

1 Floral resources provided by the new energy crop, *Silphium perfoliatum* L. (Asteraceae)

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15 Abstract

16 Flower-visiting insects are important crop pollinators, but their populations in agricultural
17 landscapes are declining. One reason is the decreasing quantity and quality of floral resources.
18 Deterioration of the situation caused by an increasing production of energy crops like maize, a
19 pollen-only resource, may be mitigated by alternative crops such as *Silphium perfoliatum* L.,
20 which produces pollen and nectar over a long-lasting flowering period (July - September).

21 The aim of this study was to assess the floral resources of *S. perfoliatum*.

22 We calculated pollen grains and nectar sugar mass per inflorescence as well as inflorescences
23 per plant using experimental plots and commercial fields in Germany. In addition, we
24 analyzed free and protein-bound amino acids in pollen as well as sugars and free amino acids

25 in nectar.

26 The amount of pollen and nectar sugar per inflorescence decreased with increasing stem

27 branching. The production of pollen and nectar sugar, however, was greatest in the second

28 half of August due to the high number of inflorescences per plant at that stage.

29 About half of the farmers harvested *S. perfoliatum* before the end of flowering to gain higher

30 methane yields. With an early harvest around the beginning of September about one sixth of

31 total pollen and one fifth of total nectar sugar production were lost to pollinators.

32 Pollen and nectar showed high amounts of certain essential amino acids, particularly histidine,

33 but total amino acids were low in concentration. Hence, *S. perfoliatum* should be

34 complemented with different bee fodder plants to ensure a well-balanced diet.

35

36 Keywords: energy crop production, perennial crop, cup plant, pollen, nectar, bee pasture,

37 pollinator protection

38

39 **1. Introduction**

40 A major proportion of plant species worldwide are animal pollinated (Ollerton, Winfree, &

41 Tarrant, 2011). Flower-visiting insects, in particular, are important pollinators of many crops

42 (Klein et al., 2007). It was estimated that 9.5% of the economic value of global crop

43 production can be attributed to insect pollination (Gallai, Salles, Settele, & Vaissière, 2009).

44 The honeybee *Apis mellifera* L. is one of the most important pollinators of crops (see Free,

45 1993), although wild bees or other insect groups like flies are also relevant to secure crop

46 yields (Klein et al., 2007; Rader et al., 2016; Ssymank, Kearns, Pape, & Thompson, 2008).

47 Pollen and nectar are usually the staple food of flower-visiting insects, and bees also gather

48 pollen and nectar to feed their larvae (Amiet & Krebs, 2012). Pollen is their main source of

49 proteins and amino acids, most important for brood rearing (Brodschneider & Crailsheim,
 50 2010). According to de Groot (1953), ten amino acids are essential for the growth of young
 51 honeybees. Nectar is the main source of sugars, needed for maintaining energy-consuming
 52 flying (Brodschneider & Crailsheim, 2010). Sucrose, glucose and fructose are the most
 53 abundant sugars in nectar with varying proportions (Percival, 1961). The second most
 54 abundant solutes of nectar are amino acids (Pacini & Nicolson, 2007). Consequently, both
 55 quantity and quality of nutrients in pollen and nectar influence pollinator's health and
 56 reproduction (Alaux, Ducloz, Crauser, & Le Conte, 2010; Tasei & Aupinel, 2008).
 57 Considering the importance of flower-visiting insects for crop production, floral resources
 58 have to be present in agricultural landscapes in sufficient and well-balanced quantity and
 59 quality over the entire lifetime of those insects to ensure strong populations for the time when
 60 crop pollination is required.

61 In recent years, the demand for energy crops has greatly increased due to policy targets and
 62 resulting federal programs and legal regulations (e.g. Renewable Fuel Standard in the USA,
 63 Directive 2009/28/EC in the EU, Renewable Energy Act in Germany). This led to an
 64 expansion of the acreage of cultivated maize (*Zea mays* L., Poaceae) to produce bioethanol (in
 65 the USA) or biogas (in Germany), causing direct or indirect land use changes (Britz &
 66 Delzeit, 2013; Nitsch, Osterburg, Roggendorf, & Laggner, 2012; Wright & Wimberly, 2013).
 67 Maize as a grass is a pollen-only resource except for honeydew in case of aphid infestation
 68 (Schick & Spürgin, 1997). Honeybees collect maize pollen (Keller, Fluri, & Imdorf, 2005),
 69 although its amount of amino acids is comparatively low (Wille et al., 1985). Therefore, an
 70 increasing maize cultivation in monoculture could negatively affect honeybees (Höcherl,
 71 Siede, Illies, Gätschenberger, & Tautz, 2012).

72 In the context of improving agricultural landscapes for pollinators, energy crops of higher

ecological value are required as alternatives to maize (Gardiner et al., 2010; Werling et al., 2014). One candidate used as feedstock for biogas production is the cup plant, *Silphium perfoliatum* L. (Asteraceae) (Gansberger, Montgomery, & Liebhard, 2015). After the first year of establishment, the perennial crop can be harvested at least ten years in succession (Biertümpfel & Conrad, 2013). Improvements in breeding and cultivation are expected to result in yields competitive to maize (Haag, Nägele, Reiss, Biertümpfel, & Oechsner, 2015). The transition from experimental to commercial cultivation of *S. perfoliatum* is ongoing (Biertümpfel, Köhler, & Müller, 2015; Wilken & Benke, 2013). The acreage of *S. perfoliatum* in Germany has already reached 2000 ha (Schittenhelm & Grunwald, 2018) and will most likely increase as the crop can be counted towards the Ecological Focus Area (EFA) requirement of the EU common agricultural policy (CAP) since 2018 (European Parliament, 2017; Fachverband Biogas e.V., 2018).

The cultivation of *S. perfoliatum* leads to changes in soil and crop management practices (no tillage, low herbicide input) that reduce the risk of soil erosion and nutrient loss and have positive effects on the soil humus content and the soil biodiversity (Gansberger et al., 2015; Schorpp & Schrader, 2016). With respect to pollinating insects *S. perfoliatum* has the advantage of a late and long-lasting flowering period from July to September (Gansberger et al., 2015). The inflorescences are yellow with a diameter of about 6 to 10 cm (Ladd, 1995). In contrast to maize, *S. perfoliatum* supplies both pollen and nectar. While the ray florets of *S. perfoliatum* hold the ovules, the disk florets produce the floral resources (personal observation, see also Kadereit, Körner, Kost, & Sonnewald, 2014; Ladd, 1995). In North America, its native range, *S. perfoliatum* is recommended as bee pasture (Decourtye, Mader, & Desneux, 2010).

The introduction of *S. perfoliatum* as new crop is therefore considered as beneficial for

pollinators in European agricultural landscapes (Gansberger et al., 2015). This might in particular be true because *S. perfoliatum* flowers during a crucial point in the life cycle of some important pollinators, i.e. in late summer, when honeybees rear new worker bees for overwintering (Brodschneider & Crailsheim, 2010) and bumblebees rear the males and the new hibernating queens (Amiet & Krebs, 2012). The cultivation of *S. perfoliatum* may thus contribute to stabilize insect populations and maintain pollination service for agricultural production. However, as a non-native plant to Europe, it is important to assess which insects actually use *S. perfoliatum* as food resource (see, e.g., Mueller & Dauber, 2016), as well as to assess the production of floral resources under the local growing conditions and management practices.

The objective of our study was therefore to evaluate pollen and nectar quantity and quality of the perennial energy crop *S. perfoliatum*. For this purpose, we 1) quantified the number of pollen grains, the nectar volume, and the sugar mass per disk floret and inflorescence using experimental fields of different years of establishment, 2) calculated the total amount of floral resources per hectare of *S. perfoliatum* by counting the number of inflorescences per plant using commercial fields, and 3) analyzed the free and protein-bound amino acids in pollen as well as sugars and free amino acids in nectar. We compared our results with existing results for other energy crops and related our findings to the pollen and nectar sugar mass requirements of honeybee larvae as well as worker honeybees. This comprehensive assessment can contribute to develop pollinator-friendly energy cropping systems that ensure sufficient and well-balanced pollen and nectar resources in agricultural landscapes.

2. Materials and methods

2.1 Study sites

Sampling of pollen and nectar took place at the experimental station of the Thuringian State Institute for Agriculture (Thüringer Landesanstalt für Landwirtschaft [TLL]) in Dornburg/Jena (Germany), where plots of *S. perfoliatum* from different years of establishment were available. Soil type on this station is a luvisol from loess. In the research years 2012 and 2013, the average annual temperature was 9.1°C and 8.7°C, respectively (+0.2°C above and -0.2°C below the long-term mean 1981-2010, respectively). The number of inflorescences per plant over the flowering period was counted on 15 commercial fields in Lower Saxony (mean: 1.2 ha, range: 0.4-2.8 ha), a federal state with a leading position in the biogas production in Germany (3N Centre of Experts, 2014) (for further information see Mueller & Dauber, 2016).

2.2 Flowering of disk florets and inflorescences (preparatory observations)

In Asteraceae, the stylus grows through a tube of five connate anthers, pressing the pollen out of the disk floret (Kadereit et al., 2014). The nectaries are annular and are located inside the disk florets at the style base (Bernardello, 2007). To be able to design an adequate sampling, we needed some further knowledge about flowering of *S. perfoliatum* we could not find in the relevant literature. Therefore, we performed observations prior to actual assessment in 2012 on early flowering inflorescences in July. We examined eight disk florets from different inflorescences as well as ten inflorescences at an experimental plot established in 2010. We found out that disk florets flower for one day and start to loosen and decay in late afternoon. Pollen was visible with the naked eye between about 7 AM and 10 AM. Disk florets of an inflorescence open consecutively and centripetally in several rings. The rings of disk florets that open the next morning elongate the previous day. The inflorescences opened on average 18 disk florets per day (range: 1-52). Flowering of an inflorescence lasted on average 9 days (range: 7-13).

145

146 ***2.3 Pollen grains per disk floret and disk florets per inflorescence***

147 Due to their connation, anthers of a disk floret cannot be sampled individually so that whole

148 elongated disk florets were sampled one day before opening to avoid losing pollen. Disk

149 florets were collected between 24th of July and 28th of August in 2012 on two plots

150 established in 2005 and 2010 and stored separately in 80% ethanol. Following Langenberger

151 & Davis (2002), we differentiated between branching degrees of stems: the inflorescences of

152 the main branches were called primary inflorescences (1°), the inflorescences of the first side153 branches were called secondary inflorescences (2°), and so on. While 1° inflorescences were154 disregarded because just one inflorescence is produced per stem, disk florets of 2° up to155 6° inflorescences were collected. For one sample, five disk florets per inflorescence were

156 needed. Ten inflorescences of different randomly selected plants per branching degree and

157 plot were sampled for pollen quantification (500 disk florets for 100 samples).

158 Pollen counting was performed at the Thünen Institute of Biodiversity in Braunschweig

159 (Germany). Pollen was separated from the components of the disk florets and diluted in water

160 manually as well as by vortexing (shaker 'IKA VF2'). All removed components were checked

161 for remaining pollen grains under the stereo microscope (50 x magnifications). For pollen

162 count, it was necessary that pollen grains were evenly distributed in the sample. The use of a

163 liquid other than water, namely 50-80% ethanol (Roulston, 2005), lactic acid/glycerin 3:1

164 (Lloyd, 1972) or 5% aqueous solution of Tween[®] 20 (Ferrara, Camposeo, Palasciano, &

165 Godini, 2007), did not separate the clumping pollen grains satisfactorily, but had negative

166 handling effects like vaporizing (ethanol), being sticky (lactic acid/glycerin) or foaming

167 (Tween[®] 20). Therefore, a full separation of pollen clumps in water was achieved by

168 dispersing them with a marten brush for 20 minutes. Suspension was filled up to exactly

1.5 ml of water. After centrifugation (20 min, 2000 x g), 1.4 ml supernatant was removed. The final suspension of 0.1 ml water with pollen of five disk florets had an adequate pollen concentration for haemocytometer counting (chamber type 'Neubauer improved'). To extrapolate the production of floral resources from a single disk floret to an inflorescence, we counted the number of disk florets of the same inflorescences used for pollen counting (n=100).

2.4 Nectar volume and sugar mass per disk floret

As nectar sampling had to take place directly in the field, we carried out two sampling periods in 2012 on one plot established in 2008. The first sampling took place in the first week of August, when mainly 3° inflorescences were flowering, and the second sampling in the fourth week of August, when mainly 5° inflorescences were flowering. Inflorescences were isolated a day before sampling to avoid nectar removal by insects. For this purpose, we used bags of breathable non-woven fabric. We quantified nectar volume with the help of microcapillary tubes (Drummond Microcaps®, 0.25 and 0.5 µl) from the morning to the afternoon between 7 AM and 5 PM and categorized five time categories of two hours each (TC 1 to TC 5). Because sampling could harm the small disk florets, one floret was sampled only once and several disk florets of one inflorescence were sampled during a day. To further minimize sampling errors, we measured two to three disk florets per inflorescence and time category in dependence of the total number of open disk florets (up to 15 disk florets per inflorescence and day). Sugar concentration of these nectar samples was measured afterwards using two hand-held refractometers for low volume (Eclipse Refractometer by Bellingham and Stanley 45-81 (0-50°Bx) and 45-82 (45-80°Bx)). One degree brix (°Bx) is equivalent to one gram of sucrose per 100 g of solution (1% sucrose by weight). The Brix reading was corrected for

temperature with the help of data recorded by the weather station of the experimental station situated nearby. While not all nectar samples could be transferred correctly to the refractometer we sampled 201 disk florets out of 22 inflorescences for nectar volume and 163 disk florets out of 16 inflorescences for sugar concentration. We aggregated pseudo-replicated samples from disk florets of the same inflorescence and time category which resulted in 76 nectar volume measurements and 63 sugar concentration measurements. Sugar mass per disk floret was calculated from nectar volume and sugar concentration as described in Galetto & Bernardello (2005).

2.5 Inflorescences per plant produced over the flowering period

We counted flowering and withered inflorescences per stem and stems per plant on six randomly selected plants per field. In doing so, we differentiated between the branching degrees of the inflorescences. We carried out four counts per field over the flowering period in 2012. In the first sampling period between 20th and 28th of July and in the second sampling period between 09th and 16th of August, all 15 fields were sampled. In the third sampling period between 28th of August and 07th of September, one field was harvested and so unusable. After the third sampling, farmers told us to stop counting because they were about to harvest. Hence, in the last sampling period between 14th and 20th of September, only eight fields remained for counting.

2.6 Composition of free and protein-bound amino acids in pollen

Pollen was collected in 2013 on two plots established in 2005 and 2010 (Sect. 2.3). It was hand-collected with marten brushes in the morning during pollen presentation and stored in clean microcentrifuge tubes. Five to seven disk florets of ten bagged inflorescences of

different randomly selected plants, respectively, were needed to gain enough pollen for one sample (mean amount of 2,7 mg dry wt, see below). Pollen for three samples per plot and branching degree (2°, 3° and 4° inflorescences) was collected (18 samples in total). Samples were cooled down to -21°C until analyses.

Analyses of free and protein-bound amino acids in pollen were performed at Newcastle University (United Kingdom). Before analyses, pollen was dried (40°C, 24h) and a mean amount of 2.7 mg per sample was added to a microcentrifuge tube. Free and protein-bound amino acids were extracted from each sample using the methods described in Stabler, Power, Borland, Barnes, & Wright (2018). Twenty-one amino acids were measured: alanine (ala), arginine (arg), asparagine (asn), aspartic acid (asp), cysteine (cys), gamma-aminobutyric acid (GABA), glutamine (gln), glutamic acid (glu), glycine (gly), histidine (his), isoleucine (ile), leucine (leu), lysine (lys), methionine (met), phenylalanine (phe), proline (pro), serine (ser), threonine (thr), tryptophan (trp), tyrosine (tyr) and valine (val). Tryptophan is almost completely destroyed during acid hydrolysis of protein-bound amino acids.

231

232 ***2.7 Composition of sugars and free amino acids in nectar***

Similarly to pollen sampling (Sect. 2.6), 18 nectar samples were taken in 2013 using the same plots and branching degrees. Nectar of 12 bagged inflorescences of different randomly selected plants, respectively, was extracted with microcapillary tubes (Drummond Microcaps®) for one sample. According to the results of daily nectar volume and sugar mass production (Sect. 3.2), nectar was sampled after midday, when sugar had already accumulated in the disk florets. Samples were cooled down to -21°C until analyses.

Analyses of sugars and free amino acids in nectar were also performed at Newcastle University (United Kingdom). Sugars (sucrose, glucose, fructose) were analyzed by High

Performance Ion Chromatography (HPIC). HPIC analysis was conducted by injecting 20 µl of each sample via a Rheodyne valve onto a Carbopac PA-100 column (Dionex, Sunnyvale, California, USA) fitted with a Dionex Carbopac PA-100 BioLC guard (4 x 50 mm). Sample components were eluted isocratically using 100 mM NaOH (de-gassed by helium gas) flowing at 1 ml/min for 10 min at RT. The chromatographic profile was recorded using pulsed amperometric detection with an ED40 electrochemical detector (Dionex, Sunnyvale, California, USA). Elution profiles were analyzed using Chromeleon (Thermo Fisher Scientific Inc., MA, USA) which automatically calculates solute concentrations (mol/l) based on a range of pre-programmed reference curves for each sugar.

Ultra High Performance Liquid Chromatography (UHPLC) was used to measure concentrations of the respective amino acids (Sect. 2.6), using methods described in Power, Stabler, Borland, Barnes, & Wright (2018). Two samples of free amino acids in raw nectar of 4° inflorescences could not be used (n=16 samples in total).

2.8 Floral resources of *S. perfoliatum* in comparison to other energy crops

To assess the ecological value of *S. perfoliatum* as alternative energy crop, the results of the present study on floral resources were compared with available literature for maize as well as for the nectariferous energy crops sunflower (*Helianthus annuus* L., Asteraceae) and oilseed rape (*Brassica napus* L., Brassicaceae). *S. perfoliatum* and *H. annuus* belong to the same subtribe, while *B. napus* is the second most cultivated energy crop in Germany used for biofuel production (Fachagentur Nachwachsende Rohstoffe e.V. [FNR], 2017). When data on pollen grains and nectar sugar mass was only available for the production per flower we calculated resource production for other units by using information about the number of flowers per plant and plants per hectare.

265

266 **2.9 Floral resources of *S. perfoliatum* considering honeybee requirements**

267 To relate the floral resources of *S. perfoliatum* to the needs of honeybees we considered the
 268 needs of both honeybee larvae, namely 125 to 187.5 mg pollen (Hrassnigg & Crailsheim,
 269 2005) and 59.4 mg sugar (Rortais, Arnold, Halm, & Touffet-Briens, 2005) per larva, as well
 270 as worker honeybees, namely 3.4 to 4.3 mg pollen (Crailsheim et al., 1992) and 4 mg sugar
 271 (Barker & Lehner, 1974) per worker bee and day (data reviewed in Brodschneider &
 272 Crailsheim, 2010).

273 Therefore, we had to convert the calculated number of pollen grains per *S. perfoliatum* plant
 274 into pollen mass. For this purpose, we used an approach given by Dicks et al. (2015).

275 In a first step, we estimated the number of pollen grains per corbícula (pollen basket on the
 276 hind leg of honeybees) by considering a pollen diameter of *S. perfoliatum* of 26.6 μm
 277 (Agentur für Gesundheit und Ernährungssicherheit [AGES], 2012), and hence, a pollen
 278 volume of the spherical pollen of $9.9 \times 10^{-6} \text{ mm}^3$ (compare Müller et al., 2006) as well as a
 279 corbicular volume of 4-6 mm^3 (Dicks et al., 2015). Accordingly, we estimated a number of
 280 about 4×10^5 to 6×10^5 pollen grains per corbicular volume (about 8×10^5 to 12×10^5 pollen
 281 grains per load). In a second step, we estimated the number of pollen grains per pollen mass
 282 by considering a pollen mass per corbícula of 5.5 mg given for *H. annuus* (5.15 mg dry mass
 283 and 6.6% water content, Vaissière & Vinson, 1994), that has a similar pollen to *S. perfoliatum*
 284 (AGES, 1999). Accordingly, we estimated a number of about 73,800 to 111,000 pollen grains
 285 per milligram pollen. Finally, the results on the number of pollen grains per inflorescence
 286 (Sect. 3.1) as well as inflorescences per plant (Sect. 3.3) could be used to calculate the number
 287 of pollen mass per plant of *S. perfoliatum*.

288

289 **2.10 Statistical analyses**

290 We conducted all data analyses using R, version 3.3.2 (R Core Team, 2016). For pollen
 291 production, we modeled the response variables disk florets per inflorescence, pollen grains
 292 per disk floret and pollen grains per inflorescence. We used generalized least squares models
 293 (package nlme, see Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2016) and set the factor
 294 branching degree (five levels: 2° up to 6° inflorescences), the factor plot (two levels:
 295 establishment in 2005 and 2010) as well as their interaction as explanatory variables. To
 296 account for variance heterogeneity, we allowed for different variances per stratum where
 297 needed (Zuur, Ieno, Walker, Saveliev, & Smith, 2009).

298 Regarding nectar volume and sugar mass per disk floret, we analyzed the effect of the factor
 299 time category (five levels: TC 1 to TC 5) and the factor sampling period (two levels: sampling
 300 periods at the beginning and the end of August, equivalent to differences in lower and higher
 301 degrees of stem branching). As the samples were nested (because disk florets that were
 302 measured on different time categories originated from the same inflorescences, and the
 303 measurements took place on different sampling days), we applied linear mixed-effects models
 304 (package lme4, see Bates, Maechler, Bolker, & Walker, 2015) with the factors inflorescence
 305 and day as random effects. Nectar volume was square root transformed to meet normality
 306 assumption of residuals.

307 Model selection was done using likelihood ratio test (LRT) as described in Zuur et al. (2009).
 308 At significance of a multilevel factor ($p(\text{LRT}) < 0.05$), t-tests with Bonferroni correction of
 309 p -values were performed for pairwise comparisons of different factor levels using the package
 310 predictmeans (Luo, Ganesh, & Koolgaard, 2014).

311

312 **3. Results**

3.1 Pollen grains per disk floret and inflorescence

An inflorescence of *S. perfoliatum* produced on average 122 disk florets (SD=31, range: 68-205, n=100), a disk floret on average 14,200 pollen grains (SD=3,400, range: 6,000-21,400, n=100), resulting in a mean number of 1.75×10^6 pollen grains per inflorescence (SD= 0.65×10^6 , range: 0.54×10^6 - 3.74×10^6 , n=100). The pattern in those data could best be explained by the factor branching degree, while the interaction branching degree x plot or the factor plot were not significant at the 5% level in all models (Table A.1, suppl. mat.). Figure 1 therefore shows the decreasing number of disk florets per inflorescence (Fig.1a), pollen grains per disk floret (Fig.1b) and pollen grains per inflorescence (Fig.1c) with increasing branching of the stem by including the results of the pairwise comparisons of different branching degrees.

[Figure 1]

3.2 Nectar volume and sugar mass per disk floret and inflorescence

According to the statistical models on nectar volume and sugar mass per disk floret the factor time category was significant at the 5% level due to the LRT (Table A.2, suppl. mat.). Nectar and sugar production of a disk floret started in the morning with small amounts and increased until midday. Although the results of the last three time categories after 11 AM did not differ significantly in both models (Fig.2), nectar volume per disk floret tended to decrease in the afternoon, as shown by the insignificant difference between TC 2 and the latter TC 4 and TC 5. Despite this decreasing nectar volume, sugar mass remained stable due to increasing sugar concentrations over the day being on average 19°Bx (TC 1), 44°Bx (TC 2), 55°Bx (TC 3) and 68°Bx (TC 4 and TC 5). Due to these varying sugar concentrations, the further resource quantification is based on nectar sugar mass instead of nectar volume. According to

model prediction, where nesting of data is taken into account, the accumulated nectar sugar mass was 0.09 mg per disk floret in all three time categories after 11 AM (n=44). In contrast to daily nectar production, no significant difference in nectar or sugar production per disk floret was detected between the early and late sampling as the factor had no explanatory power due the LRT (Table A.2, suppl. mat.). Highest amounts of nectar volume and sugar mass were measured in disk florets of 5° inflorescences, namely 0.38 µl of nectar (22th of August, 11:10 AM) and 0.21 mg of sugar (21th of August, 11:14 AM). However, considering that the number of disk florets per inflorescences decreased with increasing stem branching (Sect. 3.1), nectar sugar mass per inflorescences still decreased by the same factor with increasing branching degree. By using the predicted value of 0.09 mg per disk floret, we calculated a sugar mass of 12 mg per 2° inflorescence, but 9 mg per 6° inflorescence (Table A.3, suppl. mat.).

[Figure 2]

3.3 Inflorescences per plant produced over the flowering period

A single stem developed on average 1.5 inflorescences (SD=0.7, range: 0-5, n=90) until the end of July, 8 inflorescences (SD=4, range: 1-29, n=90) until mid-August, 26 inflorescences (SD=16, range: 1-121, n=84) until the end of August/ beginning of September, and 33 inflorescences (SD=19, range: 4-108, n=48) until mid-September with a mean number of about 6 stems per plant (SD=3, range: 1-15, n=312). Thus, total number of inflorescences per plant was estimated to be 10 (end of July), 55 (mid-August), 150 (end of August/ beginning of September) and 188 (mid-September). Due to ongoing stem branching over the flowering period, 3° (mid-August), 4° (end of August/beginning of September) or 5° inflorescences (mid-September) accounted on average for the largest part of all inflorescences per plant

(Fig.3).

[Figure 3]

3.4 Period of highest supply of floral resources

By multiplying the average production of pollen grains and nectar sugar mass per inflorescence and branching degree (Sect. 3.1 and 3.2) with the average number of inflorescences per plant and branching degree (Sect. 3.3) the period of highest pollen and nectar sugar supply was estimated (see Table A.3 to A.6, suppl. mat., for full estimation). Regarding pollen production, 4° inflorescences have produced the largest amount on average until both sampling periods, the one at the end of August/ beginning of September and the one in mid-September (32% and 28% of total production, respectively). Regarding nectar sugar production, 4° inflorescences produced the largest amount of sugar on average until the third sampling period (31% of total production), but until mid-September, 5° inflorescences had produced the largest amount of sugar on average (30% of total production). Considering a mean number of 314×10^6 pollen grains/plant (Table A.5, suppl. mat.), a mean nectar sugar mass of 2 g/plant (Table A.6, suppl. mat.) and a recommended stand density of 4 plants/m² (TLL, 2015), it can be roughly estimated that *S. perfoliatum* supplied on average 12.5×10^{12} pollen grains/ha and 80 kg sugar/ha until mid-September. While the flowering period had just started at the end of July, when about 3% of the total average amount of pollen and nectar sugar was already produced, about 29% and 26%, respectively, was produced from the first to the second sampling period, 51% and 52%, respectively, from the second to the third sampling period, and 17% and 19%, respectively, from the third to the last sampling period.

384 **3.5 Free and protein-bound amino acids in pollen**

385 In pollen, the mean total of free amino acids was 1.16 µg/mg (range: 0.09-2.23, n=18) and the
 386 mean total of protein-bound amino acids was 79.5 µg/mg (range: 70.6-88.4, n=18) (Table 1),
 387 corresponding to a protein content of about 8% (dry wt). Histidine and arginine were the most
 388 abundant amino acids in pollen. This resulted in high average proportions of essential amino
 389 acids of about 88% of total free amino acids and 81% of total protein-bound amino acids.

391 **3.6 Sugars and free amino acids in nectar**

392 The average amount of sucrose in nectar was 0.252 mol/l (SD=0.125, range: 0.029-0.468,
 393 n=18), that of glucose 0.868 mol/l (SD=0.426, range: 0.054-1.515, n=18) and that of fructose
 394 1.043 mol/l (SD=0.513, range: 0.079-1.850, n=18).

395 Regarding free amino acids in nectar, the mean total was 1.11 µmol/ml (range: 0.10-3.03,
 396 n=16). In contrast to amino acids in pollen, the amount and composition of free amino acids
 397 in nectar varied much more between samples (Table 1). Again, histidine accounted for the
 398 largest amount, but it dominated six samples only. The essential amino acids was 61% of the
 399 mean total amino acids measured, due to the larger amounts of histidine and lysine.

400 [Table 1]

402 **3.7 Floral resources of *S. perfoliatum* in comparison to other energy crops**

403 The number of pollen grains as well as nectar sugar mass of *S. perfoliatum* in comparison to
 404 other energy crops is shown in Table 2. Mean estimated pollen production per hectare of
 405 *S. perfoliatum* is higher than estimated maximum pollen production per hectare of maize,
 406 sunflower and oilseed rape. The ranges between potential minimum and maximum nectar
 407 sugar production per hectare of all nectariferous energy crops are overlapping. While nectar

sugar potential per hectare of *S. perfoliatum* exceeds that of sunflower, it does not exceed that of oilseed rape.

[Table 2]

Regarding amino acids in pollen, maize, sunflower and *Brassica* spec. showed distinctly higher amounts in a study of Wille et al. (1985) (*Z. mays*: 143.2 µg/mg, *H. annuus*: 172.2 µg/mg, *Brassica* spec.: 237.8 µg/mg, data converted from g/100 g) in comparison to *S. perfoliatum* (Sect. 3.5). When the profile of amino acids from the pollen is compared, only the concentration of the essential amino acids histidine and arginine was much higher for *S. perfoliatum* (*Z. mays*: 3.4 and 9.1 µg/mg, *H. annuus*: 9.9 and 8.3 µg/mg, *Brassica* spec.: 5.9 and 12.7 µg/mg, data converted from g/100 g, see Wille et al., 1985). It should be mentioned that Wille et al. (1985) used pollen pellets collected from honeybees instead of hand-collected pollen taken directly from the flower, as it was done in our study. Roulston, Cane, & Buchmann (2000) showed that protein content of hand-collected pollen tends to be higher than that of bee-collected pollen, since honeybees add nectar to the pollen pellets. Hence, the amounts of amino acids in pollen given by Wille et al. (1985) may still be underestimated. Regarding sugars and amino acids in nectar of *B. napus*, Lohaus & Schwerdtfeger (2014) reported mean amounts of 0.006 mol/l sucrose, 0.858 mol/l glucose and 0.782 mol/l fructose, that is quite similar to *S. perfoliatum*, but 2.0 µmol/ml amino acids (data converted from mM), that is about two-fold higher than that of *S. perfoliatum*.

427

3.8 Floral resources of *S. perfoliatum* considering honeybee requirements

The number of honeybee larvae, that could have been provided by the floral resources of one plant of *S. perfoliatum*, are shown in Table. 3. The number of worker honeybees, that could have been provided by the floral resources of one plant of *S. perfoliatum* per day, are shown in

432 Table 4.

433 [Table 3]

434 [Table 4]

435

436 4. Discussion

437 The amount of floral resources per inflorescence decreased with ongoing stem branching, but
438 the number of inflorescences per plant increased with ongoing stem branching up to

439 5° inflorescences. It was shown that of these opposing effects the increasing number of

440 inflorescences per plant and branching degree tended to have a stronger influence on total

441 production of floral resources. On average, 4° inflorescences produced the highest amount of

442 pollen and 5° inflorescences the highest amount of nectar sugar until mid-September.

443 Consequently, pollen and nectar sugar supply by *S. perfoliatum* was highest when those

444 higher branching inflorescences were flowering mostly in the second half of August.

445 One farmer harvested after the second sampling in mid-August, six farmers after the third

446 sampling at the end of August/beginning of September and eight farmers after the fourth

447 sampling in mid-September. These different harvesting dates reflect two contradicting

448 requirements. On the one hand, the optimal time for harvest to reach the highest methane

449 yield per hectare for energy production is between the last ten days of August and the first ten

450 days of September (Biertümpfel & Conrad, 2013). On the other hand, farmers growing

451 *S. perfoliatum* on single small fields are advised to harvest *S. perfoliatum* late together with

452 maize around the end of September, because for them, labor and machinery cost savings from

453 a joint harvest are higher than the returns from a separate early harvest of *S. perfoliatum* at

454 highest methane yield (Wilken & Benke, 2013). According to our estimates, about one sixth

455 of the potential pollen and about one fifth of the potential nectar sugar production were lost in

456 the study region with a harvest around the beginning of September. There is reason for
 457 concern that with an expansion of *S. perfoliatum* acreage, an early harvest could become
 458 economically viable and thus common practice. This would mean that pollinators that rely on
 459 floral food sources through late summer would not benefit from a cultivation of
 460 *S. perfoliatum*, e.g. hoverflies like *Eristalis tenax* L., that visited the remaining commercial
 461 fields mostly in mid-September (Mueller & Dauber, 2016). To enhance floral rewards for
 462 pollinators in agricultural landscapes, we suggest that the eligibility of *S. perfoliatum* as EFA
 463 crop should be on condition of a late harvest after the flowering period.

464 Regarding pollen, it was shown that the estimated mean number of pollen grains per hectare
 465 of *S. perfoliatum* was higher than that of the common energy crops maize, sunflower and
 466 oilseed rape. In contrast, mean total amount of amino acids in the pollen of *S. perfoliatum* was
 467 much lower compared to the pollen of other energy crops. Due to the fact that pure pollen
 468 diets of maize and sunflower were shown to negatively affect reproduction and development
 469 of pollinators (Höcherl et al., 2012; L.S. Schmidt, Schmidt, Rao, Wang, & Xu, 1995; Tasei &
 470 Aupinel, 2008), a monofloral diet with the pollen of *S. perfoliatum* may also be detrimental.

471 However, by providing high amounts of the essential amino acids histidine and arginine, we
 472 estimate that the pollen of *S. perfoliatum* can still be a valuable part of the honeybee diet. This
 473 might particularly be true because *S. perfoliatum* is cultivated in bioenergy crop dominated
 474 regions, where maize is an abundant pollen resource of low histidine content in particular
 475 (Höcherl et al., 2012). In addition, arginine is important for brood rearing (Génissel, Aupinel,
 476 Bressac, Tasei, & Chevrier, 2002; Herbert, Bickley, & Shimanuki, 1970; Loper & Berdel,
 477 1980). Nevertheless, a cultivation of *S. perfoliatum* should be combined with other agri-
 478 environmental measures like organic farming, diversification of crops and crop rotation, and
 479 the implementation of flower strips as well as measures outside the farmland like the

480 protection of semi-natural habitats, not only to guarantee the provision of floral resources
 481 outside the flowering period of *S. perfoliatum*, but also to guarantee that pollinators can
 482 acquire all the nutrients they need from floral pollen (see Vaudo, Tooker, Grozinger, & Patch,
 483 2015).
 484 Regarding nectar, a replacement of maize as a pollen-only resource by *S. perfoliatum* would
 485 increase its supply by 100%. It was further shown that the production of nectar sugar mass of
 486 *S. perfoliatum* was within the same range of other nectariferous energy crops. Nectar of
 487 *S. perfoliatum* is hexose rich, which is typical for exposed nectar of open flowers
 488 (Gottsberger, Schrauwen, & Linskens, 1984; Percival, 1961) and very common for Asteraceae
 489 (H. G. Baker & Baker, 1983; Torres & Galetto, 2002), including sunflower (Vear et al., 1990),
 490 and Brassicaceae (H. G. Baker & Baker, 1983), including oilseed rape (Lohaus &
 491 Schwerdtfeger, 2014). However, the amount of free amino acids in nectar of *S. perfoliatum*
 492 was much smaller than that given for oilseed rape. Although honeybees forage different nectar
 493 types, ranging from sucrose to hexose dominant nectars (Percival, 1961), it was shown that
 494 honeybees at least prefer sucrose-rich solutions compared to hexose-rich solutions (Waller,
 495 1972), as well as sugar-amino acid solutions over sugar-only solutions (Alm, Ohnmeiss,
 496 Lanza, & Vriesenga, 1990). Hence, nectar of *S. perfoliatum* might not be honeybees' first
 497 choice.
 498 Finally, we linked pollen and nectar sugar production of *S. perfoliatum* to the requirements of
 499 honeybee larvae as well as worker honeybees. We could show that by one plant of
 500 *S. perfoliatum*, up to 34 honeybee larvae could be provided with both pollen as well as nectar
 501 sugar. However, the number of worker honeybees that could be provided daily by
 502 *S. perfoliatum* with pollen was much higher than the number that could be provided with
 503 nectar sugar. With a high supply of floral resources especially in the second half of August,

504 *S. perfoliatum* may therefore serve as a pollen and sugar resource for larval rearing to develop
 505 winter bees in late summer. Those winter bees, that have a longer lifespan than summer bees,
 506 can use *S. perfoliatum* pollen to store proteins in the body for overwintering (Brodschneider &
 507 Crailsheim, 2010). Nevertheless, when beekeepers want to use *S. perfoliatum* for winter bee
 508 feeding they also have to consider limited nectar sugar resources for worker bees. In the first
 509 half of September, nectar sugar amount of one plant would have sufficed for about 6 worker
 510 bees per day without considering sugar needed for larval feeding. It was shown that honeybee
 511 colonies collected nectar mainly for their own direct requirements and stored only little or no
 512 honey in the supers when placed on *S. perfoliatum* fields (six colonies per either 1.0, 1.2 or
 513 3.0 hectares) and a few colonies even needed supplementary feed (Heidinger, 2016). This
 514 shows that beekeepers have to consider factors as colony strength, flowering time, and field
 515 size, when they decide on the number of colonies placed next to a field of *S. perfoliatum*. In
 516 addition, they need to carefully control sugar supply of the bee hives. Furthermore, late
 517 flowering crops with good nectar sources could be fostered to complete the food supply by
 518 *S. perfoliatum* as a good pollen source, e.g. green manure like *Melilotus* Mill. species or
 519 *Phacelia tanacetifolia* Benth. (Schick & Spürgin, 1997).

520

521 **5. Conclusion**

522 Diversifying the production of maize as a pollen-only resource by *S. perfoliatum* would in any
 523 case result in a surplus regarding nectar resources for flower-visiting insect. In addition,
 524 *S. perfoliatum* has a greater pollen potential than maize and other nectariferous energy crops.
 525 Considering the needs of worker honeybees, it seems to be a better pollen, rather than nectar
 526 sugar resource. *S. perfoliatum* may therefore be of particular importance to rear winter bees in
 527 late summer and, for those bees, to build storage proteins for overwintering, but not to store

honey in appreciable amounts. Furthermore, *S. perfoliatum* provides high amounts of the essential amino acids histidine and arginine, but contents of other amino acids in pollen are low. Both results – that on the one hand *S. perfoliatum* has a greater pollen than nectar sugar potential, but that pollen on the other hand supplies high amounts of only some of the essential amino acids – show that *S. perfoliatum* can be a valuable food resource in agricultural landscapes, but its cultivation has to be combined with other measures that enhance different bee fodder plants with supplementary nutritional values.

Our study showed that total pollen and nectar production of *S. perfoliatum* depends on harvesting time. Harvest is often realized in a late joint harvest with maize when field sizes are small to save labor costs, although this is too late to gain the maximum methane yield for biogas production. Such a late harvest at the end of the flowering period might not gain acceptance by farmers in the long term, because with increasing acreage and field sizes, harvesting dates might progressively be orientated on yield maximization. Such a potential development to an early harvest within the flowering period should be taken into consideration when developing regulations for *S. perfoliatum* counted as EFA.

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Appendix A. Supplemental material

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Table 1 Free and protein-bound amino acids in pollen, as well as free amino acids in nectar of *S. perfoliatum*

Amino acid	Free amino acids in pollen (n=18)			Protein-bound amino acids in pollen (n=18)			Free amino acids in nectar (n=16)		
	mean [µg/mg]	SD [µg/mg]	Freq [x/18]	mean [µg/mg]	SD [µg/mg]	Freq [x/18]	mean [µmol/ml]	SD [µmol/ml]	Freq [x/16]
ALA	0.000	0.000	0	0.13	0.23	18	0.062	0.214	7
ARG ^(a)	0.037	0.011	18	22.24	6.67	18	0.038	0.054	14
ASN	0.000	0.000	18	0.00	0.00	17	0.000	0.000	15
ASP	0.047	0.044	12	1.97	1.27	18	0.017	0.026	15
CYS	0.002	0.001	17	5.45	2.16	18	0.026	0.021	15
GABA ^(b)	0.000	0.000	18	0.00	0.00	14	0.000	0.000	16
GLN	0.000	0.000	18	0.00	0.00	9	0.000	0.000	16
GLU	0.053	0.042	17	1.38	1.19	18	0.078	0.121	12
GLY	0.000	0.001	5	0.06	0.24	2	0.027	0.046	7
HIS ^(a)	0.931	0.211	18	37.54	14.68	17	0.249	0.150	15
ILE ^(a)	0.001	0.002	18	0.10	0.16	12	0.057	0.073	14
LEU ^(a)	0.007	0.004	18	1.81	0.64	18	0.020	0.031	13
LYS ^(a)	0.003	0.001	18	0.74	0.27	18	0.198	0.136	15
MET ^(a)	0.005	0.002	18	0.16	0.15	18	0.022	0.056	4
PHE ^(a)	0.007	0.002	18	1.42	0.65	18	0.023	0.016	14
PRO	0.002	0.001	18	0.07	0.10	16	0.098	0.229	11
SER	0.029	0.014	18	4.84	3.26	14	0.113	0.084	15
THR ^(a)	0.019	0.006	18	0.05	0.16	4	0.023	0.045	10
TRP ^(a, c)	0.000	0.000	7	0.00	0.00	13	0.000	0.000	5
TYR	0.004	0.001	18	0.96	0.36	18	0.016	0.022	15
VAL ^(a)	0.016	0.007	18	0.64	0.29	18	0.046	0.087	10
Sum (all)	1.16	0.26	18	79.5	26.2	18	1.11	0.73	16

(a) Essential for the growth of young honey bees of *A. mellifera* after de Groot (1953), shaded grey; (b) GABA is a non-proteinogenic amino acids; (c) Tryptophan is almost completely destroyed during acid hydrolysis of protein-bound amino acids

Table 2 Number of pollen grains and nectar sugar mass of *S. perfoliatum* in comparison to other energy crops

Crop	Pollen				Nectar sugar			
	grains/ florete	grains/ inflorescence [x 10 ⁶]	grains/plant [x 10 ⁶]	grains/ha [x 10 ¹²]	mg/ disk floret and day	mg/inflo- rescence	g/plant	kg/ha
<i>Silphium perfoliatum</i>	14,200 (6,000- 21,400) ^(a)	1.8 (0.5-3.7) ^(a)	314 (109-627) ^(b)	12.5 (4.4-25.1) ^(b)	0.09 ^(c)	11 9-12 ^(d)	2 (0.6-4,2) ^(e)	80 (25-170) ^(e)
<i>Zea mays</i>	6,000- 22,500 ^(f)	-	14-50 ^(f)	0.8-6.0 ^(f, g)	-	-	-	-
<i>Helianthus annuus</i>	22,000- 50,000 ^(h)	35-90 ^(h, i)	35-90 ^(h, i)	2.0-5.0 ^(h, i)	0.097- 0.27 ^(j)	640- 1,080 ^(i, k)	0.6-1.1 ^(i, k)	13-140 ^(j) ; 18-47 ^(l)
<i>Brassica napus</i>	64,900 ^(m) ; 96,700 ⁽ⁿ⁾	-	11-41 ^(m, n, o)	5.7- 11.6 ^(m, n, o) ; 5 ^(p)	0.29-0.90 ^(q)	-	0.1-0.8 ^(o, q)	40-200 ^(q) ; 131 ^(l)

(a) Sect. 3.1; (b) Sect. 3.1, 3.3, Table A.5, suppl. mat.; (c) Sect. 3.2; (d) Sect. 3.1, 3.2; (e) Sect. 3.2, Sect. 3.3, Table A.6 suppl. mat.; (f) Miller (1982) and references therein: three anthers per floret, 2000 to 7500 pollen grains per anther; (g) Deutsches Maiskomitee e.V. (2009): 6-12 maize plants/m²; (h) Astiz & Hernández (2013); (i) Lindström, Pellegrini, Aguirrezábal, & Hernández (2006): about 1,600-1,800 disk florets per inflorescence at a stand density of 5.6 plants/m². They used two sunflower cultivars, therefore the number of inflorescences per plant is supposed to be one. Wild sunflowers produce one to several inflorescences (Ladd, 1995), but smaller numbers of disk florets (50-400) (Minckley, Weislo, Yanega, & Buchmann, 1994); (j) Schick & Spürgin (1997); (k) Free (1993): nectar secretion of *H. annuus* lasts three days, highest rate at second day. Thus, multiplying the amount of 24h by 3 might create wrong values. Burmistrov (1965, cited by Free, 1993) gives values of total sugar amount per floret of 0.4 to 0.6 mg; (l) Thom et al. (2016): spring oilseed rape; (m) Cresswell (1999); (n) Cresswell, Hagen, & Woolnough (2001); (o) Kołtowski (2005): 175 flowers/plant at a stand density of 50 plants/m² and 427 flowers/plant at a stand density of 28 plants/m²; (p) Chèvre, Eber, Renard, & Darmency (1999); (q) Maurizio & Schaper (1994): nectar secretion of *B. napus*: two times per flower

Table 3 Number of honeybee larvae that can be provided by one plant of *S. perfoliatum* with pollen and nectar sugar

	Pollen			Nectar sugar	
	grains/plant [x 10 ⁶] ^(a)	mass/plant [g] ^(b)	larvae provided/ plant ^(c)	mass/plant [g] ^(d)	larvae provided/ plant ^(e)
mean values	314	2.8-4.2	15-34	2.0	34
minimum values	109	1.0-1.5	5-12	0.6	10
maximum values	627	5.7-8.5	30-68	4.2	71

(a) Sect. 3.1, 3.3; Table. A.5 suppl. mat; (b) considering 74 x10⁶ to 111 x10⁶ pollen grains per gram pollen, Sect. 2.9; (c) considering a pollen requirement per larva of 125-187.5 mg (Hrassnigg & Crailsheim, 2005, cited in Brodschneider & Crailsheim, 2010); (d) Sect. 3.2, 3.3; Table. A.6 suppl. mat.; (e) considering a sugar requirement per larva of 59.4 mg (Rortais, Arnold, Halm, & Touffet-Briens, 2005, cited in Brodschneider & Crailsheim, 2010)

Table 4 Number of worker honeybees that can be provided by one plant of *S. perfoliatum* with pollen and nectar sugar per day

The requirements are given in pollen and sugar mass per worker bee *and day*. Therefore, we had to consider the days between samplings. As we do not know how many days *S. perfoliatum* was already in bloom until the first sampling period, we only consider the time between the first and the last sampling (97% of total pollen and nectar sugar production).

time period	mean number of days between samplings	Pollen				Nectar sugar			
		share of total pollen production [%] ^(a)	pollen mass/ plant and time period [g] ^(b)	pollen mass/ plant and day [mg]	worker bees provided/plant and day ^(c)	share of total sugar production [%] ^(a)	sugar mass/ plant and time period [g] ^(b)	sugar mass/ plant and day [mg]	worker bees provided/plant and day ^(d)
first half of August (1 st to 2 nd sampling) [mean]	19	29	0.8-1.2	43-65	10-19	26	0.5	28	7
second half of August (2 nd to 3 rd sampling) [mean]	21	51	1.4-2.2	69-103	16-30	52	1.0	50	12
first half of September (3 rd to 4 th sampling) [mean]	15	17	0.5-0.7	32-48	7-14	19	0.4	25	6
July – Sept. (1 st to 4 th sampling) [mean]	55	97	2.7-4.1	50-75	12-22	97	2.0	35	9
July – Sept. (1 st to 4 th sampling) [minimum]	55	97	1.0-1.4	17-26	4-8	97	0.6	11	3
July – Sept. (1 st to 4 th sampling) [maximum]	55	97	5.5-8.2	100-150	23-44	97	4.1	75	19

(a) Sect. 3.4; (b) Considering the pollen mass and nectar sugar mass production until mid-September (Table 3); (c) considering a pollen requirement of 3.4-4.3mg/worker bee and day (Crailsheim et al., 1992, cited in Brodschneider & Crailsheim, 2010); (d) considering a nectar sugar requirement of 4mg/worker bee and day (Barker & Lehner, 1974, cited in Brodschneider & Crailsheim, 2010)

Figure captions

Figure 1: Pollen production of *S. perfoliatum*

Mean number and standard deviation for disk florets per inflorescence (a), pollen grains per disk floret (b) as well as pollen grains per inflorescence (c) of *S. perfoliatum* (n=100 each), divided by branching degrees (2° to 6° inflorescences). Letters show significant differences of the groups according to pairwise comparisons ($p<0.5$).

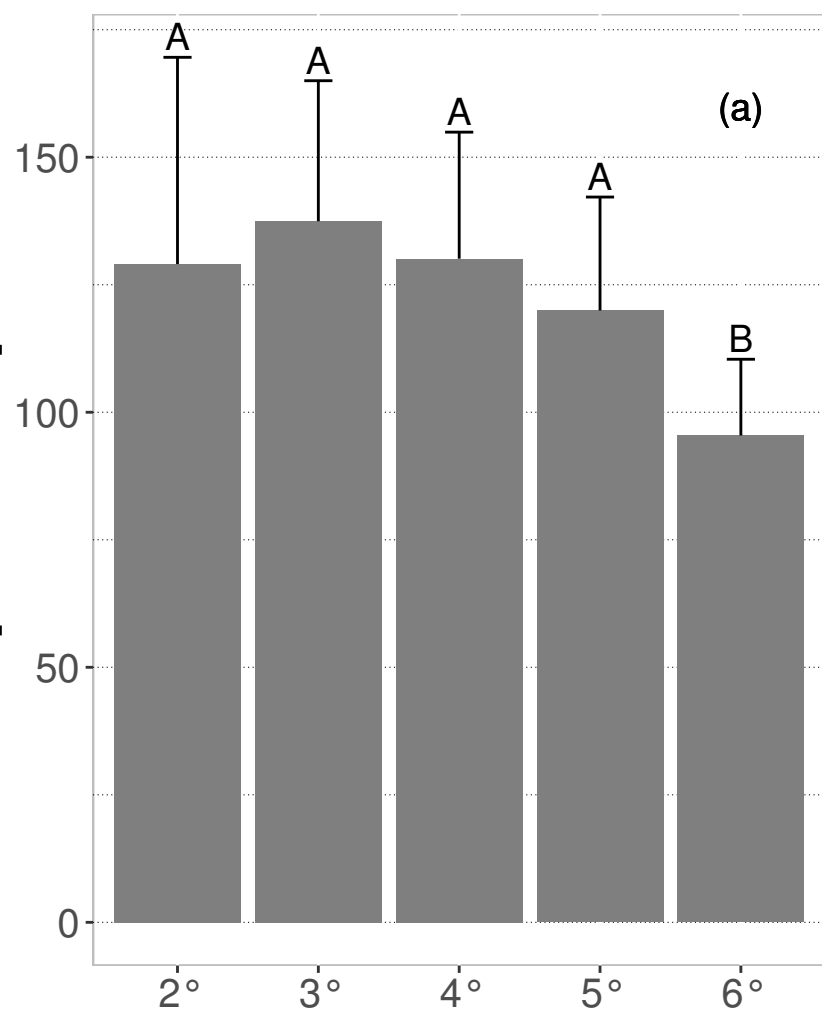
Figure 2: Nectar volume and sugar mass per disk floret of *S. perfoliatum*

Mean number and standard deviation for accumulated nectar volume (n=76) and sugar mass (n=63) per disk floret of isolated inflorescences. Samples were grouped to five time categories between 7 AM and 5 PM. Letters show significant differences of the groups according to pairwise comparisons ($p<0.5$).

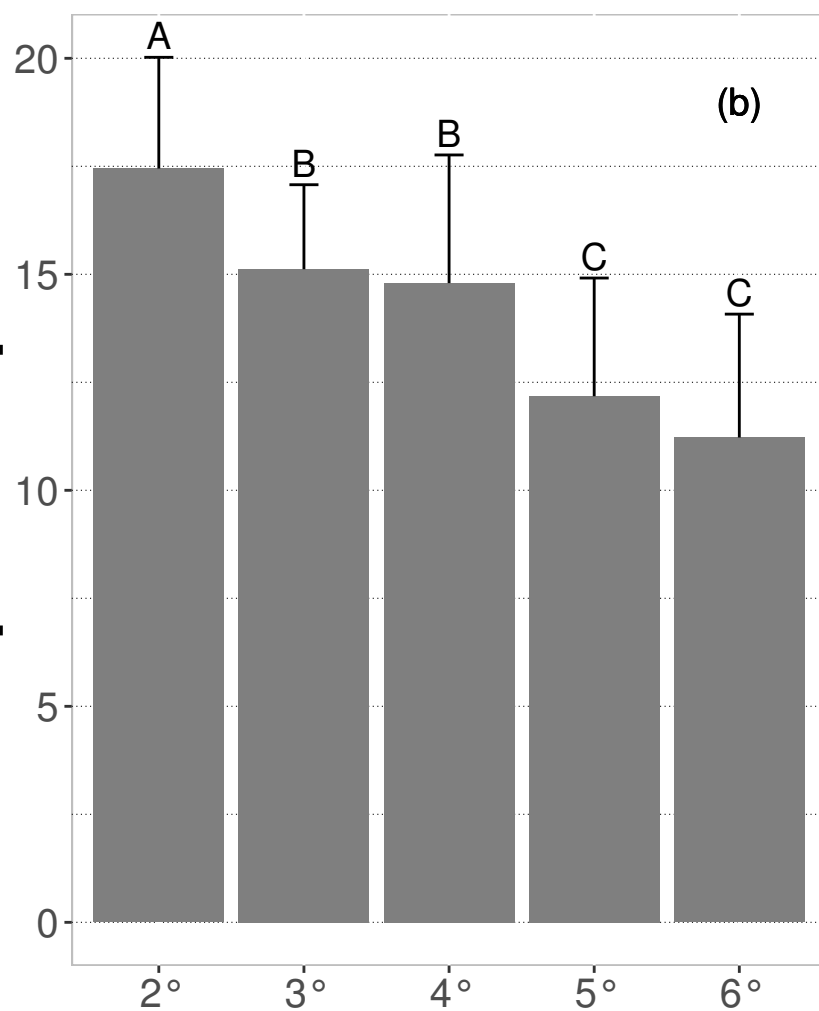
Figure 3: Inflorescences per plant of *S. perfoliatum*

Mean number and standard deviation of flowering and withered inflorescences per plant of *S. perfoliatum* measured at commercial fields in Lower Saxony (Germany) during four sampling periods between July and September, divided by branching degrees (1° to 7° inflorescences).

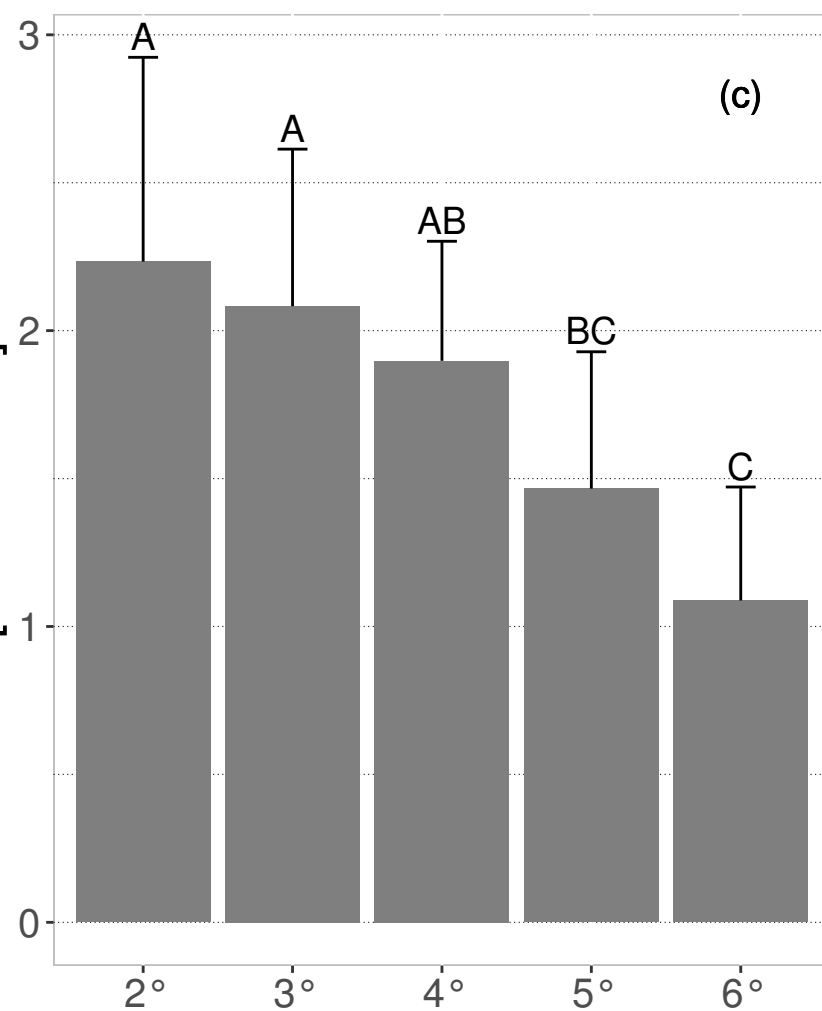
disk florets per inflorescence
[mean + SD]



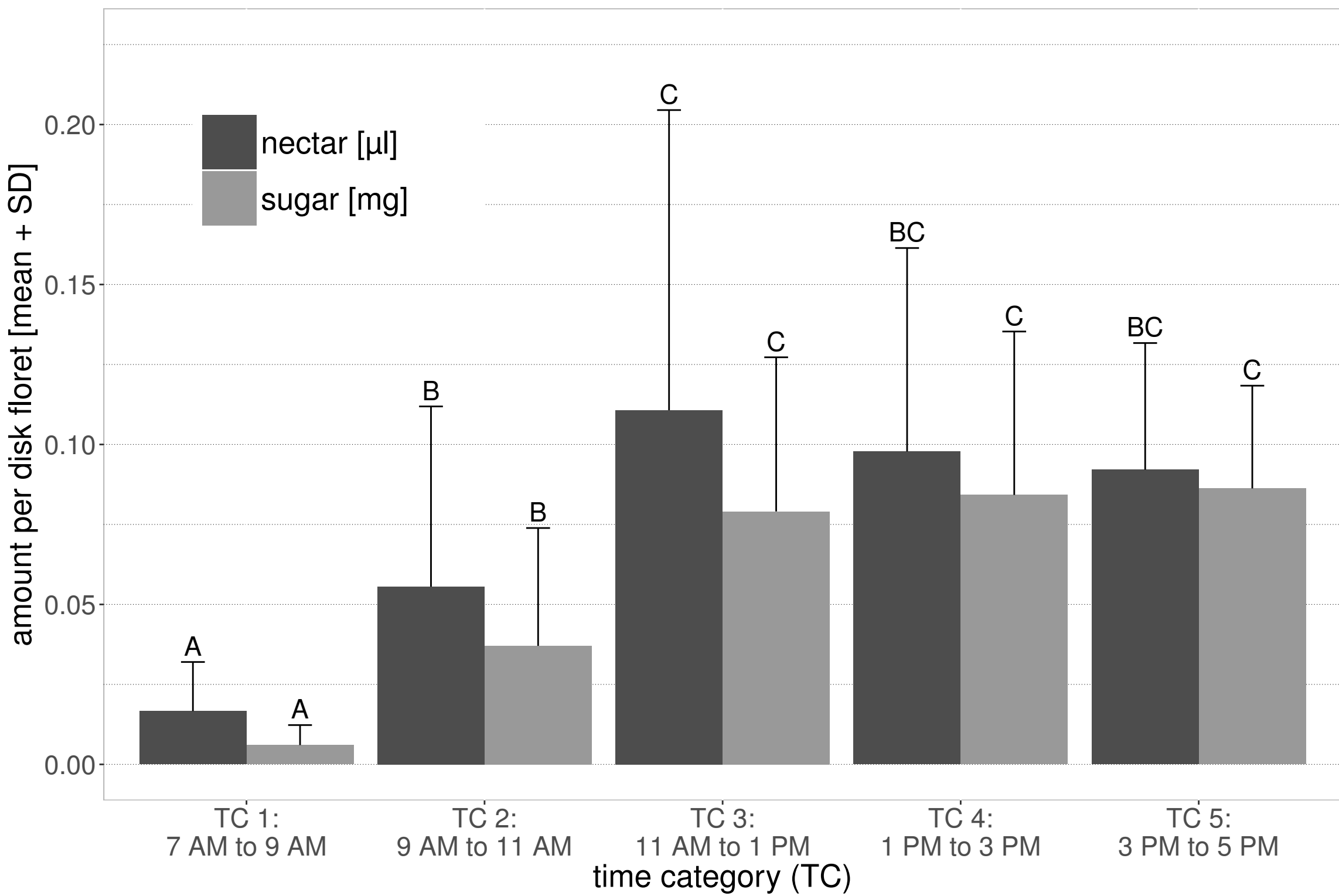
pollen grains per disk floret ($\times 10^3$)
[mean + SD]



pollen grains per inflorescence ($\times 10^6$)
[mean + SD]



secondary (2°) to senary (6°) inflorescences according to stem branching



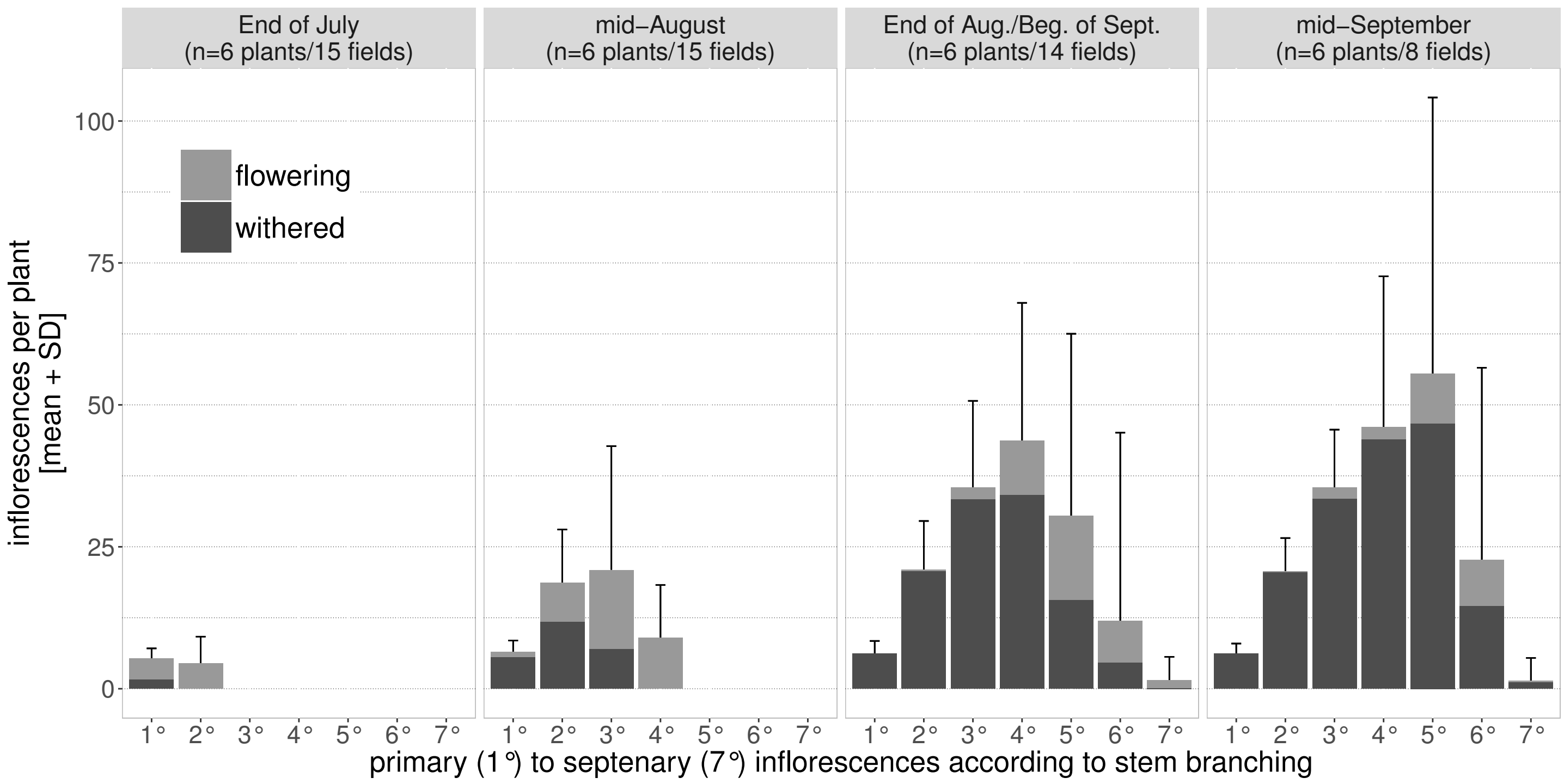


Table A.1 Model selection process of models on disk florets per inflorescence, pollen grains per disk floret as well as pollen grains per inflorescence of *Silphium perfoliatum* L.

Samples were taken from two plots (establishment in the years 2005 and 2010) and from five branching degrees (secondary [2°] to senary [6°] inflorescences) at the experimental station of the Thuringian State Institute for Agriculture (Thüringer Landesanstalt für Landwirtschaft, TLL) in Dornburg/Jena (Germany) in 2012. Generalized least squares fit by maximum likelihood was used for the comparison of models via likelihood ratio test (LRT). For all response variables, the comparison of model 2 (containing both factors) and model 3b (without factor branching degree) shows that dropping the factor branching degree worsens the explanatory power significantly. In contrast, the factor plot and its interaction with branching degree are statistically not significant.

df=degree of freedom, AIC=Akaike information criterion

model nr.	explanatory variables	df	AIC	test	L.Ratio	p-value
response variable: disk florets per inflorescence						
variance structure of the model:						
different variances per stratum for branching degree, levels: 2°, 3°, 4°, 5°, 6° inflorescences						
1	plot + branch. degree + plot:branch. degree	15	945.4			
2	plot + branch. degree	11	938.6	1 vs 2	1.228	0.874
3a	branch. degree	10	940.1	2 vs 3a	3.464	0.063
3b	plot	7	969.3	2 vs 3b	38.664	<0.001
4	without expl. variable	6	970.0	3a vs 4	37.935	<0.001
response variable: pollen grains per disk floret						
variance structure of the model:						
different variances per stratum for plot, levels: establishment in the years 2005 and 2010						
1	plot + branch. degree + plot: branch. degree	12	1865.7			
2	plot + branch. degree	8	1860.0	1 vs 2	2.379	0.666
3a	branch. degree	7	1858.1	2 vs 3a	0.033	0.856
3b	plot	4	1916.0	2 vs 3b	64.001	<0.001
4	without expl. variable	3	1914.1	3a vs 4	63.987	<0.001
response variable: pollen grains per inflorescence						
variance structure of the model:						
different variances per stratum for plot, levels: establishment in the years 2005 and 2010						
1	plot + branch. degree + plot: branch. degree	12	2919.5			
2	plot + branch. degree	8	2914.1	1 vs 2	2.636	0.621
3a	branch. degree	7	2915.3	2 vs 3a	3.156	0.076
3b	plot	4	2966.4	2 vs 3b	60.238	<0.001
4	without expl. variable	3	2966.2	3a vs 4	58.904	<0.001

Table A.2 Model selection process of models on nectar volume and sugar mass of *Silphium perfoliatum* L.

Samples were taken during two periods (beginning and end of August) and at different daytimes (five time categories of two-hour intervals between 7 AM and 5 PM) in a middle-aged plot (establishment in 2008) at the experimental station of the Thuringian State Institute for Agriculture (Thüringer Landesanstalt für Landwirtschaft, TLL) in Dornburg/Jena (Germany) in 2012. Linear mixed model fit by maximum likelihood was used for the comparison of models via likelihood ratio test (LRT). The inflorescences to whom the disk florets (that were sampled in different time categories) belonged to as well as the day of sampling formed the random structure. For both response variables, the comparison of model 1 (containing both factors) and model 2b (without factor time category) shows that dropping the factor time category worsens the explanatory power significantly. In contrast, the factor sampling period is statistically not significant.

df=degree of freedom, AIC=Akaike information criterion

model nr.	explanatory variables	df	AIC	test	L.Ratio	p-value
response variable: nectar volume per disk floret [µl] (square root transformed)						
1	sampl. period + time cat.	9	-140.2			
2a	time category	8	-141.8	1 vs 2a	0.449	0.503
2b	sampling period	5	-99.8	1 vs 2b	48.476	<0.001
3	without expl. variable	4	-101.2	2a vs 3	48.589	<0.001
response variable: sugar mass per disk floret [mg]						
1	sampl. period + time cat.	9	-238.9			
2a	time category	8	-240.5	1 vs 2b	0.414	0.520
2b	sampling period	5	-196.5	1 vs 2b	50.468	<0.001
3	without expl. variable	4	-197.9	2a vs 3	50.638	<0.001

Table A.3 to A.6 Estimating mean, minimum and maximum production of floral resources of *Silphium perfoliatum* L. over the flowering period

Numbers in bold indicate the branching degree that produced the highest number of inflorescences, number of pollen grains or nectar sugar mass, respectively, to a given sampling period.

Table A.3 Pollen grains and nectar sugar mass per inflorescence and branching degree of *S. perfoliatum*

The number of pollen grains per inflorescence and branching degree was calculated by multiplying the counted numbers of pollen grains per disk floret and disk florets per inflorescence. Nectar sugar mass per inflorescence was calculated by multiplying the predicted nectar sugar mass per disk floret with the counted number of disk florets per inflorescence.

branching degree* * primary (1°) and septenary (7°) inflorescences are disregarded	mean number of pollen grains per inflorescence	mean sugar mass per inflorescence [mg]
2°	2.2 x 10 ⁶	12
3°	2.1 x 10 ⁶	12
4°	1.9 x 10 ⁶	12
5°	1.5 x 10 ⁶	11
6°	1.1 x 10 ⁶	9

Table A.4 Inflorescences per plant and branching degree over the flowering period of *S. perfoliatum*

Six plants of *S. perfoliatum* were sampled per commercial field in Lower Saxony (Germany) and sampling period. In addition to the average number of all study sites, the number of inflorescences per plant and branching degree are given for the sites with the lowest and highest average number of inflorescences per plant in mid-September.

branching degree* * primary (1°) and septenary (7°) inflorescences are disregarded	mean number of inflorescences per plant (n=6 plant per commercial field)				mimum of sites (n=6)	maximum of sites (n=6)
	end of July (n=15 fields)	mid-August (n=15 fields)	end of Aug./ beg. of Sept. (n=14 fields)	mid-September (n=8 fields)	mid-September	mid-September
2°	4	19	21	21	21	24
3°	0	21	35	35	25	44
4°	0	9	44	46	5	83
5°	0	0	30	55	0	145
6°	0	0	12	23	0	103
sum	4	49	143	180	51	399

Table A.5 Estimated number of pollen grains per plant and branching degree over the flowering period
Numbers were calculated by considering values of table A3 and A4.

branching degree* * primary (1°) and septenary (7°) inflorescences are disregarded	estimated pollen supply [grain number/plant]				estimated minimum pollen supply	estimated maximum pollen supply
	end of July	mid-August	end of Aug./ beg. of Sept.	mid-September	mid-September	mid-September
2°	9.9 x 10⁶	42 x 10 ⁶	47 x 10 ⁶	46 x 10 ⁶	47 x 10 ⁶	53 x 10 ⁶
3°	0	44 x 10⁶	74 x 10 ⁶	74 x 10 ⁶	52 x 10 ⁶	92 x 10 ⁶
4°	0	17 x 10 ⁶	83 x 10⁶	87 x 10⁶	9 x 10 ⁶	157 x 10 ⁶
5°	0	0	45 x 10 ⁶	81 x 10 ⁶	0	213 x 10 ⁶
6°	0	0	13 x 10 ⁶	25 x 10 ⁶	0	112 x 10 ⁶
sum	9.9 x 10 ⁶	102 x 10 ⁶	261 x 10 ⁶	314 x 10 ⁶	109 x 10 ⁶	627 x 10 ⁶
plants/ha	40,000	40,000	40,000	40,000	40,000	40,000
estim. pollen supply [grain number/ha]	0.4 x 10 ¹²	4.1 x 10 ¹²	10.5 x 10 ¹²	12.5 x 10 ¹²	4.4 x 10 ¹²	25.1 x 10 ¹²
Increase [pollen grains/ha]		3.7 x 10 ¹²	6.4 x 10 ¹²	2.1 x 10 ¹²		20.7 x 10 ¹²
[% on total supply]		29	51	17		

Table A.6 Estimated nectar sugar mass per plant and branching degree over the flowering period
Amounts were calculated by considering values of table A3 and A4.

branching degree* * primary (1°) and septenary (7°) inflorescences are disregarded	estimated sugar supply [mg/plant]				estimated minimum sugar supply	estimated maximum sugar supply
	end of July	mid-August	end of Aug./ beg. of Sept.	mid-September	mid-September	mid-September
2°	51	217	244	240	247	276
3°	0	259	439	439	309	547
4°	0	105	512	539	59	969
5°	0	0	329	599	0	1,566
6°	0	0	103	195	0	886
sum	51	580	1,627	2,012	615	4,244
plants/ha	40,000	40,000	40,000	40,000	40,000	40,000
estim. sugar supply [kg/ha]	2	23	65	80	25	170
increase [kg/ha]		21	42	15		145
[% on total supply]		26	52	19		