grain and dual-purpose sorghum types with a high pollen shedding potential, and not for photosensitive or self-sterile biomass types. Extending the duration of flowering in sorghum would further enhance its agro-ecological value. In practice, this could be achieved by mixed planting of varieties with consecutive flowering dates, which would probably also have a positive impact on yield stability. Intercropping approaches, such as the combination of sorghum with flowering undersown species, could add even more ecological value to bioenergy crop rotations, but are more challenging from an agronomic point of view.

5 | CONCLUSIONS

Sorghum represents a promising novel bioenergy crop for Central Europe. Also there, foragers collect readily its pollen. Sorghum pollen is not harmful or toxic to bees. It supports brood rearing. The productivity of colonies fed with sorghum pollen is slightly but statistically not significantly superior to colonies feeding on maize. Integrating sorghum in classical bioenergy crop rotations might be promising as it broadens the diversity of the offered pollen specimen and extends the time slot in late summer with pollen plants. Pollen-collecting bees did not reduce sorghum seed yield. Under adverse environmental conditions for sorghum, bees helped to stabilize seed yield.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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