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'Coloring Foods' - development of a suitable cultivation and harvesting system for florets of safflower (Carthamus tinctorius L.)

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by

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List of Abbreviations

°C	degree Celcius
a.m.	ante meridiem, before midday
B.C.	before Christ
BMEL	Bundesministerium für Ernährung und Landwirtschaft, Federal Ministry of Food and Agriculture
CROPGRO	template crop module in DSSAT
d-index	Willmot Agreement Index
DLG	Deutsche Landwirtschafts-Gesellschaft, German Society of Agriculture
DSSAT	decision support system for agrotechnology transfer
EEA	European Environment Agency
e.g.	exempli gratia, for example
et al.	et alii, and others
E-Number	number for an authorized additive in the EU
EU	European Union
F1	first generation in breeding
F2	second generation in breeding
FAO	Food and Agriculture Organization of the United Nations
FNR	Fachagentur Nachwachsende Rohstoffe, Agency for Renewable Resources
FSA	Food Standards Agency
ha	hectare
HIPIN	initial pod harvest index
HIPMX	maximum pod harvest index
PETALX	ratio of floret weight to capitulum weight
p.m.	post meridian, afternoons
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RMSE	root mean square error
SME	Small and Medium Enterprise
SDLIP	potential seed lipid (g (oil) g (seed)$^{-1}$)
SDPRO	Potential seed protein (g (protein) g (seed)$^{-1}$)
t	ton
USA	United States of America
USD	US-Dollar

UV-B	ultraviolet B-rays

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1. Introduction

1.1 Natural colorants

The term 'natural colorant' is not legally accepted or exactly defined in the USA or the EU (Lehto et al., 2017; Viera et al., 2019). In general, natural colorants are defined as pigments derived from renewable resources such as plants, minerals, fungi, animals or micro-organisms (Adeel et al., 2017; Hendry, 1996). The classification of natural colorants is either based on their sources, their color, their application or their chemical structure, which is the most recognized classification method (Adeel et al., 2017; Kumar and Sinha, 2004; Yusuf et al., 2017). The classification according to the chemical structure includes e.g. the plant based carotenoids, chlorophylls, indigoids, tannins or flavonoids (Adeel et al., 2017; Kumar and Sinha, 2004; Yusuf et al., 2017). The largest group of plant colorants are the flavonoids, which are mainly known for their yellow/orange coloration, but also for their pharmaceutical relevance (Adeel et al., 2017; Delshad et al., 2018; Kumar and Sinha, 2004; Yusuf et al., 2017).

Natural colorants have been used for dyeing and painting for a long time (Adeel et al., 2017; Cordon, 2009; Kumar and Sinha, 2004; Patel, 2011). As an example already around 1000 B.C. berries or roots were used for dyeing fibers (Sequin-Frey, 1981). Natural colorants from insects, plants, minerals and molluscs used to be the only way to color cosmetics, clothing, pharmaceuticals or food (Glover, 1998; Melo, 2009; Shahid et al., 2013; Yusuf et al., 2017). This changed in 1856 with the invention of the synthetic colorant ‘Mauveine’ by William Henry Perkin (Adeel et al., 2017; Cordon, 2009; Garfield, 2002). Due to the cost factor, the lightness of dyeing, the reproducibility of many shades and the broad field of application, the use of natural colors decreased rapidly (Holme, 2006; Kumar and Sinha, 2004; Shahid et al., 2013). In recent decades, various studies have linked synthetic colorants more and more to the fact that they are, for example, harmful to the environment, carcinogenic or allergy triggers, which has reduced their application e.g. in the food as well as in the textile sector (Kumar and Sinha, 2004; Shahid et al., 2013; Yusuf et al., 2017).

To protect the environment and human health, some synthetic colorants, for example, azo colorants, are restricted by the REACH regulation of the EU (European Chemicals Agency, 2020). This and the fact that the public interest in healthy and biodegradable goods is increasing, the trend is towards natural products, such as natural colorants (Coultate and Blackburn, 2018; Kumar and Sinha, 2004; Shahid et al., 2012; Shahid et al., 2013). This is shown, for example, in a pan-European study by the GNT Group in 2016, which showed that over 30% of those surveyed believe that natural colorings are important and that 46% would be more willing to pay more for snacks, if they contained no artificial colors (GNT Group B.V.,

2016). Furthermore, 50% of respondents stated that they would buy snacks more often, if they were produced with natural colorants (GNT Group B.V., 2016). Another study showed in 2016 that artificial colors are rejected by consumers in dairy products (Ingredion Germany GmbH, 2016). According to a study by the Nielsen company with more than 30.000 participants around the world, 61% of respondents said they endeavor to avoid food with artificial colorings (The Nielsen Company, 2016). The revival of natural colorants is reflected in the rising of global revenues, which market research institutes estimate will grow to USD 5 billion by 2024 (Arizton Advisory & Intelligence, 2019).

Besides the many advantages of natural colorants, such as being eco-friendly or non-toxic, there are also some disadvantages (Shahid et al., 2013; Sigurdson et al., 2017; Yusuf et al., 2017). These are for example their price, colour yield, availability, cultivation and dyeing efficiency or their limitation of shades and stability (Glover, 1998; Kumar and Sinha, 2004; Rodriguez-Amaya, 2016; Wrolstad and Culver, 2012).

1.2 Natural food colorants

A large share of the natural colorants are natural food colorants, which, according to a market analysis report in 2016, account for over 80% of revenue (Grand View Research, 2018). The demand for natural food colorants is increasing worldwide, which is reflected in the market size of USD 1.32 billion in 2015 and a predicted rapid growth until 2025 (Grand View Research, 2017). According to the report, the food industry, but above all the beverage industry, is expected to play a decisive role in the natural food colorant market in Europe in the forecast period (Grand View Research, 2017). Other market research institutes have come up with similar predictions and expect the market for natural colorants to grow at a rate of 6.3% from 2020 onwards, so that by 2024 sales of USD 1.5 billion are likely to be achieved (Research and Markets, 2020; vegconomist, 2019).

The increasing revenues in the natural food coloring sector reflect the importance of colors for the marketing of foods. Color alone is responsible for 62–90% of consumer evaluations (Singh, 2006). According to the DLG, the appearance of a product, which includes color, is usually the first sensory characteristic that is registered (Derndorfer and Gruber, 2017). This color aspect is the most important sensory impression, which directly affects the acceptance, preferences and selection of a food product of the consumers (Delgado-Vargas and Paredes-Lopez, 2003; Martins et al., 2016; Shim et al., 2011). Another important aspect is that customers connect food safety with the color of the products, which should have specific colors, e.g. bananas should be yellow (Attokaran, 2017; Delgado-Vargas and Paredes-Lopez, 2003). Especially in products for children, such as sweets, color plays an important role (Attokaran, 2017). The association of color with safety as well as, for example, the

increasing environmental awareness, led to the fact, that consumers, producers as well as the government tend towards natural food colorants (Shahid et al., 2013; Viera et al., 2019; Yusuf et al., 2017).

This can be seen in the now small proportion of artificial colorings used in food, which is still 12–32% in North America and only 3–16% in Europe (Mintel Group Ltd., 2016; Simon et al., 2017; Witham, 2016). This data also results from the regulations for color additives, which exist, for example, in the EU. The EU, Regulation (EU) No. 1333/2008 mainly determines which food colors are allowed and under which conditions they can be applied, although there are other regulations for example for the labelling (European Parliament, Council of the European Union, 2008; Lehto et al., 2017; Oplatowska-Stachowiak and Elliott, 2017). In 2013, the European Commission adopted the guideline ‘Guidance notes on the classification of food extracts with coloring properties’, which distinguishes between foods with coloring properties (‘Coloring Foods’) and colorants, which are classified as additives (European Commission, 2013; GNT Group B.V., 2013). Additives require a legal authorization and must be listed in the list of ingredients as ‘Colorant’ with e.g. their E-number, whereas ‘Coloring Foods’ do not require such approval (European Commission, 2013; GNT Group B.V., 2013). This guideline had an impact on the food coloring industry, which was reflected in the fact that already in 2016 14% of food colorants were covered by ‘Coloring Foods’ (Mintel Group Ltd., 2016; Simon et al., 2017). Therefore, the food coloring industry is now attaching great importance to 'Coloring Foods', such as colorants from black carrots, spirulina, radishes or annatto (Chr. Hansen Holding GmbH, 2014; Colourfood Professional, 2020; GNT Group B.V., 2018).

1.3 Safflower as natural food colorant

Safflower (*Carthamus tinctorius* L.) is a plant that is used as a food colorant and belongs to the group of 'Coloring Foods'. Safflower is an annual or winter-annual thistle-like plant and belongs to the family of *Asteraceae* (Emongor, 2010). The plant size can vary between 0.3 and around 2.0 m and produces branches (up to tertiary), each with a globular flower head (capitulum) with flowers (florets) from white to yellow, orange to red (Dajue and Mündel, 1996; Emongor, 2010; Weiss, 2000). It has a deep taproot up to 3 m deep, which makes it tolerant to water stress (Emongor, 2010; Sirel and Aytac, 2016). Safflower is also characterized by the fact that it tolerates salty soil conditions and a wide temperature range from -7 to 40 °C (Bassil and Kaffka, 2002; Emongor, 2010; Emongor et al., 2015; Khalili et al., 2014; Mündel et al., 2004). Due to this, safflower is cultivated all over the world, mainly in regions with low rainfall (Arnon, 1972; Singh and Nimbkar, 2016; Sirel and Aytac, 2016).

Safflower has many applications, e.g. as medicinal plant, tea, livestock forage, birdseed, or cut flower (Ekin, 2005; Emongor, 2010). The latinized name of safflower (*Carthamus*) already indicates the original use of safflower with the derivation from the Arabic ‘quartum’, which is related to the pigment obtained from safflower flowers (Singh and Nimbkar, 2007). In the field of garment coloring, as in cosmetics and also in the industry of carpet-weaving safflower played an important role (Dajue and Mündel, 1996; Weiss, 2000).

Due to the high price of saffron, safflower is known as a food colorant substitute, which is also reflected in the well-known name of safflower as 'false saffron’ (Nobakht et al., 2000; Weiss, 2000). It was used e.g. in soups, rice or bread to give them a more appetizing color (Weiss, 2000). Before the 18th century, safflower from Egypt was utilized to color mainly cheese and sausage in Great Britain, France and Italy (Dajue and Mündel, 1996). From the middle of the 19th century, with the invention of 'Mauveine' as an artificial coloring agent, the use of natural food colorants slumped almost completely in the 20th century (Emongor and Oagile, 2017; Garfield, 2002; Singh and Nimbkar, 2007).

Due to the increasing interest in ‘Coloring Foods’, the demand for safflower florets is rising (Singh and Nimbkar, 2007). Before the 'Guidance notes on the classification of food extracts with coloring properties', spinach or stinging nettle, for example, were often used for green coloring, which according to the Guidance notes do not meet the criteria of a 'Coloring Food' (European Commission, 2013; Wiley, 2015). Since then a combination of spirulina and safflower has been used to achieve green tones (Colourfood Professional, 2020; Viera et al., 2019; Wiley, 2015). In the Guidance notes, the definition of 'Coloring Food' is based, for example, on the 'enrichment factor' (selective or non-selective extraction) (European Commission, 2013). Thus, in addition to spinach and stinging nettle, the previously available curcuma or paprika are also included to the ones which do not meet the criteria, making safflower also an attractive alternative for various shades of yellow and orange (Colourfood Professional, 2020; Wiley, 2015).

1.3.1 Coloring pigments in safflower

Until now, more than 200 compounds have been extracted from safflower, most of which are flavonoids (Guo et al., 2017; Salem et al., 2011; Tu et al., 2015). The flavonoids are divided into further subclasses such as flavones, isoflavones, anthocyanins or chalcones, which are the major compounds in safflower (Adeel et al., 2017; Kumar and Sinha, 2004; Salem et al., 2011).

Safflower has both red and yellow pigments, which are almost all classified as C-glycosyl quinochalcone (Asgarpanah and Kazemivash, 2013; Kazuma et al., 2000; Yue et al., 2013; Yue et al., 2014). Among the red pigments, carthamin is the predominant pigment (Bernard

et al., 2011; Kazuma et al., 2000; Obara and Onodera, 1979). It is mainly used for the cosmetics, textile and medicine industry, but because of its low solubility in water it is of minor importance for the food industry and is mainly used for coloring of chocolate, for example (Bernard et al., 2011; Hanagata et al., 1992; Meselhy et al., 1993; Watanabe et al., 1997). The yellow pigments of safflower, on the other hand, are water-soluble, which is why they can and are used in a variety of foods, such as for coloring beverages, sweets, yogurt (Bernard et al., 2011; Francis, 1996; Henry, 1996; Watanabe et al., 1997; Yoon et al., 2003).

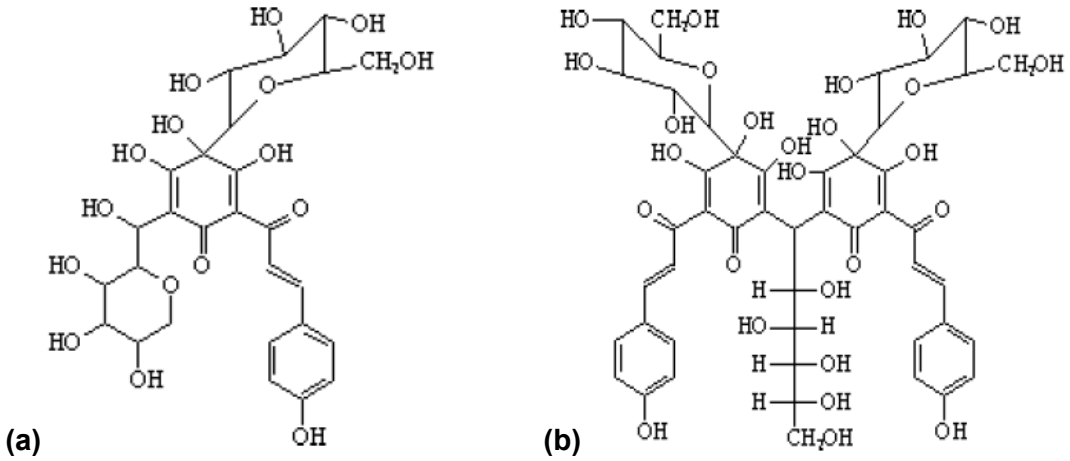

Figure 1: Structural formula of hydroxysaffor yellow (a) and safflor yellow B (b). Reference: (FAO, 1998)

The yellow pigments in safflower are, for example, safflor yellow A, safflomin C, tinctormin or precarthamidin (Kumazawa et al., 1994; Meselhy et al., 1993; Onodera et al., 1989; Takahashi et al., 1982). Hydroxysafflor A and safflor yellow B are the main coloring matters of carthamus yellow and can be grouped and extracted as carthamidin (Figure 1) (FAO, 1998; Meselhy et al., 1993; Mohammadi and Tavakoli, 2015; Takahashi et al., 1984).

Its advantages in comparison to other colorants such as stable to light in aqueous solution, the use in different pH-values and temperatures, make the yellow pigments of safflower an attractive alternative compared to other colorants (Shin and Yoo, 2012; Yoon et al., 2003). As a result, the yellow pigments of safflower are increasingly used in the food industry, e.g. in cheese, fruit syrups or pastries, especially as it can be used as an alternative for the colorants tetrazine (E102) and quinoline yellow (E104) (Dajue L., 1993; Ekin, 2005; FSA, 2018; Fusaro, 2010; Wiley, 2015).

1.3.2 Safflower cultivation worldwide

Safflower is one of the oldest crops used by humans which was already used in China 2200 years ago (Emongor, 2010). Safflower seeds were found in Egyptian graves 4000 years ago (Emongor, 2010; Gyulai, 1996). Originally, safflower was cultivated as a natural colorant in

Africa from the Nile valley to Ethiopia and from China to the Mediterranean region (El Bassam, 2010; Emongor, 2010; Singh and Nimbkar, 2016; Weiss, 1971). For its medicinal and coloring aim it is now only cultivated in a few countries like in Turkey, Iran and China, but only with around 2000 t of flowers in e.g. China (Zhaomu and Lijie, 2001). Currently safflower is mainly cultivated for its seeds containing high quality oil e.g. in Canada, India, Australia, Russia, Spain or Turkey with a worldwide harvested area of about 700.000 ha (Chakradhari et al., 2020; FAO, 2018; Singh and Nimbkar, 2016; Sirel and Aytac, 2016).

The first historically mentioned cultivation sites of safflower in Germany are in Bad Boll (Baden-Württemberg) and in Eichstätt (Bavaria) in the 16th century (terra fusca GbR, 2005). In the 17th and 18th century safflower was cultivated on a large scale as a coloring plant in Thuringia, Alsace and partly also in the Palatinate (Dallinger, 1800; Körber-Grohne, 1988; Pude et al., 2012; terra fusca GbR, 2005). From the middle of the 18th century onwards, cultivation in Germany declined due to imports from the East and finally came to a total stop with the invention of aniline colors around 1850 (Garfield, 2002; Pude et al., 2012; terra fusca GbR, 2005).

Due to the increasing demand for safflower florets, especially in the food and pharmaceutical industries, the existing cultivation area and obtained yields will be no longer sufficient (Bernard et al., 2011; FAO, 2018; Fatahi et al., 2009; Singh and Nimbkar, 2007; Zhaomu and Lijie, 2001). In addition, the growing interest in regional foods could lead to the fact, that ‘Coloring Foods’ should in future also be grown in the country where they are processed. As shown in a study by the German Federal Ministry of Food and Agriculture, which confirmed that 83% of those questioned, requested it (BMEL, 2020). The cultivation of safflower florets in Europe could contribute to a more beautiful landscape, an increasing biodiversity and crop rotation and also could be an alternative in the organic agriculture (terra fusca GbR, 2005). As safflower has already been cultivated in Germany in the past as a coloring plant, the FNR regarded safflower as suitable for cultivation and coloring and as the demand for both regional and ‘Coloring Foods’ is increasing, safflower florets cultivation would be interesting again in Europe, e.g. in Germany (Biertümpfel et al., 2013; BMEL, 2020; Mintel Group Ltd., 2016; Simon et al., 2017; terra fusca GbR, 2005).

However, cultivation guidelines and mechanical harvesting must be developed in order to make the growing supply in Europe sustainable and economical (terra fusca GbR, 2005; Yun et al., 2016)

1.4 Cultivation parameters and harvest of safflower for florets

For the optimization of floret yield and therefore also the color content and color yield, several factors are decisive, such as the cultivar, the cultivation system (e.g. row spacing and sowing density), the harvest date and also the general environment (Azari and Khajehpour, 2005; Camaş and Esendal, 2006; Kizil et al., 2008; Mohammadi and Tavakoli, 2015). To enable a successful cultivation of safflower florets under local conditions, it is therefore necessary to investigate some of these parameters such as different cultivars, sowing densities or harvest dates.

Cultivar selection and origin are important factors that influence flower/floret color, floret yield, carthamidin content and carthamidin yield (Hamza, 2015; Kizil et al., 2008; Knowles, 1969; Mohammadi and Tavakoli, 2015). The number of branches and capitula, which in turn influence the flower yield, are also affected by the cultivar (Hamza, 2015; Marchione, 1997; Singh et al., 2008). In countries such as Iran, Turkey, India, Egypt or Tunisia, where safflower has already been grown or tested with local cultivars for floret yield, yields range, depending on cultivar, between 75 and 630 kg ha^{-1} (Hamza, 2015; Mohammadi and Tavakoli, 2015; Nagaraj, 2009; Omidi and Sharifmoghaddasi, 2010). From this list of countries it can be concluded that these are mainly countries with lower rainfall and higher temperatures, which are favored by safflower and could lead to higher yields (Armah-Agyeman et al., 2002; Arnon, 1972; Koutroubas et al., 2009; Sirel and Aytac, 2016). Reasons for this include the susceptibility of safflower to *Botrytis* or foliar diseases like *Alternaria*, which are more likely to occur in humid growing conditions (Biertümpfel et al., 2013; Emongor and Oagile, 2017; Singh and Nimbkar, 2007). Due to the different susceptibility of the cultivars to diseases, their different yield, which in turn depends on the environment, the testing of different cultivars under local conditions is indispensable to prepare the cultivation for farmers in an attractive way (Camaş and Esendal, 2006; Elfadl et al., 2012; Hamza, 2015).

Another decisive factor in the cultivation of safflower for the production of florets is the cultivation system. As an example the selection of the correct row spacing and sowing density can be mentioned. Both parameters influence the number of branches and the number of capitula and therefore determine the floret yield (Köse and Bilir, 2017; Sharifmoghaddasi and Omidi, 2009; Singh et al., 2008; Zheng et al., 1993b). Most studies show that plants with more space (lower sowing density and larger row spacing) produce more branches and more capitula (Caliskan and Caliskan, 2018; Hamza, 2015; Sharifmoghaddasi and Omidi, 2009). Most studies test the cultivation of safflower in row spacing between 15–60 cm, a sowing density between 8–50 plants m^{-2} (Azari and Khajehpour, 2005; Caliskan and Caliskan, 2018; Hamza, 2015; Patanè et al., 2020). The

environment has a decisive influence on the floret yield and cultivation practices such as row spacing and sowing density, which should be chosen according to location and growing conditions (Caliskan and Caliskan, 2018; Köse and Bilir, 2017; Mohammadi and Karimizadeh, 2013). Therefore, it is important to test and to adapt them depending on the cultivation conditions in Europe.

In addition, the optimal harvest date has to be evaluated, as this has an impact on the floret yield, the color content and therefore also on the color yield (Kizil et al., 2008; Mohammadi and Tavakoli, 2015). Moreover, the optimal harvest date also depends on the cultivar (Kizil et al., 2008). Depending on the cultivar, the peak of the floret yield is reached when most of the capitula are flowering, therefore mid-season harvests are more likely to achieve the highest and best yield, which could be due to the successive flowering of the secondary and tertiary capitula (Dajue and Mündel, 1996; Emongor and Oagile, 2017; Kizil et al., 2008). In contrast, the highest carthamidin contents are reached at the beginning of flowering, when the yellow colorant has not yet been oxidatively degraded (Ghorbani et al., 2015; Mohammadi and Tavakoli, 2015). Due to the strong influence of the environment on the flowering time and thus also the possible optimal harvest date (Dajue and Mündel, 1996; Emongor and Oagile, 2017), cultivation of safflower florets should be tested under the respective cultivation conditions.

In the countries where safflower is cultivated for floret production, e.g. Iran or China, the florets are harvested by hand, which is time and personnel intensive and expensive (Azimi et al., 2012). Since there is no industrially produced harvesting machine for harvesting safflower florets, harvesting and a large-scale production in e.g. Germany would be not economical (Azimi et al., 2012; terra fusca GbR, 2005; Yun et al., 2016). In general, due to the increasing demand for ‘Coloring Foods’ in the EU, there is a need to test the cultivation and harvesting of safflower florets in Europe.

1.5 Aim of study and objectives

The overall aim of this work was to contribute to the expansion of the natural raw material supply for food colorants. This was tested on the example of safflower to check the potential of this plant and to show the possible limitations, which lead to the fact that the plant is not yet used for the production of florets in Europa. The aim of this work was therefore to identify the existing barriers that limit its cultivation in Europe and to show which developments are necessary to remove them.

The main limitations are on the one hand the lack of cultivation guidelines for other regions like Europe and on the other hand the lack of technology for mechanical harvesting to be able to make the cultivation economical. Therefore, one focus of this work was on the cultivation method in order to test various parameters that are crucial for successful cultivation under different climatic conditions. For this purpose, different cultivars, row spacing and sowing densities were tested at different harvest dates. In addition to the growing conditions, there is also the economic aspect, which is mainly limited by the lack of mechanical harvesting, which plays a decisive role in establishing the cultivation of safflower for florets in Europe. Therefore, in order to keep the development costs low and increase the attractiveness for farmers, the mechanical floret harvest was carried out with a combine harvester with the parameters that are decisive such as different cultivars, harvest times and threshing parameter settings.
New production systems or new directions of use, like the cultivation of safflower for floret production, create many uncertainties regarding the development and yield of a crop. In order to promote the cultivation of safflower for floret production in Europe in the future and to make it attractive for farmers, long-term cultivation and yield estimates would be required. As these are very time and labor intensive and yet very specific to the tested location and season, crop growth modeling tools, such as DSSAT CROPGRO, have been developed which can be used to simulate for example the cultivation of safflower under European conditions.

Based on the given challenges for the cultivation of safflower as food colorant the specific objectives were:

(1) to examine the effect of different cultivars, row spacing, sowing densities and harvest dates on yield parameters for safflower floret production under European conditions,

(2) to investigate a mechanical harvest with a combine harvester with regard to quality parameters, threshing and carthamidin yield,

(3) to evaluate and modify the DSSAT CROPGRO safflower model to simulate floret yield under European conditions.

Two field experiments were conducted, each for two years in 2017 and 2018 at the experimental station ‘Ihinger Hof’ of the University of Hohenheim. One field trial aimed at the general parameters that influence the cultivation system, while the other field experiment focused on the development of a mechanical harvesting system. The project was funded by the German Federal Ministry for Economic Affairs and Energy within the Central Innovation Program for SMEs. This led to the cooperation with the mid-sized enterprise Zürn Harvesting

GmbH & Co. KG, who carried out the mechanical harvest with their combine harvester. With the results of the two field experiments three publications could be achieved, which are presented in the following three chapters of this thesis. **Publication I** covers different cultivation parameters like cultivar, sowing density and row spacing and their effect on number of branches and capitula. Due to the different development of cultivars and colorants, the harvest date (five per period) was added as an additional parameter to further investigate the influence of this parameter on yield traits such as floret yield, carthamidin content and carthamidin yield. In **Publication II** the performance of different threshing parameter settings of a combine harvester on two cultivars, which were harvested on different harvest dates was tested and evaluated. In addition, the aim was to give some preliminary recommendations on the cultivar properties which could be important for mechanical harvesting. **Publication III** deals with the modification of the plant simulation model DSSAT CROPGRO for the simulation of safflower yield (seeds) and the integration of a new subroutine to predict the floret yield of safflower for two different cultivars and years.

The results of the present thesis are represented in publication I–III, which are integrated in sections 3–5. These three publications are already published in peer-reviewed journals. As an extension to the points already discussed in the publications, further aspects are discussed in the general discussion, which is included in section 6. General aspects such as climate change and how it could affect the cultivation of safflower in Europe and also the possibility of growing cultivars of other origins in Europe are discussed. Breeding is also discussed as a way to find more suitable, more resistant cultivars for both cultivation and mechanical harvesting. Also modeling as a tool to test different cultivars, different regions and also the possibility to use modeling and the new subroutine for other coloring foods or other substances of plants are discussed. Also the potential of other colorants is discussed and an outlook on further needed fields of research is given. Finally, section 7 contains the summary of the entire study.

2. Publications

The current thesis includes three scientific articles, which have been published in peer-reviewed journals. These three articles are the main part of this thesis. A list of further publications from conferences can be found in the appendix (Table A1). To cite these three articles, please use the references below.

Publication I (published, Impact Factor 2.603):

Steberl, K., Hartung, J., Munz, S., Graeff-Hönninger, S. (2020): Effect of Row Spacing, Sowing Density, and Harvest Time on Floret Yield and Yield Components of Two Safflower Cultivars Grown in Southwestern Germany. Agronomy 10 (5), 664

DOI: 10.3390/agronomy10050664

Publication II (published, Impact Factor 2.603):

Steberl, K., Hartung, J., Graeff-Hönninger, S. (2020): Impact of Cultivar, Harvest Date and Threshing Parameter Settings on Floret and Carthamidin Yield of Safflower. Agronomy 10 (9), 1272

DOI: 10.3390/agronomy10091272

Publication III (published, Impact Factor 2.603):

Steberl, K., Boote, K.J., Munz,S., Graeff-Hönninger, S. (2020): Modifying the CROPGRO Safflower Model to Simulate Growth, Seed and Floret Yield under Field Conditions in Southwestern Germany. Agronomy 10 (1), 11

DOI: 10.3390/agronomy10010011

3. Effect of Row Spacing, Sowing Density, and Harvest Time on Floret Yield and Yield Components of Two Safflower Cultivars Grown in Southwestern Germany

Publication I:

Steberl, K., Hartung, J., Munz, S., Graeff-Hönninger, S. (2020): Effect of Row Spacing, Sowing Density, and Harvest Time on Floret Yield and Yield Components of Two Safflower Cultivars Grown in Southwestern Germany. Agronomy 10 (5), 664

Although the demand of the industry for natural colorants such as safflower is increasing, there is currently no cultivation of safflower florets in Germany due to a lack of cultivation recommendations. Therefore the current demand of safflower florets is mainly covered by deliveries from Asia. In order to be able to cover the future rising demand for safflower florets on a regional and sustainable way, basic cultivation guidelines are needed. According to this, the publication "Effect of row spacing, sowing density, and harvest time on floret yield and yield components of two safflower cultivars grown in southwestern Germany" examined two different row spacing and sowing densities at five harvest dates and their influence on parameters such as floret yield and its carthamidin content. Carthamidin yield in particular is highlighted as a decisive marketing parameter.

agronomy

MDPI

Article

Effect of Row Spacing, Sowing Density, and Harvest Time on Floret Yield and Yield Components of Two Safflower Cultivars Grown in Southwestern Germany

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Abstract: The current demand for safflower florets (*Carthamus tinctorius* L.) in the food-coloring industry, especially in Europe, is rising. The present production, mainly located in China, is not sufficient. Unlike for the production of seeds, there are currently no recommendations for the cultivation of safflower for floret production in Germany. Therefore, field experiments were conducted at the experimental station Ihinger Hof, Southwestern Germany, in 2017 and 2018. The aim was to evaluate yield and yield parameters, such as number of capitula, floret yield, and carthamidin content for (i) two cultivars grown with (ii) two row spacing (12 and 33 cm) using (iii) two sowing densities (40 and 75 plants m^{-2}), and (iv) five harvest dates. Results showed that lower sowing densities resulted in a significantly larger number of branches and capitula per plant and higher yields of florets and carthamidin. Harvesting two to three weeks after flowering resulted in the significantly highest floret and carthamidin yields. More capitula per plant, higher carthamidin contents, and higher floret and carthamidin yields were obtained with the Chinese cultivar. In general, yields of flowering florets (2.30–468.96 kg ha^{-1}), carthamidin contents (2.53–8.29%), and carthamidin yields (0.04–37.86 kg ha^{-1}) were comparable to or higher than in other studies. In conclusion, this study showed that safflower has great potential for the production of florets in Southwest Germany, for the food-color industry.

Keywords: *Carthamus tinctorius* L.; safflower; row spacing; sowing density; harvest time; floret yield; carthamidin yield

1. Introduction

Safflower (*Carthamus tinctorius* L.) belongs to the Asteraceae family and is one of the oldest crops used by humans [1–3]. It is an annual thistle-like plant, which can reach a height between 0.3 and 2.1 m [1,3,4]. Safflower produces primary, secondary, and tertiary branches, each with a globular flower head (capitulum) at its end, with yellow, orange, or red flowers (florets) [1,3,4]. Safflower is a multifunctional plant used, for example, as bird seed, a medicinal plant, livestock forage, tea, or as cut flowers [1,5–7]. Currently, safflower is mainly grown for its oil, which is rich in bioactive compounds and highly polyunsaturated fatty acids [1,7–9]. In addition, safflower has traditionally been used for its flowers, which were applied as colorant for textiles and foods [1,2,7,9]. After the invention of Mauveine in 1856, which is derived from aniline and therefore cheaper, the need for natural dyes decreased [4,10]. Nowadays, cultivation of safflower as colorant take place, e.g., in China, but other uses were more important globally [4,11].

However, the demand for healthier, safer food due to allergic or carcinogenic effects which can be caused by artificial colorants and the need for biodegradable colorants increased the interest in natural

food colorants [12–15]. Since 2013, the new EU directives "Guidance notes on the classification of food extracts with coloring properties" distinguish between "dyes", which are regarded as additives and require a legal approval and "Coloring Foods", which are not subject to any additional approval [9,16–18]. Therefore, interest in natural dyes increased even more [9,16,17,19]. According to the directives of the EU the food-coloring industry considers safflower as a suitable yellow- and orange-coloring alternative because it has a low enrichment factor compared to the conventionally used curcuma or paprika extracts [16,20]. The yellow colorants of safflower are increasingly used in cheese, sausages, pastries, candies, fruit syrups, and fruit juices [5,21]. Safflower can be used, for example, as a natural replacement for two of the "Southampton Six" (connection to hyperactivity in children) color additives for confectionery products, Tartrazine (E102) and Quinoline Yellow (E104) [22,23]. The yellow pigment of safflower has many advantages compared to other colorants, e.g., highly soluble in water, cheaper than saffron, stable to light in aqueous solutions, and it can be used at different temperatures and pH values [24,25].

So far, the demand for safflower florets is mainly met by deliveries from Asia [4,26]. However, the existing cultivation area is not sufficient to meet the increased demand [9,19,27,28]. Therefore, cultivation guidelines for other regions must be developed to cover the demand regionally and sustainably.

Many factors, such as environment, cultivar, harvest time, and cultivation system, play an important role in optimizing floret yield and their color content, finally determining color yield [29–32]. One of the major factors is the selection of cultivar and its origin, which influences the flower color and the number of capitula and branches [29,30,33,34]. The number of capitula and branches, in turn, depends on agronomic practices like sowing density and row spacing and thus impacts the final floret yield [35–38]. The numbers of capitula and branches are further influenced by environmental conditions, as it was shown in a study of Kizil et al. [30] in which more rain resulted in an increased number of these. On the other hand, drier conditions could also have a positive effect, with an increase in secondary phytochemicals, including colorants [39,40]. Agronomic practices like sowing density and row spacing play an important role for growth and productivity of safflower, and it is essential to determine them for different locations and growing conditions [37,38,41,42]. Furthermore, the color content and the floret yield varies with the cultivar and also depends on the harvest time [29,30,34,36].

The cultivation under Southwestern Germany conditions for floret yield has not been assessed yet and therefore requires testing of the harvest dates and cultivation methods compared to oil production. Therefore, the objectives of the present study were to identify the impact of different (i) row spacing, (ii) sowing densities, and (iii) harvest times on yield parameters like number of capitula and branches, floret yield, color content, and color yield of two different safflower cultivars under the pedoclimatic conditions of Southwestern Germany.

2. Materials and Methods

2.1. Plant Material

Two different cultivars of *Carthamus tinctorius* L. were used in two field trials over two years. Both cultivars are used especially for floret production, but show differences in flower color and origin. The German cultivar cv. "Goldschopf" (Gartenland Produkion GmbH, Aschersleben, Germany) was used. The seeds of the Chinese cultivar were provided by a food-coloring company. The German cultivar (C1) is characterized as spiny, with primarily yellow florets, while the Chinese cultivar (C2) is thornless and has orange florets (Figure 1).

(**a**) (**b**)

Figure 1. The two cultivars of *Carthamus tinctorius* L. used in the field trials: (**a**) German, spiny cultivar; (**b**) Chinese, thornless cultivar.

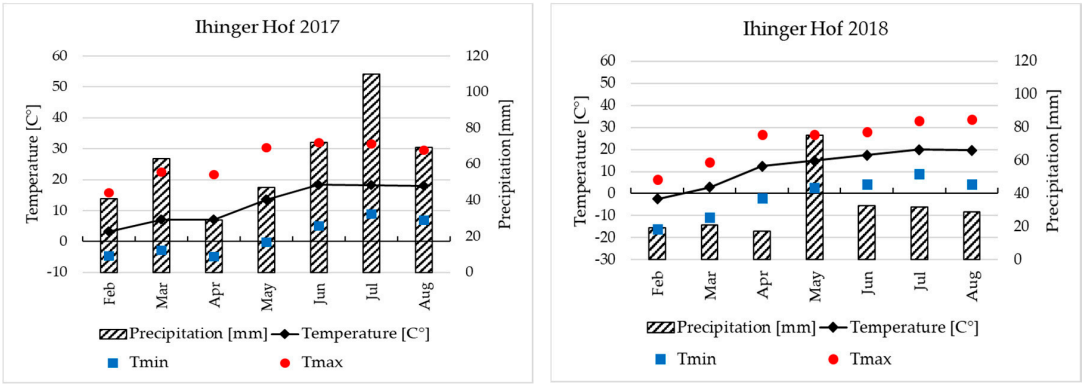
2.2. Field Site Characteristics

The field trials were conducted at the experimental station Ihinger Hof (48°44′ N, 8°55′ E, 478 m a.s.l.) of the University of Hohenheim, in Southwestern Germany, in 2017 and 2018.

According to the World Reference Base, the soils can be classified as vertic Luvisol in 2017 and vertic Cambisol in 2018 [43,44]. Both soil types are known to be fertile and appropriate for growing many different types of crops [43,44]. Soil textures were determined in the depths of 0–30, 30–60, and 60–90 cm, according to Köhn [45]. The clay content was on average 30.5%, the sand content 3%, and the silt content 66.5%. The mineral nitrogen content of the soil (N_{min}) was measured according to Thun and Hoffmann [46], using a flow injection analyzer (FIAstar 5000 Analyzer, FOSS GmbH, Hamburg, Germany). The mineral nitrogen contents of the two years were different and resulted in 124 kg ha^{-1} in 2017 and 45 kg ha^{-1} in 2018 for 0–90 cm depth.

The average long-term (8 years) temperature at the experimental site is 9.6 °C, with an average annual precipitation of 683.4 mm. In comparison, the experimental year 2017 had both a lower annual average temperature of 9.2 °C and a lower annual precipitation of 653.9 mm. In 2018, however, the annual rainfall was even lower with 525.9 mm, while the average temperature of 10.2 °C was higher than the long-term average. Comparing single months, maximum temperatures were higher in May and June 2017, while in July and August, the maximum temperatures were higher in 2018, respectively.

Weather data were recorded by an automatic weather station at the experimental station. When comparing the vegetation period, we saw there was less rainfall and higher temperatures in 2018 compared to 2017 (Figure 2).



(**a**) (**b**)

Figure 2. Average temperature (°C, connected points), mean monthly maximum (T_{max}, °C, red points) and minimum temperature (T_{min}, °C, blue points), and accumulated monthly precipitation (mm, bars) during the field experiments in (**a**) 2017 and (**b**) 2018.

2.3. Field Experiments

The field trials were conducted as randomized, complete block designs with three replicates. Each plot within a replicate was further subdivided into five sub-plots and harvested separately. The two safflower cultivars C1 and C2 were grown in two different row spacings (12 (S1) and 33 cm (S2)) and two different sowing densities (40 (D1) and 75 plants m^{-2} (D2)), resulting in eight treatment combinations (Table 1). Sequence of harvested sub-plot within a plot was randomized according to a randomized complete block design. A total of 24 plots per year were arranged. Plot size was 32 m^2 (8 m × 4 m). Treatments and harvest dates were randomly assigned to plots and sub-plots, using CycDesigN 5 (VSNI, Hemel Hemstead, Unites Kingdom).

Table 1. Experimental treatments and abbreviations: two cultivars (C1 = German, C2 = Chinese cultivar), two row spacings (S1 = 12 cm, S2 = 33 cm), and two sowing densities (D1 = 40, D2 = 75 plants m^{-2}).

Abbreviation Treatment	Origin and Cultivar	Row Spacing (cm)	Sowing Density (plants m^{-2})
C1 S1 D1	Germany (C1)	12 (S1)	40 (D1)
C1 S1 D2	Germany (C1)	12 (S1)	75 (D2)
C1 S2 D1	Germany (C1)	33 (S2)	40 (D1)
C1 S2 D2	Germany (C1)	33 (S2)	75 (D2)
C2 S1 D1	China (C2)	12 (S1)	40 (D1)
C2 S1 D2	China (C2)	12 (S1)	75 (D2)
C2 S2 D1	China (C2)	33 (S2)	40 (D1)
C2 S2 D2	China (C2)	33 (S2)	75 (D2)

The previous crop was wheat and triticale in 2017 and 2018, respectively. With the cultivator "POM Meteor" (MEZGER Landtechnik GmbH & Co. KG, Ditzingen, Germany), the residues of the previous crops were incorporated six months before sowing to a depth of 3–5 cm. At the end of November, the experimental fields were ploughed with "Juwel 8 TCP V" (LEMKEN GmbH and Co. KG, Alpen, Germany) to a depth of around 25 cm. Before sowing, the seedbed was prepared in both years to a depth of 5–8 cm, with the rotary harrow "HRB 403" (Kuhn Maschinen-Vertrieb GmbH, Genthin, Germany) and the prism roller "Simplex" (Güttler GmbH, Kirchheim/Teck, Germany). Sowing was carried out with a plot driller "Deppe D82" (Agrar-Markt DEPPE GmbH, Bad Lauterberg-Barbis, Germany), at a depth of 2 cm, on 25 April 2017 and on 19 April 2018. The target soil mineral nitrogen content was 80 kg N ha^{-1}. Since this value was exceeded in 2017, no fertilizer was applied in that

year. In 2018, 40 kg N ha^{-1} was applied as calcium ammonium nitrate shortly after sowing, using the fertilizer broadcaster "UKS 230" (RAUCH Landmaschinenfabrik GmbH, Sinzheim, Germany). Manual weeding was done twice in 2017 (31 May and on 7 June, 35 and 43 days after sowing (DAS)) and due to high weed pressure eight times in 2018 between 25 April and 30 May (6 to 41 DAS) until safflower plants reached the branching stage at which the plants are no longer susceptible to weeds [47–49]. In 2017, symptoms of Alternaria leaf spot disease were observed for the cultivar C1 in July, but no disease or pest management was applied.

2.4. Data Collection

2.4.1. Destructive Harvests

Harvesting started when most plants had reached the principal growth stage 6 (flowering following the BBCH scale) [49], and were then carried out once a week between 18 July and 15 August 2017, and between 10 July and 7 August 2018 (Table 2). At each harvest, all plants from an area of 0.25 m^2 within each sub-plot were cut manually at the soil surface in the center rows, and the fresh matter of the whole sample was recorded. Samples were then separated into capitula, florets, and residual plant parts. The number of capitula was recorded, and florets were removed from the capitula and separated into the categories "flowering" and "withered". The number of primary branches was recorded on the first and on the third harvest. Afterward, no further change in the number of primary branches was observed. Fresh weights of the mentioned plant parts were determined before drying them to constant weight to record dry matter. The dry matter content was determined by dividing the dry matter by the fresh matter. Florets were dried according to Mohammadi and Tavakoli [29], at 40 °C, and the remaining samples at 100 °C.

Table 2. Sowing dates and the number and date of harvests (days after sowing (DAS)) in the experimental years 2017 and 2018.

Year	Sowing Date	Number of Harvest	Date of Harvest (DAS)
2017	25.04.2017	1	18.07 (84)
		2	25.07 (91)
		3	01.08 (98)
		4	08.08 (105)
		5	15.08 (112)
2018	19.04.2018	1	10.07 (82)
		2	17.07 (89)
		3	24.07 (96)
		4	31.07 (103)
		5	07.08 (110)

2.4.2. Determination of Color Content (Carthamidin Content)

The carthamidin content (yellow pigment) of the flowering florets was determined spectrophotometrically according to Mohammadi and Tavakoli [29] and the method of the FAO [50], with minor modifications. For the extraction of carthamidin, 0.015–0.018 g of dried florets was put into a 50 mL screw cab falcon tube. These were filled to a volume of 50 mL with a citric acid/disodium hydrogen phosphate buffer solution (pH 5.0), which was made after McIlvain [51]. Samples were shaken at room temperature for 90 min, with a frequency of 100 rpm, in the laboratory shaker 'Swip SM25' (Edmund Bühler GmbH, Bodeshausen, Germany). Then, 1.5 mL of the sample was filled into single-use fully UV-transparent plastic cuvettes 'Semi-micro cuvette PS' (nerbe plus GmbH, Winsen/Luhe, Germany). The cuvettes were placed in the spectrophotometer 'Ultrospec 3100 pro' (GE Healthcare Europe GmbH, Freiburg im Breisgau, Germany), and the absorption (A) was identified at an absorption maximum of 400 nm [52,53].

By using the buffer as a blank sample, the percentage of carthamidin content (P) could be determined mathematically by using the following formula [29,50]:

$$p_i = \frac{A_i}{487} \cdot \frac{50}{W_i} \quad (1)$$

where p_i is the percentage of carthamidin content of sample i; A_i and W_i are the maximum absorption (range of 400–408 nm) and weight (in g) of sample i, with 487 being the specific absorption of carthamidin (in g mL^{-1}) and 50 being the volume of mL the sample i was filled to with the buffer solution.

The color yield (carthamidin yield) was calculated according to Mohammadi and Tavakoli, by the multiplication of the floret yields with their carthamidin content [29].

2.5. Statistical Analysis

Data were analyzed by a mixed-model approach. The model can be represented in the syntax of Piepho [54] as follows:

$$Y \times B + Y \times C \times S \times D \times H : Y \cdot B \cdot H + \underline{B \cdot Y \cdot C \cdot S \cdot D \cdot H} \quad (2)$$

where Y, B, H, C, S, and D denoted effects for year, block, harvest date within a year, cultivar, row spacing, and sowing density, respectively. Moreover, × is the crossing operator, e.g., Y × B expands to Y + B + Y · B. Furthermore Y · B and Y · B · H correspond to complete block effects within years across harvest dates and for each harvest date. As years were very different from each other, interest was in the optimal agronomic strategy for both years. Thus, effects for years were taken as fixed. Main effects for complete block were taken as fixed. In contrast, harvest-date-specific block effects were assumed as random denoted by writing these effects behind the colon. All other factors were taken as fixed. Note that block effects across harvest dates and error (=$\underline{B \cdot Y \cdot C \cdot S \cdot D \cdot H}$) effects are potentially correlated within a year, as data were repeatedly taken from the same plot. Different variance–covariance structures were tested for both effects, and the best model was selected via AIC [55]. The tested variance–covariance structures are the first order autoregressive with homogeneous or heterogeneous variances (with harvest date-by-year specific variances) and an unstructured variance–covariance matrix. Residual plots of each trait were visually checked for homogeneous variance and normal distribution. If one or both of these two assumptions were fulfilled, a logarithmic transformation was performed to meet the assumptions. For traits floret yield and carthamidin yield, some values were below the detection boundary. These values were replaced prior to analysis by half of the minimum value measured. Results of multiple comparisons were presented as letter display [56], using the Fisher´s Least Significant Difference test, at $\alpha = 5\%$, after finding significant corresponding F-tests. In the case of multiple relevant two-, three-, and four-way interactions, displaying significant differences for all relevant marginal means get complicated. In this case, simple means or margin means for a single higher-way interaction are presented to simplify presentation. For instance, if interactions Y·C·S, Y·C·D, and Y·D·S were significant, marginal means of Y·C·S·D are presented within the main text dropping results from the letter display. Note that, in this case, all relevant means and their comparisons for all significant interactions (including a letter display) were additionally shown in the Appendix A.

In case of logarithmical transformed data, means were back-transformed and denoted as median. Standard errors were back-transformed using the delta method. In this case, letter display was omitted. All analyses were performed within the statistical software SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

3. Results and Discussion

3.1. Yield Parameters

3.1.1. Primary Branches per Plant

The mean number of primary branches is presented for sowing density and year-by-harvest date combinations, as only these two model terms showed significance (Tables 3 and 4).

Table 3. Mean values ± standard error and significant differences of the Fisher's LSD test ($\alpha = 0.05$) for number of primary branches for the factor year (2017 and 2018) and harvest date (Harvest 1 (2017: 84, 2018: 82 DAS) and 3 (2017: 98, 2018: 96 DAS) (DAS, days after sowing). Mean values with at least one identical lowercase letter are not significantly different within a column. Mean values with at least one identical capital letter are not significantly different within a row.

Year	Harvest Date	
	1	3
2017	10.38 ^{bA} ± 0.53	9.54 ^{aA} ± 0.53
2018	14.15 ^{aA} ± 0.60	11.02 ^{aB} ± 0.60

Table 4. ANOVA table of the significant terms and interactions in number of primary branches and capitula per plant.

Trait	ANOVA Table of the Significant Terms and Interactions		
	Model Term	Degree of Freedom	*p*-Value [1]
Branches	Sowing density	1	<0.0001
	Year * Harvest date	1	0.0477
Capitula	Sowing density	1	<0.0001
	Year * Cultivar	1	<0.0001

[1] *p*-value of an F-test for differences between levels of the corresponding factor or factor combinations.

The highest number of primary branches per plant was observed on the first harvest date in 2018 (14.15), while the lowest number was achieved on the third harvest date in 2017 (9.54; Table 3). The number of primary branches in 2017 did not differ significantly between harvest dates, whereas on the first harvest date in 2018, plants had more primary branches (14.15) than on the third harvest date (11.02). Plants sown at a lower sowing density (D1) produced a significantly higher number of primary branches per plant (13.0 ± 0.40) than at the higher sowing density (D2) (9.54 ± 0.40) (Table A2), which is in line with other studies [35,57–59]. The total number of primary branches is within the range [60,61]. The number varied between 4.0 and 24.8 and indicated, in most cases, that less than 20 primary branches, depending on cultivars, were formed [60,61]. In the current study, effects of row spacing and cultivar were not significant. In contrast, Oad et al. showed that, for larger inter- and intra-row spacing, the number of primary branches increased from around 7 to 10 [62].

Furthermore, the number of branches varied between years, probably due to differences in temperatures and precipitation [63]. Numbers of branches were higher in years with cooler growing conditions [64,65]. In this study, however, a higher number of branches was found in the warmer, but drier year, 2018 (Figure 2). This might be explained by the susceptibility to diseases under humid conditions [66,67], and therefore a higher productivity of, e.g., branches under dry and warm conditions like in 2018. Moreover, the lower maximum temperatures in May and June, when the branches are formed, could explain the delay in growth and, thus, the higher number of branches in 2018 (Figure 2).

3.1.2. Number of Capitula per Plant

Significant differences for the number of capitula were found for sowing density and year-by-cultivar interactions (Tables 4 and 5).

Table 5. Mean values ± standard error and significant differences of the Fisher's LSD test (α = 0.05) for number of capitula for the factor year (2017 and 2018) and cultivar (C1 and C2). Mean values with at least one identical lowercase letter are not significantly different within a column. Mean values with at least one identical capital letter are not significantly different within a row.

Year	Cultivar	
	C1	C2
2017	8.97 ^{aB} ± 0.27	12.46 ^{bA} ± 0.37
2018	8.61 ^{aB} ± 0.60	16.32 ^{aA} ± 0.60

The highest number of capitula was produced with cultivar C2 in both years (2017: 12.46; 2018: 16.32) (Table 5). At a lower sowing density (D1), more capitula per plant (14.35 ± 0.36) were observed compared to the higher sowing density (D2) (8.73 ± 0.22) (Table A2).

The number of capitula per plant is within the range of other studies demonstrating 9.7–20.3 capitula per plant depending on cultivar, row spacing, sowing density, and year [61,68,69]. The higher number of capitula at lower sowing densities could be explained by several studies [31,58,70,71], showing correlations between the number of branches and number of capitula per plant [61,64,72,73]. In our study, a higher number of branches and capitula was obtained at a lower sowing density. Effects of cultivars were shown in several studies [30,61,74,75]. One possible reason for the higher productivity of C2 in both years in the current study could be that this cultivar is explicitly used in colorant production in China and is therefore designed for high productivity. The slightly longer time to flowering and, thus, the delay in development could also explain the higher number of capitula of cultivar C2. The delay in 2018 could also be explained by the lower maximum temperatures in June, when the capitula were formed (Figure 2).

3.2. Yield and Quality Parameters

3.2.1. Yield of Flowering Florets

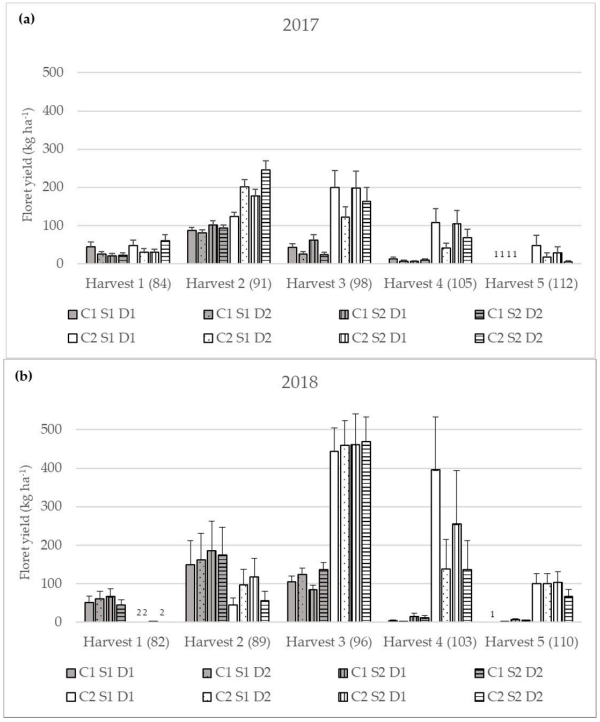
The simple least square means of flowering floret yields are presented, as model terms showed significant differences for several three-way interaction terms (Figure 3 and Table 6).

The yield of flowering florets ranged from 2.30 to 468.96 kg ha^{-1} (Figure 3) and was comparable to studies with average floret yields between 168 and 188 kg ha^{-1}, depending on year, cultivar, and sowing density [30,57,76].

The cultivar C1 achieved its highest floret yield earlier compared to C2, which can be explained by an earlier start of flowering. Furthermore, in all years and most harvest dates, C2 achieved higher floret yields than C1 (Table A1). The highest yield for C1 was obtained at the second harvest date (2017: 90.64; 2018: 167.81 kg ha^{-1}), and the lowest yields were obtained on the two last harvest dates (2.36–8.89 kg ha^{-1}) (Table A1). For C2, highest yields of flowering florets were harvested in 2017, at the second and third harvest dates (181.82 and 167.56 kg ha^{-1}), and in 2018, at the third harvest date (458.70 kg ha^{-1}) (Table A1). The lowest yields of flowering florets were achieved, depending on year, at the first (2018: 0.67 kg ha^{-1}) or at the last harvest date (2017: 18.98 kg ha^{-1}), which could be explained by the fact that it was not yet flowering or had already withered toward the end.

In 2017, most flowering florets were harvested on the second harvest date (D1: 118.27; D2: 139.35 kg ha^{-1}), while in 2018, most flowering florets were harvested on the third harvest date (D1: 206.71; D2: 245.37 kg ha^{-1}) (Table A2). On these two harvest dates (second harvest date in 2017 and

third harvest date in 2018), D2 achieved higher floret yields than D1. In 2018, however, no significant influence on the yield of flowering florets between the sowing densities were found.



[1] No longer harvested, because all florets were withered. [2] Not flowering yet.

Figure 3. Yield of flowering florets (kg ha^{-1}) for the two years ((**a**) 2017 and (**b**) 2018) and the eight different treatments (German and Chinese cultivar (C1 and C2), row spacing of 12 and 33 cm (S1 and S2), and sowing densities of 40 and 75 plants m^{-2} (D1 and D2) of the five harvests (Harvest 1–5) (DAS, days after sowing) represented as mean values ± standard error.

Table 6. ANOVA table of the significant terms and interactions in yield of flowering florets.

ANOVA Table of the Significant Terms and Interactions		
Model Term	**Degree of Freedom**	***p*-Value [1]**
Harvest date * Cultivar * Row spacing	4	0.0027
Year * Harvest date * Cultivar	3	<0.0001
Year * Harvest date * Sowing density	4	0.0258

[1] *p*-value of an F-test for differences between levels of the corresponding factor or factor combinations.

The year-effect on the floret yield is also reflected in other studies that reported higher floret yields at higher temperatures and lower precipitation [77–79]. The lower disease infestation with lower precipitation [66] could explain the higher floret yields in the hotter and drier year, 2018, in this study (Figures 2 and 3). Moreover, the lower maximum temperatures in May and June 2018 may have led to a delayed development (Figure 2). Furthermore, the higher maximum temperatures during flowering in 2018 (July and August) may have had a positive effect on the floret yields. Other studies also reported differences in floret yield of cultivars, harvest dates, and years [29,30,57,76]. Yields peaked in the middle of the flowering period (Harvest 2 to 3), which could be explained by the successive flowering of the secondary and tertiary capitula [3,4]. A reason for the higher floret yields of C2 could be the higher number of capitula, as it determines final floret yield [36,80]. Additionally, the effect of row spacing and sowing density on floret yield is in line with studies of Azari et al. and Hamza et al. showing higher floret yield with lower sowing density and lower row spacing [31,57].

3.2.2. Carthamidin Content of Flowering Florets

For the carthamidin content, the model showed significant differences for all two-way interactions of year, harvest date, and cultivar. Therefore, least square means of the corresponding three-way interactions are presented (Table 7).

Table 7. Carthamidin content of flowering florets (%) for the two years (2017 and 2018) and the two cultivars (German and Chinese cultivar (C1 and C2)) of the five harvests (Harvest 1–5) (DAS, days after sowing) represented as mean values ± standard error.

	Cultivar	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
		84 DAS	91 DAS	98 DAS	105 DAS	112 DAS
2017	C1	5.72 ± 0.32	3.43 ± 0.32	4.08 ± 0.32	2.68 ± 0.31	[1]
	C2	6.58 ± 0.32	7.29 ± 0.32	6.51 ± 0.32	5.97 ± 0.31	5.91 ± 0.34
		82 DAS	89 DAS	96 DAS	103 DAS	110 DAS
2018	C1	3.40 ± 0.14	3.54 ± 0.14	3.41 ± 0.14	3.19 ± 0.18	[1]
	C2	[2]	8.12 ± 0.14	7.55 ± 0.14	7.14 ± 0.14	6.98 ± 0.14

ANOVA table of the significant terms and interactions.

Model term	Degree of freedom	*p*-value [3]
Harvest date * Cultivar	4	0.0006
Year * Cultivar	1	<0.0001
Year * Harvest date	4	0.0007

[1] No longer harvested, because all florets were withered. [2] Not flowering yet. [3] The *p*-value of an F-test for differences between levels of the corresponding factor or factor combinations.

The cultivar C2 reached higher carthamidin contents in 2018 (6.98%–8.12%) compared to 2017 (5.91%–7.29%) (Table 7). In addition, cultivar C2 had higher carthamidin contents (6.45%–7.71%) than C1 (2.94%–4.56%) on all existing harvest dates and in both years (Table A1).

The range of carthamidin contents was comparable to a study of Mohammadi and Tavakoli in which the carthamidin contents were between 4.60% and 5.93% [29]. This study showed the difference in carthamidin contents between harvest dates, cultivars, and their interaction, which was also shown in other studies [29,30,34,81]. The higher carthamidin content at earlier harvest dates in this study (Tables 7 and A1) can be explained by the presence of an oxidative enzyme (β-glucose oxidase) which leads to a color change from yellow to red during ripening [82–84]. The carthamidin content is also affected by the environmental weather conditions, wherefore the harvest date is a determining factor for the carthamidin content [29,66,81,82]. As it was shown for number of capitula per plant and for floret yield (Table 4 and Figure 3), C2 is better adapted to the hot and dry weather conditions, and this could also explain the higher carthamidin contents. These conditions, and also the highest maximum temperatures in July and August 2018, are advantageous during the flowering period, for good development and less disease formation in safflower (Figure 2) [66,77–79].

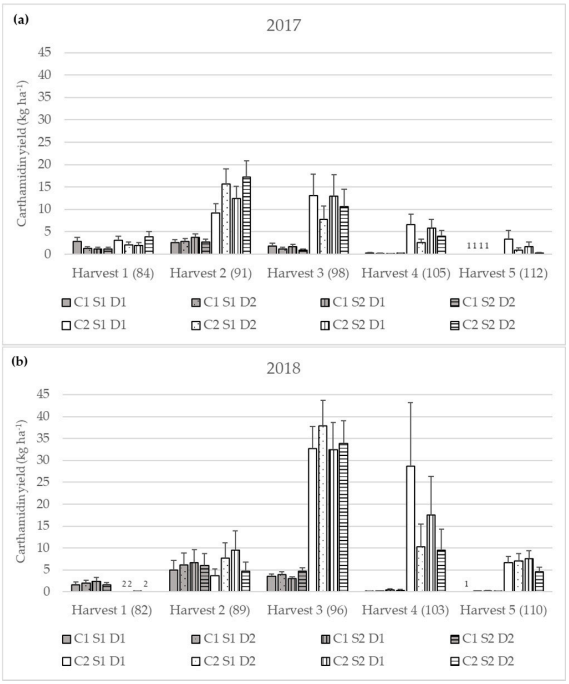
3.2.3. Carthamidin Yield

As the analysis showed significant differences between sowing density as well as several three-way interactions of cultivar, row-spacing, harvest date and year, means for sowing density, and the corresponding four-way interactions are presented (Figure 4 and Table 8).

In both years, C1 produced the highest carthamidin yields on the second harvest date (2017: 2.94; 2018: 5.93 kg ha^{-1}) (Table A1). C2 had the highest carthamidin yield on the second harvest date in 2017 and on the third harvest date in 2018 (2017: 13.28; 2018: 34.13 kg ha^{-1}). The product of the factors floret yield and carthamidin content (Figure 3 and Table 7) in the carthamidin yield explains the peak at the beginning of the flowering period (Figure 4).

The highest carthamidin yields were achieved with C2 on the third harvest date (S1: 18.86; S2: 19.69 kg ha^{-1}) (Table A3). Furthermore, it could be shown that, in 2018, S2 produced higher carthamidin yields (2.18 kg ha^{-1}) compared to S1 (1.58 kg ha^{-1}) (Table A3).

The carthamidin yield ranged between 0.04 and 37.86 kg ha^{-1} (Figure 4), which is comparable to a study of Mohammadi and Tavakoli, with carthamidin yields depending on cultivars and harvest dates between 26.33 and 36.62 kg ha^{-1} [29].



[1] No longer harvested, because all florets were withered. [2] Not flowering yet.

Figure 4. Carthamidin yield (kg ha^{-1}) for the two years ((**a**) 2017 and (**b**) 2018) and the eight different treatments (German and Chinese cultivar (C1 and C2), row spacing of 12 and 33 cm (S1 and S2), and sowing densities of 40 and 75 plants m^{-2} (D1 and D2) of the five harvests (Harvest 1–5) (DAS, days after sowing) represented as mean values ± standard error.

Table 8. ANOVA table of the significant terms and interactions in carthamidin yield.

ANOVA Table of the Significant Terms and Interactions		
Model Term	**Degree of Freedom**	***p*-Value [1]**
Sowing density	1	0.0026
Harvest date * Cultivar * Row spacing	4	0.0242
Year * Row spacing	1	0.0299
Year * Harvest date * Cultivar	3	<0.0001

[1] *p*-value of an F-test for differences between levels of the corresponding factor or factor combinations.

In both years, cultivar C2 produced the highest carthamidin yields, which could be due to the origin and its genetic potential, because it is designed for colorant production in China. Both the higher floret yields and the higher carthamidin contents of C2 led to the highest carthamidin yields in 2018, which could be explained by the drier and hotter weather conditions and the higher maximum temperatures during flowering in 2018 (Figure 2). Disease infestation is less likely to occur under these conditions (Figures 2–4 and Table 7) [66,77–79]. Moreover, the drier conditions by which secondary phytochemicals can be increased according to different studies could be the reason for the higher carthamidin yields in 2018 [39,40]. Environmental differences between years and agronomic practices (e.g., row spacing and sowing density) can affect competition for nutrients, light, and water [3,64]. This could be a possible explanation even for differences in carthamidin yield.

4. Conclusions

In general, the results of the study show that the experimental factors year, harvest time, cultivar, and sowing density had significant influences on most traits. Row spacing indicated a significant impact on two traits and just in interactions with other effects, wherefore further experiments should be carried out. Based on this study, the Chinese cultivar (C2), a plant density of 40 plants m^{-2} (D1), and a harvest time of two to three weeks after flowering can be recommended to achieve maximum floret and carthamidin yields under the conditions in Southwest Germany.

The most critical factor for safflower cultivation in Southwest Germany seems to be the impact of diseases under rainy conditions during flowering, wherefore selection of resistant accessions should be carried out in a first step. Further studies should also test the cultivation of safflower at warmer and drier locations in Southwest Germany.

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Appendix A

Table A1. Mean values ± standard error and significant differences of the Fisher's LSD test ($\alpha = 0.05$) for the factors year (2017 and 2018), harvest date (Harvest 1–5), and cultivar (C1 and C2). Mean values with at least one identical lowercase letter are not significantly different between harvest dates (within columns). Mean values with at least one identical capital letter are not significantly different between cultivars (within rows).

Parameter	Year	Harvest Number	Cultivar	
			C1	C2
Yield of flowering florets (kg ha^{-1})	2017	Harvest 1	26.76 ^{bA} ± 3.86	40.30 ^{cA} ± 5.81
		Harvest 2	90.64 ^{aB} ± 4.28	181.82 ^{aA} ± 8.59
		Harvest 3	36.41 ^{bB} ± 4.06	167.56 ^{aA} ± 18.68
		Harvest 4	8.89 ^{cB} ± 1.49	74.99 ^{bA} ± 12.56
		Harvest 5	n.d.	18.98 ^{dA} ± 5.22
	2018	Harvest 1	55.38 ^{bA} ± 8.70	0.67 ^{dB} ± 0.11
		Harvest 2	167.81^{aA} ± 34.83	73.30 ^{cB} ± 15.21
		Harvest 3	110.57 ^{aB} ± 7.58	458.70 ^{aA} ± 33.75
		Harvest 4	5.47 ^{cB} ± 1.50	209.34 ^{bA} ± 57.27
		Harvest 5	2.36 ^{dB} ± 0.31	91.22 ^{cA} ± 12.12
Carthamidin content of flowering florets (%)		Harvest 1	4.56 ± 0.17	n.d.
		Harvest 2	3.48 ^{B} ± 0.17	7.71 ^{A} ± 0.17
		Harvest 3	3.75 ^{B} ± 0.17	7.03 ^{A} ± 0.17
		Harvest 4	2.94 ^{B} ± 0.17	6.56 ^{A} ± 0.17
		Harvest 5	n.d.	6.45 ± 0.17

Table A1. *Cont.*

Parameter	Year	Harvest Number	Cultivar	
			C1	C2
Carthamidin yield (kg ha^{-1})	2017	Harvest 1	1.51 ^{bB} ± 0.23	2.65 ^{cA} ± 0.40
		Harvest 2	2.94 ^{aB} ± 0.32	13.28 ^{aA} ± 1.45
		Harvest 3	1.30 ^{bB} ± 0.24	10.88 ^{aA} ± 2.00
		Harvest 4	0.19 ^{cB} ± 0.03	4.46 ^{bA} ± 0.75
		Harvest 5	n.d.	1.09 ^{dA} ± 0.32
	2018	Harvest 1	1.88 ^{bA} ± 0.33	0.04 ^{dB} ± 0.01
		Harvest 2	5.93 ^{aA} ± 1.33	5.94 ^{cA} ± 1.33
		Harvest 3	3.76 ^{aB} ± 0.29	34.13 ^{aA} ± 2.81
		Harvest 4	0.19 ^{cB} ± 0.05	14.92 ^{bA} ± 3.75
		Harvest 5	0.08 ^{dB} ± 0.01	6.34 ^{cA} ± 0.73

Table A2. Mean values ± standard error and significant differences of the Fisher's LSD test ($\alpha = 0.05$) for the factors year (2017 and 2018), harvest date (Harvest 1–5), and sowing density (D1 and D2). Mean values with at least one identical lowercase letter are not significantly different between harvest dates (within columns). Mean values with at least one identical capital letter are not significantly different between sowing densities (within rows).

Parameter	Year	Harvest Number	Sowing Density	
			D1	D2
Number of primary branches per plant			13.0 ^{A} ± 0.40	9.54 ^{B} ± 0.40
Number of capitula per plant			14.35 ^{A} ± 0.36	8.73 ^{B} ± 0.22
Yield of flowering florets (kg ha^{-1})	2017	Harvest 1	33.93 ^{A} ± 4.89	31.79 ^{A} ± 4.59
		Harvest 2	118.27 ^{B} ± 5.59	139.35 ^{A} ± 6.58
		Harvest 3	101.57 ^{A} ± 11.32	60.06 ^{B} ± 6.70
		Harvest 4	31.57 ^{A} ± 5.29	21.12 ^{A} ± 3.54
		Harvest 5	n.d.	n.d.
	2018	Harvest 1	6.87 ^{eA} ± 1.08	5.41^{dA} ± 0.85
		Harvest 2	110.19 ^{bA} ± 22.29	111.63 ^{bA} ± 23.17
		Harvest 3	206.71 ^{aA} ± 15.21	245.37 ^{aA} ± 16.82
		Harvest 4	49.57 ^{cA} ± 13.56	23 08 ^{cA} ± 6.31
		Harvest 5	14.22 ^{dA} ± 1.89	15.15 ^{cA} ± 2.01
Carthamidin yield (kg ha^{-1})			n.d.	n.d.

Table A3. Mean values ± standard error and significant differences of the Fisher's LSD test ($\alpha = 0.05$) for the factors cultivar (C1 and C2), year (2017 and 2018), harvest date (Harvest 1–5), and row spacing (S1 and S2). Mean values with at least one identical lowercase letter are not significantly different between harvest dates (within columns). Mean values with at least one identical capital letter are not significantly different between row spacing (within rows).

Parameter	Cultivar	Year	Harvest Number	Row Spacing	
				S1	S2
Carthamidin yield (kg ha^{-1})	C1	2017	Harvest 1	1.85 ^{A} ± 0.30	1.53 ^{A} ± 0.25
			Harvest 2	3.87 ^{A} ± 0.68	4.51 ^{A} ± 0.79
			Harvest 3	2.33 ^{A} ± 0.33	2.10 ^{A} ± 0.30
			Harvest 4	0.15 ^{A} ± 0.03	0.25 ^{A} ± 0.05
			Harvest 5	n.d.	n.d.
	C2	2018	Harvest 1	0.28 ^{dA} ± 0.05	0.37^{dA} ± 0.06
			Harvest 2	7.95 ^{bA} ± 1.40	9.88 ^{bA} ± 1.74
			Harvest 3	18.86 ^{aA} ± 2.66	19.69 ^{aA} ± 2.83
			Harvest 4	8.42 ^{bA} ± 1.80	7.90 ^{bA} ± 1.68
			Harvest 5	3.46 ^{cA} ± 0.77	2.00 ^{cA} ± 0.44
Carthamidin yield (kg ha^{-1})		2017		n.d.	n.d.
		2018		7.9^{B} ± 0.7 [1]	10.9^{A} ± 0.95 [1]

[1] Here are presented values over all harvest dates.

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4. Impact of Cultivar, Harvest Date and Threshing Parameter Settings on Floret and Carthamidin Yield of Safflower

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The so far imported safflower florets from the Asian countries, where these are harvested by hand due to the low wages, will not be able to cover the future rising demand, particularly in Europe. Harvesting by hand is very slow, expensive, labor and time consuming and would not be economical in Europe. Therefore, the cultivation of safflower for the production of florets is not economical at present, since there is still no possibility of harvesting them mechanically on a large scale. According to this, in the following publication the mechanical harvest with different threshing parameter settings at several harvest dates and the influence of two cultivars on the threshed floret yield, their carthamidin content and yield were studied. In particular, the study of cultivar-specific characters, that could influence the selection of cultivars for mechanical harvesting, should be emphasized.

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Impact of Cultivar, Harvest Date and Threshing Parameter Settings on Floret and Carthamidin Yield of Safflower

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Abstract: The industrial need for safflower (*Carthamus tinctorius* L.) increased over the last decade due to its potential use as food colorant. Safflower is mainly cultivated in Asia for its use as floret. In Germany, an economically attractive cultivation for floret use would require a mechanization of harvest. In order to develop a mechanical harvesting system, field experiments were conducted at the experimental station Ihinger Hof of the University Hohenheim in 2017 and 2018. Safflower was harvested with a combine harvester to obtain the florets. Two safflower (i) cultivars were harvested with (ii) three threshing parameter settings on (iii) five harvest dates to evaluate threshed floret yield, dry matter and carthamidin content, and carthamidin yield. Results showed that the maximum threshed floret yield was achieved at the latest harvest date (784.78–1141.76 kg ha^{-1}), while the highest carthamidin contents were observed depending on cultivar on the first two harvest dates (0.53–3.14%). The decisive and resulting amount of carthamidin yield reached its maximum with the Chinese cultivar and the threshing parameter setting P3 between the fourth and fifth harvest date in 2018 (19.05–19.36 kg ha^{-1}). Highest dry matter contents were achieved at the last harvest date (62.67–77.77%). Individual capitula weight and carthamidin content decreased with later harvest dates. Further investigations should clarify whether the individual capitula weight and carthamidin content correlate with each other or are independent of the date of harvest. This could be a decisive criterion for the selection of cultivars for harvesting florets with a combine harvester. Reduced costs of machine harvesting compared to hand harvesting will make the cultivation of safflower for the food coloring industry in Germany more attractive in the future.

Keywords: *Carthamus tinctorius* L.; safflower; threshing parameters; combine harvester; carthamidin content; carthamidin yield; mechanization; harvest

1. Introduction

Safflower (*Carthamus tinctorius* L.), a member of the Asteraceae family, has a deep-rooting system and a strong taproot [1–4]. Safflower is an annual, thistle-like plant with many spines on its bracts and leaves [1,2]. It branches out to tertiary branches [5]. Each one has a spherical flower head (capitulum) containing the white, yellow, orange or red petals (florets) [1,2,5]. It has many purposes, such as vegetable, animal feed, tea, cut flowers or as a medicinal plant [1,2,6,7]. It has been used by humans for over 2200 years [1,5]. The main benefit of safflower currently is its use as oil, which is regarded as a healthy alternative to sunflower oil due to its oil composition [1,2,8,9]. Traditionally, safflower florets were used for coloring food and textiles [1–3]. However, this traditional use receded into the background when cheaper, synthetic aniline colors were invented in 1856 [2,10].

Different studies claim that these artificial food colorants negatively influence the behavior of children or may cause carcinogenic or allergic effects [11–14]. Therefore, and also due to the growing

awareness of environmentally conscious, safe and healthy consumption, the attention to natural colorants has increased [13–17]. The 2013 EU directive "Guidance notes on the classification of food extracts with colouring properties" distinguishes between "dyes" and "coloring foods" [18,19]. "Dyes" are defined as additives requiring legal admission, which is not the case for "coloring foods" [18,20]. Due to the lower enrichment factor (variable to indicate accumulation of a substance in a living organism) of safflower compared to paprika and curcuma, it is considered as an appropriate yellow- and orange-coloring substitute, which is increasingly used in, e.g., ice cream, candies, fruit syrups and juices [7,18,21,22]. Safflower has a number of advantages compared to other colorants, especially in the processing properties regarding light, temperature and pH value, and it is cheaper than saffron [23,24].

On an international level, cultivation of safflower for the use of florets is currently limited and is still mainly practiced in Asia [1,2,25]. However, this will no longer be sufficient to meet the rising demand of the food coloring industry, especially in Europe and other Western countries [7,13,26–32]. With the expansion of the cultivation of safflower to other regions, an adaption of cultivar choice and cultivation methods to regional conditions is required. Several studies have shown that there are suitable cultivars of safflower for oil and seed production in Central Europe [33–37]. There have also been tests on the cultivation of safflower with the aim of gaining florets in southwest Germany, which showed that the cultivation is possible and that the floret yields, colorant contents and colorant yields (specified on carthamidin as yellow colorant) can compete with other international studies [28,38–43], but can vary between years, harvest dates and cultivars.

Safflower for floret production is mainly harvested by hand, which is very slow, labor- and time-intensive and expensive [44–46]. Due to the fact that there is no industrially produced harvesting machine for this type of use so far [44,45], the production efficiency is very low, which means that no economical, large-scale production of florets is possible currently [45]. Therefore, a suitable method for the mechanization of harvesting should be developed and tested.

Within the study, mechanized harvesting was tested. In order to reduce costs and offer an economically attractive way to produce florets in Germany, a combine harvester was chosen as a harvesting machine due to its availability to every farmer. The threshing efficiency is influenced by many variables: for example, threshing drum speed, crop moisture (or dry matter content) and concave setting [47,48]. Furthermore, the cleaning wind as well as the sieves have to be adapted to the crop [49].

The objectives of the present study were to (i) test different cultivars, (ii) assess harvest dates and (iii) investigate threshing parameter settings on threshed floret yield, dry matter content, carthamidin content and yield, when safflower is harvested with a combine harvester. Furthermore, it was investigated whether (iv) relationships between plant-specific characteristics (e.g., capitulum weight) and carthamidin content exist that impact the suitability of cultivars for mechanized harvesting.

2. Materials and Methods

2.1. Description of the Experimental Site and Design

Two field experiments were conducted at the experimental station Ihinger Hof of the University of Hohenheim in southwestern Germany (48°44′ N, 8°55′ E, 478 m a.s.l.) in 2017 and 2018. Annual temperature in 2018 (10.2 °C) was higher than in 2017 (9.2 °C) and the annual rainfall was lower in 2018 (525.9 mm) than in 2017 (653.9 mm). Detailed weather and soil conditions were described in Steberl et al. [38].

For the first experiment and for both experimental years 2017 and 2018, the field trial was arranged in a kind of split plot design with three replicates. The main plat factor was cultivar. The two levels of cultivar were the Chinese (C2) and German Cultivar (C1). In contrast to a common split-plot design with one main-plot per cultivar and replicate, the replicate was split into four main-plots. This increased the efficiency of cultivar mean estimates and comparison. The subplot factor is allocated to plot within main-plots according to an α-design with two incomplete blocks (=main-plots). The plots had a size of 30 m^2 (2 × 15 m) each containing four rows in the center with a row spacing of 0.33 m.

A sowing density of 40 plants m^{-2} was used to enable mechanical hoeing. This is essential due to a slow development of the plant up to the rosette stage [50], and the potential high weed pressure after emergence [51]. The row orientation was north–south in 2017 and east–west in 2018.

Information on previous crop, tillage, sowing and fertilization is similar to the experiment described in Steberl et al. [38]. In contrast to Steberl et al. [38] the sowing took place on 25 April 2017 and 20 April 2018. In 2017, manual weeding was done once 43 days after sowing (DAS). Because of high weed density in 2018, weeding was carried out three times (6, 33 and 48 DAS). Afterwards, no additional weeding was required because plants reached the branching stage, where they are no longer susceptible to weeds [50,52,53].

For the relationship between individual head weight and carthamidin content, data from the second field experiment was used, which is described in all details in Steberl et al. [38]. Thus, all remaining parts in the Materials and Methods section focus on the first experiment, except if stated differently.

2.2. Treatments

For both experiments, two different cultivars with three different threshing parameter settings were studied and harvested at different dates. The two safflower cultivars were a German (C1) and a Chinese cultivar (C2). These two cultivars are described in detail in Steberl et al. [38]. Due to the morphological differences, the growth habit and height, differences were assumed with regard to the threshed floret yield, the carthamidin content and, accordingly, the carthamidin yield.

For the first experiment, different threshing parameters for the setup of the combine harvester were selected based on a previous test in 2016. A range of combinations of top and bottom sieve, wind, threshing drum rpm (rounds per minute), concave setting and the use of a different number of rub bars or omission of them was selected. Threshing parameters in 2017 and 2018 were chosen from these combinations (P1–P3) (Table 1).

Table 1. List of threshing parameter settings (P1–P3) tested in the experimental years 2017 and 2018.

Characteristics	P1	P2	P3
Top sieve (lamella sieve)	15 mm opened	9 mm opened	15 mm opened
Bottom sieve (round hole sieve)	16 mm	10 mm	16 mm
Wind	400 min^{-1}	400 min^{-1}	500 min^{-1}
Threshing drum	1200 min^{-1}	1200 min^{-1}	700 min^{-1}
Concave setting	Step 1	Step 1	Step 3
Rub bars	-	-	3

Harvest of the florets took place at different dates representing different dry matter contents and different stages of development. The first harvest was carried out when the majority of the plants of the cultivar had reached flowering (BBCH stage 65) [50]. Due to the fact that C1 flowers earlier than C2, the first harvest was planned when C1 started to flower. Thus, C2 was not yet flowering at that date (see BBCH stages in Table 2).

The second harvest took place when both cultivars were in full bloom. The third harvest was scheduled when flowering went towards the end for both cultivars. The fourth was planned when C1 was withered and C2 was still slightly flowering. The last harvest took place when both cultivars were already withered. At that date, plants were drier and therefore a higher threshability was expected [48,54–56]. Due to changing weather conditions, some of the planned harvests could not be realized. Table 2 gives an overview of all harvest dates in both years.

Table 2. Sowing dates, and the number (BBCH stage C1/C2 (Chinese/German cultivar)) and date of harvests (days after sowing (DAS)) in the experimental years 2017 and 2018.

Year	Sowing Date	Harvest Time	Date of Harvest (DAS)
2017	25 April 2017	3 (69/67)	03.08 (100)
		4 (71/69)	14.08 (111)
2018	20 April 2018	1 (61/59)	13.07 (84)
		2 (67/65)	24.07 (95)
		4 (71/69)	09.08 (111)
		5 (75/71)	16.08 (118)

2.3. Data Collection

2.3.1. Harvesting and Post-Harvest Procedures

At each harvest date, plots were harvested with a plot combine harvester "Zürn 150" (Zürn Harvesting GmbH and Co. KG, Schöntal-Westernhausen, Germany). Fresh and dry matter weight was recorded for these samples of harvested florets. The drying temperatures were 40 °C, according to Mohammadi and Tavakoli [39], in order to prevent destruction of ingredients. Dry matter content was the result of the division of dry by fresh matter.

Despite the different threshing parameters, the threshed material still contained many coarse parts (leaves, branches, etc.) (Figure 1).

(**a**) (**b**) (**c**)

Figure 1. Photos of (**a**) threshed sample immediately after harvesting, (**b**) residue remaining in the sieve after sieving, (**c**) sample that was sieved and then used for further laboratory analysis.

In order to obtain a higher proportion of florets, a subsample of each plot was sieved with a 3 mm sieve as a post-harvest procedure, which is typical in flower production [57]. Data analysis was concentrated on sieved samples only. Therefore, they differed from the pure florets by still containing, e.g., small leaf parts, which is why they are called threshed florets in the following in order to avoid confusion with the pure florets.

In addition, number and weight (fresh and dry) of the capitula were recorded, as described in Steberl et al. [38].

2.3.2. Laboratory Analyses for the Determination of Carthamidin Content

Carthamidin content of the threshed florets was measured spectrophotometrically at the University of Hohenheim as described in [39,58] with minor modification. Determination of the carthamidin content was described in detail in [38]. The carthamidin yield was calculated as the product of the threshed floret yield and carthamidin content [39]. The carthamidin contents in Section 3.5 were determined from pure florets, as described in Steberl et al. [38].

2.4. Statistical Analysis

Data were analyzed using a mixed model approach. In the syntax of Piepho [59], the model can be shown as:

$$Y/R + Y \times C \times P \times H: B{\cdot}R{\cdot}Y + B{\cdot}R{\cdot}H{\cdot}Y + R{\cdot}C{\cdot}P{\cdot}Y + \underline{R{\cdot}C{\cdot}P{\cdot}Y{\cdot}H}$$

where Y, R, B, C, P and H indicate the effects for year, replicate, incomplete block or main-plot within replicate, cultivar, threshing parameter settings and harvest dates. The nesting operator / expands, for example, Y/R to Y + Y·R. The crossing operator × expands, for example, Y × C to Y + C + Y·C. Complete replicate effects within years are achieved through Y·R in the model. Due to the weather conditions, the years were very different. Since the interest was to find the best treatment for both years, the effect of the year was assumed to be fixed. In contrast, incomplete blocks within a replicate and year (B·R·Y), harvest date-specific incomplete block effects (B·R·Y·H) as well as plot effects (R·C·P·Y) were assumed as random. A colon is used to separate fixed effects and random effects (behind the colon) in the model. The residual error effects (R·C·P·Y·H) are underlined. A year-specific variance was fitted to these error effects. The assumptions of normal distribution residuals with homogenous variance were checked graphically via residual plots for all traits. In case of rejection of at least one assumption, a logarithmic or a square root transformation was performed to meet the criteria. In this case, the estimated means were back-transformed for presentation purpose only. Furthermore, standard errors were back-transformed by using the delta method. After finding significant effects via the F-test, significant differences were evaluated using the multiple Fisher´s least significant difference test at a significance level of $\alpha = 5\%$. Using the %mult macro in SAS [60], a letter display was generated to show the results of the multiple comparison. In case of multiple (two or three way) interactions, letter displays for all significant differences become complicated. Therefore, simple or marginal cultivar-by-threshing parameter settings-by-year-harvest date means of single higher-level interactions were presented in the result and discussion part to simplify the presentation of the results of the manuscript. Additionally, all relevant means were compared within the Appendix A.

The statistical analysis was performed using PROC MIXED of the statistical software SAS 9.4. (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Threshed Floret Yield

The analysis showed significant differences for year and for several two-way interactions of cultivar, threshing parameter settings and harvest date (Figure 2 and Table 3).

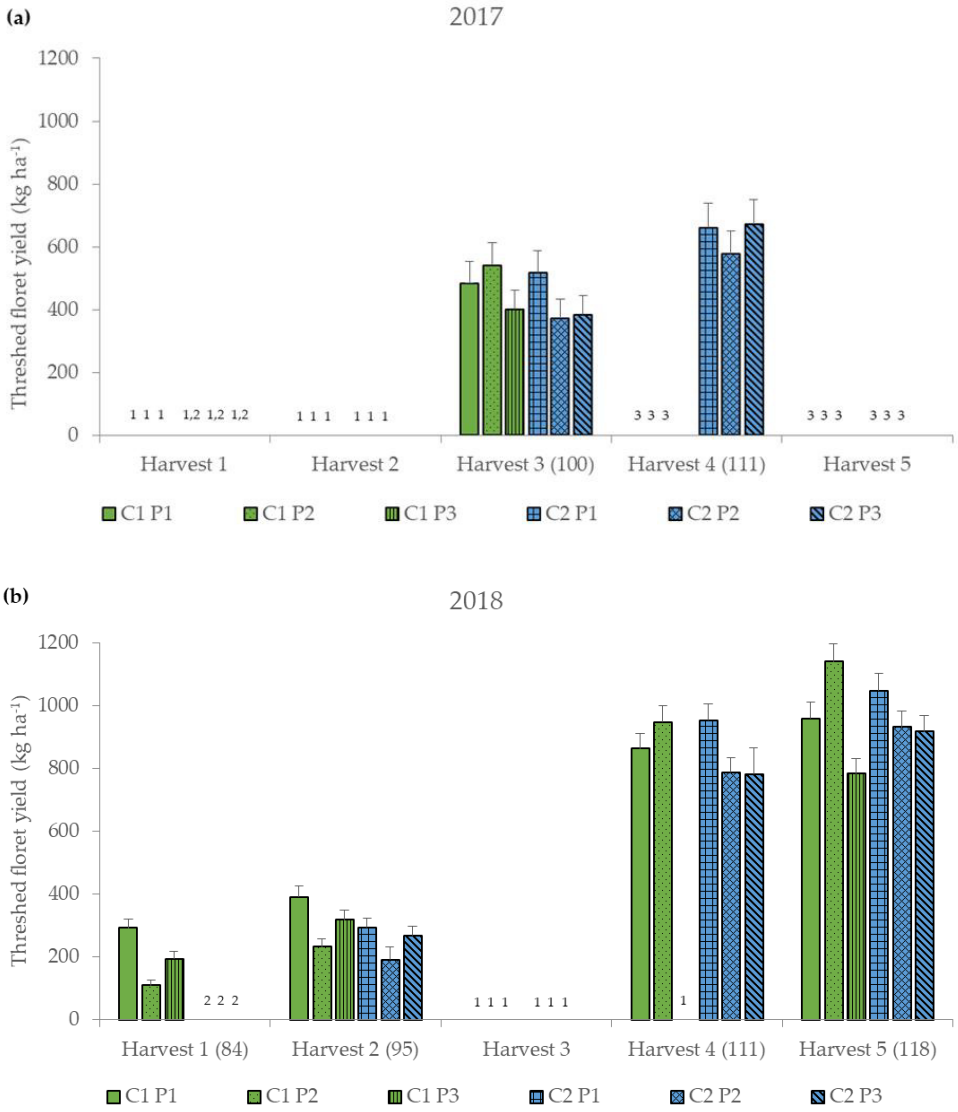

Figure 2. Threshed floret yield (kg ha^{-1}) for the six different treatment combinations of two safflower cultivars (German and Chinese cultivar, C1 and C2) and three threshing parameter settings (P1–P3) (Table 1) at five harvest dates (Harvest 1–5) (DAS, days after sowing) for the two years (**a**) 2017 and (**b**) 2018, represented as mean values ± standard error. [1] Harvest was not possible. [2] Not yet harvested because not yet flowered. [3] No longer harvested because no longer flowered.

Table 3. ANOVA table of the significant terms and interactions of threshed floret yield.

ANOVA Table of the Highest Significant Terms and Interactions		
Model Term	**Degrees of Freedom**	***p*-Value [1]**
Year	1	0.0015
Cultivar * Threshing parameter setting	2	0.0284
Harvest date * Threshing parameter setting	8	<0.0001

[1] *p*-value of an F-test for differences between levels of the corresponding factor or factor combinations.

Therefore, marginal means for years and cultivar-by-threshing parameter settings-by-harvest date of threshed floret yields are presented. On average, the threshed floret yield was higher in 2018 (621.58 kg ha^{-1}) than in 2017 (512.14 kg ha^{-1}) (Figure 2 and Table A1). Threshed floret yield increased at later harvest date (Figure 2 and Table A1). C1 achieved the highest threshed floret yield with the threshing parameter setting P2 (1141.76 kg ha^{-1}), while C2 achieved the maximum yields with the threshing parameter setting P1 (1048.63 kg ha^{-1}) at the fifth harvest (Figure 2 and Table A1). The lowest yields were obtained from both cultivars with P2 at the first or second harvest date (C1: 110.46 kg ha^{-1}; C2: 192.05 kg ha^{-1}). For C2, threshing parameter setting P1 led to the highest yield at all harvest dates (294.95–1048 kg ha^{-1}). C1, on the other hand, had the highest yields at the early harvest dates with P1 (Harvest date 1: 293.64; Harvest date 2: 392.67 kg ha^{-1}). For the third to fifth harvest date, the settings of P2 were more favorable (540.51–1141.76 kg ha^{-1}). Threshed floret yield ranged between 110.46 and 1141.76 kg ha^{-1} (Figure 2). Other studies in which only the petals were harvested by hand showed that the yields ranged between 2.30 and 647.53 kg ha^{-1}, depending on cultivar, harvest date and year [3,28,38–40,42,43,61,62]. The higher yields in threshing compared to hand harvesting (pure florets) can be explained by the fact that, despite sieving, the threshed material still contained other plant parts, e.g., small leaves or seeds (Figure 2).

Other studies indicated higher floret yields in warmer and drier years [38,63–65]. In this study, higher yields were achieved in 2018, the year with higher temperatures and more importantly less rainfall, which could also be related to lower susceptibility to diseases in drier conditions [51]. Furthermore, floret yields depended on year, harvest date and cultivar [28,31,38–40,42]. The highest threshed floret yields were obtained for both cultivars at the last harvest date in 2018, which could be described by the consecutive flowering of the secondary and tertiary capitula [2,5], and the increasing maturity of the plant which resulted in drier, and thus better threshing conditions. This increasing maturity and drying of the crop (Table 4) could also be a reason for the significant interactions of harvest date and threshing parameters.

In contrast to another study, in which marigold was harvested by machine and in which the highest yields were achieved at earlier harvest dates [66], this study did not only focus on the inflorescence. Safflower was harvested with a combine harvester instead of a virtual rotating comb-type chamomile harvester, which is specifically designed for flower harvesting. Therefore, in this study the yields were higher at the end of the harvest dates, when the plant has more biomass and it is more important that the threshed material is drier. C2 achieved the highest threshed floret yields at all harvest dates and C1 at the first two harvest dates with P1 (Figure 2 and Table A1), which could be explained by the larger openings of the top and bottom sieve (Table 2). Due to these larger sieve diameters, they do not clog quickly by the wet threshing material, therefore there is less threshing loss and a higher threshed floret yield. C1 has a developmental advantage compared to the other cultivar results in more mature, drier plants and florets at an earlier date. This explains why P2 for drier material properties no longer clogged the smaller sieve diameters. Hence, the highest threshed floret yields could be achieved by low threshing losses (Figure 2 and Table A1). In addition, the higher threshing drum speeds of P1 and P2 compared to P3 potentially led to a higher threshing efficiency and therefore to higher threshed floret yields under these parameter settings. This was also shown in several studies with different crops [48,54,56]. The lowest threshed floret yields at the third and fifth harvest date were achieved with P3. The reasons could be the higher wind, the lower threshing drum rpm or the inserted rub bars, which may have led to more material being transported out of the combine harvester as threshing loss. The fact that P3 performed worse than P1 and P2 could also be due to the wider concave setting. That wider concave setting led to a lower threshing efficiency with a higher floret loss. This was also shown in a study by Pragalyaashree et al. [67] where onion florets were separated.

Table 4. Dry matter content (%) for the six different treatment combinations of the two cultivars (German and Chinese cultivar, C1 and C2) and three threshing parameter settings (P1–P3) (Table 1) at five harvest dates (Harvest 1–5) (DAS, days after sowing) for the two years (2017 and 2018) represented as mean values ± standard error.

Year	Treatment	Harvest Date				
		1	**2**	**3**	**4**	**5**
				100 DAS	111 DAS	
2017	C1 P1	[1]	[1]	28.47 ± 0.70	[3]	[3]
	C1 P2	[1]	[1]	31.23 ± 0.70	[3]	[3]
	C1 P3	[1]	[1]	32.50 ± 0.70	[3]	[3]
	C2 P1	[1,2]	[1]	28.47 ± 0.70	36.77 ± 0.70	[3]
	C2 P2	[1,2]	[1]	31.20 ± 0.70	40.13 ± 0.70	[3]
	C2 P3	[1,2]	[1]	30.73 ± 0.70	42.23 ± 0.70	[3]
		84 DAS	95 DAS		111 DAS	118 DAS
2018	C1 P1	34.22 ± 0.90	33.12 ± 0.89	[1]	51.84 ± 0.90	65.38 ± 0.90
	C1 P2	33.39 ± 0.90	32.16 ± 0.90	[1]	58.48 ± 0.89	70.86 ± 0.90
	C1 P3	29.61 ± 0.90	32.88 ± 0.90	[1]	[1]	77.77 ± 0.91
	C2 P1	[2]	27.82 ± 0.90	[1]	52.09 ± 0.91	62.67 ± 0.91
	C2 P2	[2]	30.11 ± 1.50	[1]	55.42 ± 0.90	68.31 ± 0.91
	C2 P3	[2]	29.71 ± 0.91	[1]	57.21 ± 1.50	72.53 ± 0.91

ANOVA table of the significant terms and interactions.

Model term	**Degrees of freedom**	***p*-value** [4]
Year	1	<0.0001
Harvest date * Cultivar * Threshing parameter setting	3	0.0188

[1] Harvest was not possible. [2] Not yet harvested because not yet flowered. [3] No longer harvested because no longer flowered. [4] The *p*-value of an F-test for differences between levels of the corresponding factor or factor combinations.

3.2. Dry Matter Content

The analysis showed significant differences for year and for the three-way interactions of cultivar, threshing parameter setting and harvest date. Therefore marginal means of the four-way interactions are presented (Table 4).

In 2017, the average dry matter content of 33.53% was less than the average achieved dry matter content in 2018 (48.78%) (Tables 4 and A1). Further, a higher the dry matter content was achieved at later harvest dates (Tables 4 and A1). Both cultivars C1 and C2 achieved the highest dry matter contents at the last harvest date (C1: 77.77%; C2: 72.53%) (Tables 4 and A1). For cultivar C1 the lowest dry matter contents were obtained at the third harvest date in 2017 (28.47%), while C2 reached the lowest dry matter contents at the second harvest date in 2018 (27.82%) (Tables 4 and A1). In general, comparison of the threshing parameters demonstrated that at the earlier harvest dates (harvest date 1 and 2), with P1 the highest threshed floret yields could be achieved with C1 (Figure 2), which could be explained by the highest dry matter contents (34.22 and 33.12%) and the resulting better threshability (Table 4). At later harvest dates (harvest date 4 or 5), P3 was the setting with the lowest threshed floret yields (Figure 2). This could be due to the too high dry matter contents (Table 4), which with the higher wind setting of P3 led to higher losses and thus to lower yields (Table 1 and Figure 2). Further, on mostly all harvest dates cultivar C1 showed higher dry matter contents than C2 (C1: 28.47–77.77%; C2: 27.82–72.53%). Comparing this with values of chamomile or marigold flowers [49,68], for example, where the dry matter content at harvest is about 26–30%, the values of this study are higher, i.e., the

crop is drier, which could also be due to the fact that it is not only the pure product of the flowers. Therefore, the values of dry matter contents can be better compared with late cuts for ensiling or hay purposes, when the whole plant is harvested. In these cases, the dry matter contents at the beginning of flowering were around 16–38% [69–72]. These values are comparable to the dry matter contents at the first and second harvest date in this study. These studies also showed the increasing dry matter content with increasing maturity (later harvest dates), which was also observed in this study [69–72]. The higher dry matter contents in 2018 could be due to the weather, which was warmer and drier. Higher temperatures lead to an earlier maturity of the crop [73,74], which could explain the earlier increased dry matter contents in 2018. The higher dry matter contents of C1 could be explained by the developmental advantage of about one week compared to C2. The highest threshed floret yields at the last harvest date (Figure 2 and Table A1) could be explained by the high dry matter contents. Higher dry matter contents (lower moisture contents) increase the threshing efficiency [48,54–56], and thus the threshed floret yield.

3.3. Carthamidin Content

As the analysis showed significant differences between years and for the three-way interactions of cultivars, threshing parameter setting and harvest date, medians of the four-way interactions are presented in Table 5.

Table 5. Carthamidin content (%) for the six different treatment combinations of the two cultivars (German and Chinese cultivar, C1 and C2) and three threshing parameter settings (P1–P3) (Table 1) at five harvest dates (Harvest 1–5) (DAS, days after sowing) for the two years (2017 and 2018) represented as median values ± standard error.

Year	Treatment	Harvest Date				
		1	**2**	**3**	**4**	**5**
				100 DAS	111 DAS	
2017	C1 P1	[1]	[1]	0.30 ± 0.03	[3]	[3]
	C1 P2	[1]	[1]	0.23 ± 0.02	[3]	[3]
	C1 P3	[1]	[1]	0.41 ± 0.04	[3]	[3]
	C2 P1	[1,2]	[1]	0.48 ± 0.04	0.31 ± 0.03	[3]
	C2 P2	[1,2]	[1]	0.44 ± 0.04	0.27 ± 0.02	[3]
	C2 P3	[1,2]	[1]	0.96 ± 0.09	0.35 ± 0.03	[3]
		84 DAS	95 DAS		111 DAS	118 DAS
2018	C1 P1	0.87 ± 0.05	0.53 ± 0.03	[1]	0.59 ± 0.04	0.49 ± 0.03
	C1 P2	0.80 ± 0.05	0.54 ± 0.03	[1]	0.54 ± 0.03	0.43 ± 0.03
	C1 P3	1.40 ± 0.09	0.90 ± 0.05	[1]	[1]	0.62 ± 0.04
	C2 P1	[2]	2.42 ± 0.15	[1]	1.80 ± 0.11	1.33 ± 0.08
	C2 P2	[2]	1.90 ± 0.18	[1]	1.74 ± 0.11	1.50 ± 0.09
	C2 P3	[2]	3.14 ± 0.19	[1]	2.37 ± 0.22	2.05 ± 0.13

ANOVA table of the significant terms and interactions.

Model term	**Degrees of freedom**	***p*-value** [4]
Year	1	<0.0001
Harvest date * Cultivar * Threshing parameter setting	3	0.0016

[1] Harvest was not possible. [2] Not yet harvested because not yet flowered. [3] No longer harvested because no longer flowered. [4] The *p*-value of an F-test for differences between levels of the corresponding factor or factor combinations.

In 2018, higher carthamidin contents could be achieved (on average 1.30%), than in 2017 (0.42%) (Tables 5 and A1). Both cultivars reached the highest carthamidin content in 2018 with threshing parameter setting P3, C1 with 1.4% at the first harvest date, C2 with 3.14% at the second harvest date (Tables 5 and A1). In contrast, both cultivars achieved the lowest carthamidin contents in 2017 with P2, C1 at the third harvest date with 0.23%, C2 at the fourth harvest date with 0.27%. In general, it was shown that for both cultivars P3 achieved the highest carthamidin contents (0.35–3.14%), while P2 showed the lowest contents at most harvest dates (0.23–1.90%) (Tables 5 and A1). The carthamidin contents ranged between 0.23 and 3.14% (Table 5). The other parts in the threshing material, such as small leaves or seeds, could explain the lower carthamidin content in comparison to the pure florets, which ranged between 2.68 and 8.12% and depended on cultivars, harvest dates and years [38,39]. Different cultivars, harvest dates and their interaction could have an influence on the carthamidin content, which was also shown in this study [5,38–40,75,76]. In this study, the highest carthamidin contents were achieved at the first two harvest dates, which can be explained by the oxidative and enzymatic degradation of the yellow carthamidin to the red carthamin caused by ripening [77–79]. This was also shown in several studies where the highest carthamidin contents were achieved at earlier harvest dates [38,39]. Steberl et al. [38] also showed that year 2018 with its dry conditions had a positive influence on the carthamidin content. Reasons for this could be that safflower is adapted to warm and arid weather conditions and has a low disease incidence under these conditions [51,63–65]. This could lead to good plant development during flowering, especially in 2018 when temperatures were higher and thus to high carthamidin contents. The higher carthamidin content of C2 in contrast to C1 was shown in a study by Steberl et al. [38].

In general, the lowest carthamidin contents were achieved with P2, which could be due to the fact that this setting has the smallest sieve diameters. Therefore, sieves most likely became clogged and the small florets could not be collected. In contrast, the highest carthamidin levels were achieved with P3 for both cultivars and all harvest dates. This could be related to the wider opening of the concave and the rub bars used. They help to ensure that the florets are better rubbed off from the capitula, whereby a higher proportion of florets can be obtained and due to the generally lower threshed floret yield (Figure 2), the ratio of florets to residual material can be increased and thus explain the higher carthamidin contents. In a study by Ehlert and Beier in which a chamomile harvester was tested, it was found that at higher ground speed the lower rotation speed led to a higher proportion of capitula in the harvested goodcrop compared to higher rotation speeds [80]. This may explain why P3 with a lower threshing drum speed may have a higher proportion of capitula and thus a higher carthamidin content.

3.4. Carthamidin Yield

For the carthamidin yield, the analysis showed significant differences for year and various two-way interactions of threshing parameter setting, harvest date and cultivar (Table 6).

Table 6. ANOVA table of the significant terms and interactions of carthamidin yield.

ANOVA Table of the Highest Significant Terms and Interactions		
Model Term	**Degrees of Freedom**	***p*-Value [1]**
Year	1	<0.0001
Cultivar * Threshing parameter setting	2	0.0350
Harvest date * Threshing parameter setting	8	<0.0001

[1] The *p*-value of an F-test for differences between levels of the corresponding factor or factor combinations.

Therefore, medians of the corresponding four-way interactions are presented (Figure 3).

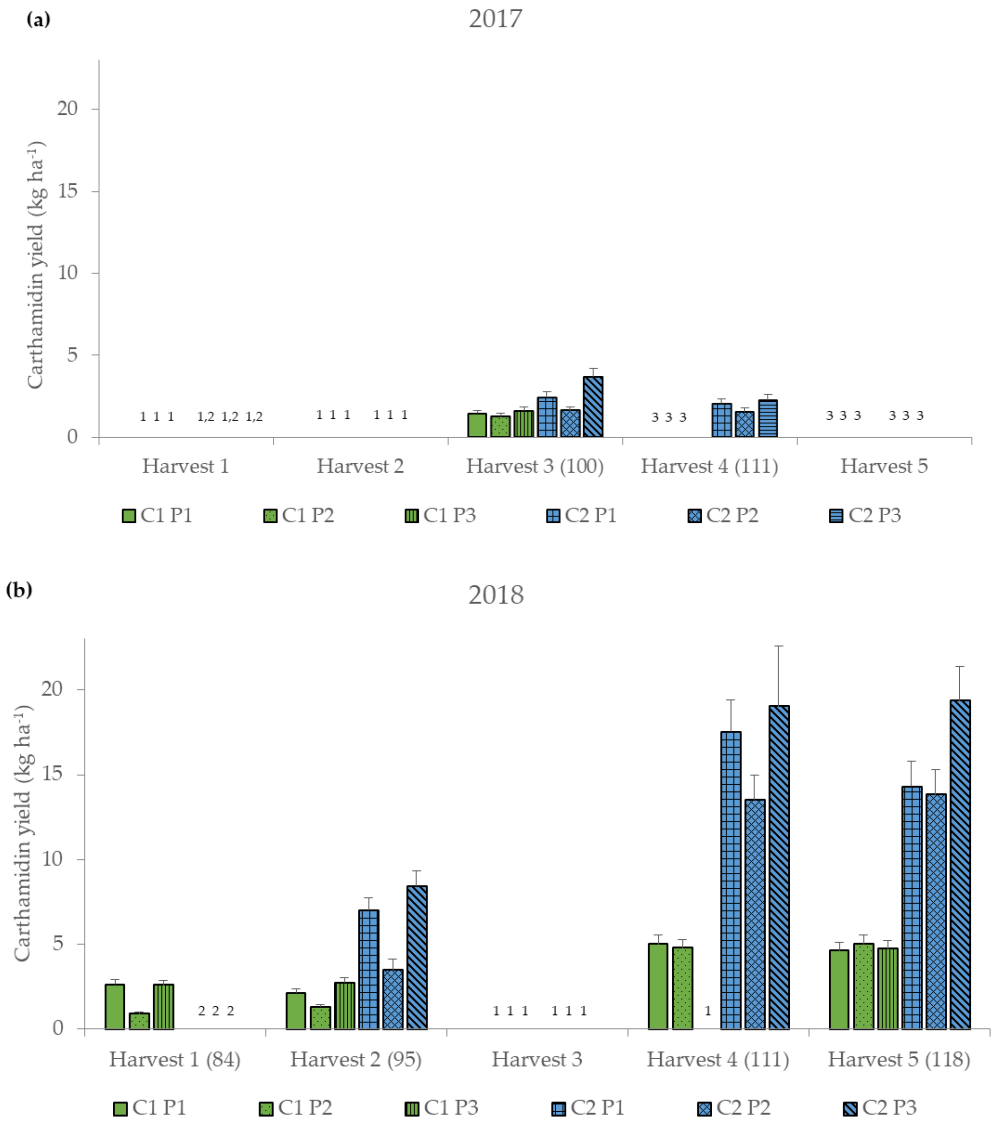

Figure 3. Carthamidin yield (kg ha^{-1}) for the six different treatment combinations of two cultivars (German and Chinese cultivar, C1 and C2) and three threshing parameter settings (P1–P3) (Table 1) at the five harvest dates (Harvest 1–5) (DAS, days after sowing) for the two years (**a**) 2017 and (**b**) 2018, represented as median values ± standard error. [1] Harvest was not possible. [2] Not yet harvested because not yet flowered. [3] No longer harvested because no longer flowered.

In 2017, an average carthamidin yield of 2.0 kg ha^{-1} was achieved, while in 2018 higher carthamidin yields were observed with around 7.7 kg ha^{-1} (Figure 3 and Table A1). Generally, C2 had higher carthamidin yields than C1, and P2 resulted in lower carthamidin yields compared to P1 and P3 (Figure 3 and Table A1). C1 produced the highest carthamidin yield with P2 at the fifth harvest (5.03 kg ha^{-1}), while at the previous harvest dates with P2 the lowest carthamidin yields were recorded (Figure 3 and Table A1). The highest carthamidin yields of C2 were reached at all harvest dates with P3 (2.27–19.36 kg ha^{-1}), whereas the least amount of yields were obtained with P2 (1.55–13.83 kg ha^{-1}). The carthamidin yields ranged from 0.91 to 19.36 kg ha^{-1} (Figure 3). In comparison to other studies, where the carthamidin yields were between 0.04 and 37.86 kg ha^{-1} depending on cultivar, harvest date and year [38,39], the carthamidin yields of the threshing samples are in the middle range, which can be

explained by the addition of other plant parts such as leaves and seeds and the lower carthamidin contents (Table 5). The highest carthamidin yields were obtained by C2 with P3 at the fifth harvest in 2018, which was mainly due to the high threshed floret yields at that date (Figure 2), and the still relatively high carthamidin contents of around 2% (Table 5). The drier and hotter weather conditions in 2018 led to a better ripening of the plants (Table 4), resulting in better threshing. Therefore, in 2018 high carthamidin contents were achieved with P3, resulting in the highest carthamidin yields.

3.5. Relationship between Individual Head Weight and Carthamidin Content

The results presented above (Sections 3.1–3.4) showed with which combine harvester parameter settings, on which harvest dates the highest yields, the highest carthamidin contents and thus also the highest carthamidin yields could be achieved. The results indicated significant differences between the two cultivars C1 and C2, especially in the carthamidin contents, which then led to higher carthamidin yields of the C2 cultivar (Table 5 and Figure 3). The data analysis revealed cultivar traits, which are important for both mechanical harvesting and carthamidin yield. As in general the threshing performance and efficiency depends, among other factors, on the crop and on the ear (spike) shape and size [47,81–83], the capitula size/weight of the tested cultivars was examined in more detail. As a study by Mozaffari and Asadi revealed that the capitulum diameter and the capitulum weight correlated significantly with each other [84], the current study considered the weight of the capitula only.

The individual capitula weights of the two cultivars C1 and C2 revealed that the weight of the capitula decreased at later harvest dates (Figure 4).

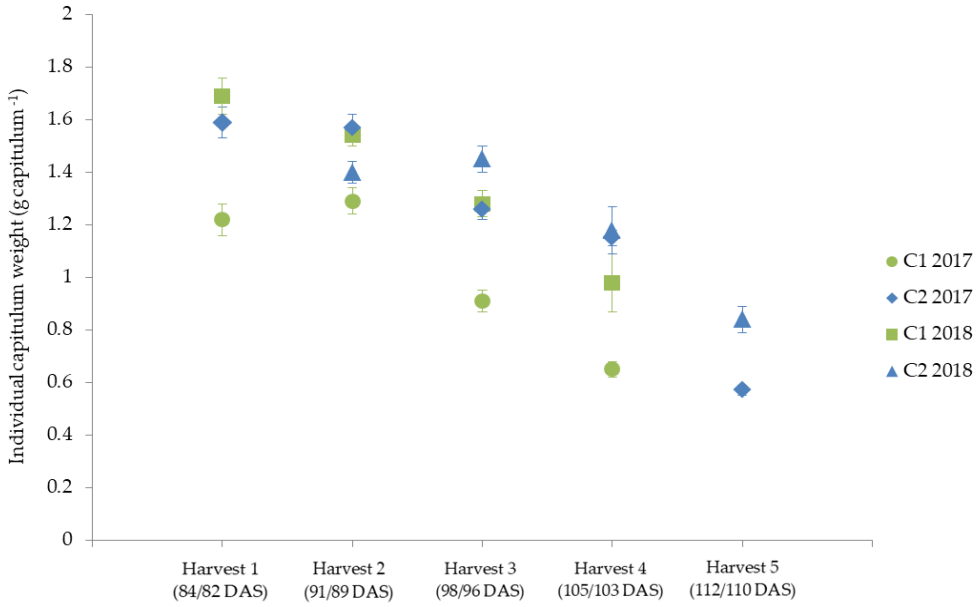

Figure 4. Individual capitulum weight (g capitulum^{-1}) for the two cultivars (C1 and C2) for the five harvest dates (Harvest 1–5) in 2017 and 2018, represented as mean values ± standard error.

C2 had a higher average individual capitula weight than C1 (Figure 4). One possible reason for the decrease in individual capitula weight could be that the primary capitula are formed first; the secondary and tertiary order capitula, which are less productive, are formed at a later stage [85]. This is in line with other studies about marigold and chamomile, which showed that flowers harvested at an earlier harvest date tend to be larger and thus have more weight [66,86]. Reasons for the cultivar differences could be the genetically influenced weight of the capitula [87,88], or, for example, the origin of the cultivar, which also has an influence on the size of the capitula [89]. The study by Knowles [89] showed that cultivars from the Middle East and Egypt had the largest capitula size, while cultivars

from Europe had average capitula size. This could explain the larger capitula size and weight of C2, which originates from China.

Since both the threshed floret yield and the carthamidin content are decisive for the carthamidin yield, the carthamidin content for the selection of cultivars was also examined more closely. For this parameter, a decrease with increasing harvest date and a cultivar difference was shown. This was already illustrated and discussed in the study of Steberl et al. [38]. Reasons for the decreasing carthamidin content at later harvest dates can be explained by the oxidative degradation of the colorant, which changes from yellow to red and therefore the yellow dye (carthamidin) decreases [77–79]. In a study by Salem et al., it was shown that in safflower the flavonoid content, to which the yellow dye of safflower belongs, both decreased with increasing developmental status and depended on the flower color [90]. It was shown that the flavonoid content is higher in orange flowers [90], which are mainly found in C2, than in yellow flowers (C1) [38]. This can also explain the higher carthamidin content of C2

Since the two factors, individual capitula weight and carthamidin content, depend both on cultivar and harvest date and decreased at later harvest date, both parameters (capitula weight and carthamidin content) are presented against each other (Figure 5).

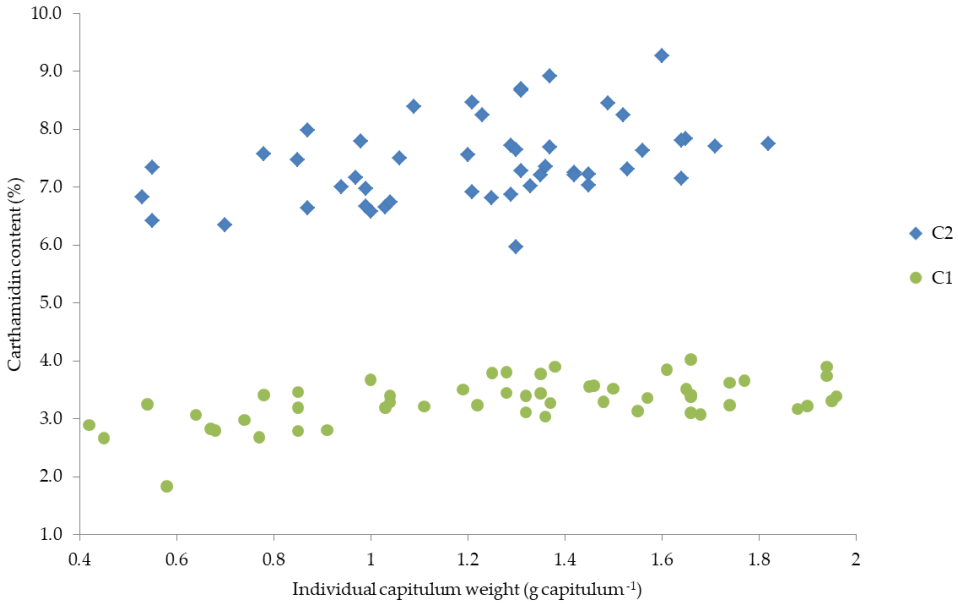

Figure 5. Relationship between individual capitulum weight and carthamidin content of the two cultivars (C1 and C2).

Figure 5 indicates a relationship between carthamidin content and individual capitula weight. However, it could not be statistically revealed whether this relationship is based only on the fact that both characteristics decreased with later harvest date or whether it is based on a correlation between the two characteristics capitula weight and carthamidin content. The dependence of the flavonoid content on the weight of the fruit and on the size of the flower/fruit has been shown in several studies [91,92]. In cranberries, an increase in anthocyanins was observed with increasing fruit weight [91], while in lemon an increasing concentration of two flavonoids was observed with increasing fruit size [92]. This is in line with the results of this study, in which the carthamidin content increased with increasing individual capitulum weight. In a study of Mohammadi and Tavakoli [39] similar results were observed, indicating that the cultivar with the smallest capitula size produced the lowest carthamidin content, while the highest carthamidin contents were obtained from medium and large capitula. This could explain the higher carthamidin contents of C2. In addition, in a study

with spelt wheat narrower ears resulted in a lower threshability [93], which could explain the better threshability of the larger capitula of C2 and thus the higher carthamidin content. Further, higher yields and carthamidin contents of C2 could be explained by the correlation of capitula size/weight and the floret yield [31]. Therefore, many studies argue in favor of a possible correlation between capitula weight and carthamidin content independent of the harvest date. Further investigations should therefore be made with both small and large capitulas and their carthamidin content should be examined separately to confirm this hypothesis.

4. Conclusions

This study tested the potential mechanization of floret harvest in safflower and revealed the impact on overall floret yield and carthamidin content. In general, to achieve highest carthamidin yields the Chinese cultivar (C2) with the threshing parameter setting P3 at the fourth or fifth harvest date (111–118 DAS (days after sowing)) can be recommended for harvesting safflower florets with a combine harvester.

As the carthamidin yields are in the middle range of those of hand harvesting, further trials should be carried out with further threshing parameter settings and with components of the combine harvester especially developed for this crop and the intended utilization of florets in the food coloring industry. Further improvements could then possibly eliminate the post-harvest sieving process. In addition, since threshability was influenced by the size/shape of the ear/capitula, which mainly depends on the cultivar, and capitula weight and carthamidin content are apparently related to each other, the capitula weight could be used as a criterion for selecting suitable safflower cultivars. The focus could be on cultivars with larger capitula, which should be further selected by breeding. When selecting cultivars, care should also be taken to assure duration to reach maturity, and thus higher dry matter contents, as higher threshed floret yields and carthamidin yields were obtained with higher dry matter contents.

Overall, threshing of florets with a combine harvester seems to be feasible. This offers the chance to reduce the high cost of manual harvests in the future, thus enabling an economic production of florets for the food coloring industry in southwestern Germany.

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Appendix A

Table A1. Simple means for the interactions of factors year (2017 and 2018), harvest date (Harvest 1–5), threshing parameter setting (P1–P3) and cultivars (C1 and C2). A letter display was added to allow for pairwise comparisons ($\alpha = 0.05$). Harvest date-by-year means with at least one identical lowercase letter are not significantly different from each other for each cultivar and threshing parameter setting combination. Threshing parameter setting-by-year means with at least one identical capital letter are not significantly different from each other for each cultivar and harvest date combination. Cultivar-by-year means with at least one identical Greek letter are not significantly different from each other for each harvest date and threshing parameter setting combination.

Parameter	Year	Harvest Date	Threshing Parameter Setting	Cultivar C1	Cultivar C2
Threshed floret yield(kg ha^{-1})	2017	3	1	484.21 $^{aA\alpha}$ ± 67.51	517.71 $^{aA\alpha}$ ± 69.80
			2	540.51 $^{aA\alpha}$ ± 71.32	372.70 $^{bA\alpha}$ ± 59.23
			3	400.91 $^{aA\alpha}$ ± 61.43	384.08 $^{bA\alpha}$ ± 60.12
		4	1	n.d.	660.91 $^{aA\alpha}$ ± 78.87
			2	n.d.	576.42 $^{aA\alpha}$ ± 73.65
			3	n.d.	671.82 $^{aA\alpha}$ ± 79.51
	2018	1	1	293.64 $^{cA\alpha}$ ± 28.67	n.d.
			2	110.46 $^{dC\alpha}$ ± 17.58	n.d.
			3	194.61 $^{cB\alpha}$ ± 23.34	n.d.
		2	1	392.67 $^{bA\alpha}$ ± 33.15	294.95 $^{bA\beta}$ ± 28.73
			2	233.66 $^{cB\alpha}$ ± 25.57	192.05 $^{cA\alpha}$ ± 40.89
			3	319.66 $^{bA\alpha}$ ± 29.91	269.67 $^{bA\alpha}$ ± 27.47
		4	1	864.74 $^{aA\alpha}$ ± 49.20	954.45 $^{aA\alpha}$ ± 51.69
			2	949.77 $^{bA\alpha}$ ± 51.56	788.98 $^{bB\beta}$ ± 46.99
			3	n.d.	783.15 $^{aAB\alpha}$ ± 82.58
		5	1	961.24 $^{aB\alpha}$ ± 51.87	1048.63 $^{aA\alpha}$ ± 54.18
			2	1141.76 $^{aA\alpha}$ ± 56.53	933.83 $^{aA\beta}$ ± 51.13
			3	784.78 $^{aC\alpha}$ ± 46.87	918.91 $^{aA\alpha}$ ± 50.72
Dry matter content (%)	2017	3	1	28.47 $^{aB\alpha}$ ± 0.70	28.47 $^{bB\alpha}$ ± 0.70
			2	31.23 $^{aA\alpha}$ ± 0.70	31.20 $^{bA\alpha}$ ± 0.70
			3	32.50 $^{aA\alpha}$ ± 0.70	30.73 $^{bA\alpha}$ ± 0.70
		4	1	n.d.	36.77 $^{aB\alpha}$ ± 0.70
			2	n.d.	40.13 $^{aA\alpha}$ ± 0.70
			3	n.d.	42.23 $^{aA\alpha}$ ± 0.70
	2018	1	1	34.22 $^{cA\alpha}$ ± 0.90	n.d.
			2	33.39 $^{cA\alpha}$ ± 0.90	n.d.
			3	29.61 $^{cB\alpha}$ ± 0.90	n.d.
		2	1	33.12 $^{cA\alpha}$ ± 0.89	27.82 $^{cA\beta}$ ± 0.90
			2	32.16 $^{cA\alpha}$ ± 0.90	30.11 $^{cA\alpha}$ ± 1.50
			3	32.88 $^{bA\alpha}$ ± 0.90	29.71 $^{cA\beta}$ ± 0.91
		4	1	51.84 $^{bB\alpha}$ ± 0.90	52.09 $^{bB\alpha}$ ± 0.91
			2	58.48 $^{bA\alpha}$ ± 0.89	55.42 $^{bA\beta}$ ± 0.90
			3	[3]	57.21 $^{bA\alpha}$ ± 1.50
		5	1	65.38 $^{aC\alpha}$ ± 0.90	62.67 $^{aC\beta}$ ± 0.91
			2	70.86 $^{aB\alpha}$ ± 0.90	68.31 $^{aB\alpha}$ ± 0.91
			3	77.77 $^{aA\alpha}$ ± 0.91	72.53 $^{aA\beta}$ ± 0.91

Table A1. *Cont.*

Parameter	Year	Harvest Date	Threshing Parameter Setting	Cultivar C1	Cultivar C2
Carthamidin content (%)	2017	3	1	0.30 $^{aB\beta}$ ± 0.03	0.48 $^{aB\alpha}$ ± 0.04
			2	0.23 $^{aB\beta}$ ± 0.02	0.44 $^{aB\alpha}$ ± 0.04
			3	0.41 $^{aA\beta}$ ± 0.04	0.96 $^{aA\alpha}$ ± 0.09
		4	1	n.d.	0.31 $^{bA\alpha}$ ± 0.03
			2	n.d.	0.27 $^{bA\alpha}$ ± 0.02
			3	n.d.	0.35 $^{bA\alpha}$ ± 0.03
	2018	1	1	0.87 $^{aB\alpha}$ ± 0.05	n.d.
			2	0.80 $^{aB\alpha}$ ± 0.05	n.d.
			3	1.40 $^{aA\alpha}$ ± 0.09	n.d.
		2	1	0.53 $^{bcB\beta}$ ± 0.03	2.42 $^{aB\alpha}$ ± 0.15
			2	0.54 $^{bB\beta}$ ± 0.03	1.90 $^{aC\alpha}$ ± 0.18
			3	0.90 $^{bA\beta}$ ± 0.05	3.14 $^{aA\alpha}$ ± 0.19
		4	1	0.59 $^{bA\beta}$ ± 0.04	1.80 $^{bB\alpha}$ ± 0.11
			2	0.54 $^{bA\beta}$ ± 0.03	1.74 $^{abB\alpha}$ ± 0.11
			3	n.d.	2.37 $^{bA\alpha}$ ± 0.22
		5	1	0.49 $^{cB\beta}$ ± 0.03	1.33 $^{cC\alpha}$ ± 0.08
			2	0.43 $^{cB\beta}$ ± 0.03	1.50 $^{bB\alpha}$ ± 0.09
			3	0.62 $^{cA\beta}$ ± 0.04	2.05 $^{bA\alpha}$ ± 0.13
Carthamidin yield (kg ha^{-1})	2017	3	1	1.45 $^{aA\beta}$ ± 0.20	2.43 $^{aAB\alpha}$ ± 0.34
			2	1.26 $^{aA\alpha}$ ± 0.18	1.64 $^{aB\alpha}$ ± 0.23
			3	1.62 $^{aA\beta}$ ± 0.23	3.68 $^{aA\alpha}$ ± 0.52
		4	1	n.d.	2.05 $^{aA\alpha}$ ± 0.28
			2	n.d.	1.55 $^{aA\alpha}$ ± 0.22
			3	n.d.	2.27 $^{bA\alpha}$ ± 0.32
	2018	1	1	2.65 $^{bA\alpha}$ ± 0.28	n.d.
			2	0.91 $^{cB\alpha}$ ± 0.10	n.d.
			3	2.62 $^{bA\alpha}$ ± 0.27	n.d.
		2	1	2.16 $^{bA\beta}$ ± 0.23	7.01 $^{bA\alpha}$ ± 0.74
			2	1.32 $^{bB\beta}$ ± 0.14	3.49 $^{bB\alpha}$ ± 0.65
			3	2.75 $^{bA\beta}$ ± 0.29	8.44 $^{bA\alpha}$ ± 0.88
		4	1	5.01 $^{aA\beta}$ ± 0.53	17.55 $^{aA\alpha}$ ± 1.84
			2	4.80 $^{aA\beta}$ ± 0.50	13.54 $^{aA\alpha}$ ± 1.42
			3	n.d.	19.05 $^{aA\alpha}$ ± 3.53
		5	1	4.65 $^{aA\beta}$ ± 0.49	14.30 $^{aB\alpha}$ ± 1.50
			2	5.03 $^{aA\beta}$ ± 0.53	13.83 $^{aB\alpha}$ ± 1.45
			3	4.74 $^{aA\beta}$ ± 0.50	19.36 $^{aA\alpha}$ ± 2.03

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5. Modifying the CROPGRO Safflower Model to Simulate Growth, Seed and Floret Yield under Field Conditions in Southwestern Germany

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Currently there is no cultivation of safflower for floret production in Germany. With a new crop or a new direction of use many uncertainties are connected, which would need to be tested in complex, time-consuming and labor-intensive field trials, which would still be very specific to the respective location and season. Plant simulation models, such as the DSSAT used in this publication, can be one way to simulate growth and potential yield. In this publication, the existing CROPGRO safflower model was evaluated against data from a two-year safflower trial in Germany. Necessary model parameters were modified and thus the model simulations were improved with regard to growth and yield. Especially the integration of a new subroutine, which allows simulating the floret yield, should be pointed out.

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Article

Modifying the CROPGRO Safflower Model to Simulate Growth, Seed and Floret Yield under Field Conditions in Southwestern Germany

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Abstract: The Decision Support System for Agrotechnology Transfer (DSSAT) currently provides a safflower model based on CROPGRO. The model was calibrated with the field data of one cultivar grown in New Mexico in 2013 and 2014. As it is rather new and has not yet been tested with other field data, it is important to evaluate the model in different environments. This study evaluated the CROPGRO safflower model for two different cultivars grown under field conditions in southwestern Germany. In addition, a new approach was added, enabling it to predict the yield of florets, which is of special interest, as these are used as a food colorant in Europe. The default model was evaluated with data from 2017 and 2018, obtained in a field trial in southwestern Germany with two cultivars, with row spacing of 12 and 33 cm and sowing densities of 40 and 75 plants m^{-2}. As the default model was not well adapted to European conditions, model modifications were implemented in the species, ecotype, and cultivar files. With these modifications, observed variables such as leaf appearance over time were well predicted (RMSE: 4.76; *d*-index: 0.88), and simulations of the specific leaf area and leaf area index were greatly improved (RMSE: 24.14 and 0.82; *d*-index: 0.78 and 0.73). Simulations of the original New Mexico data set were also improved. The newly-added approach to predict floret yield was successfully integrated into the model. Over two years and two cultivars, floret yield was simulated with a RMSE of 97.24 and a *d*-index of 0.79. Overall, the extended model proved to be useful for simulating growth, floret yield, and yield of safflower in southwestern Germany.

Keywords: safflower; *Carthamus tinctorius* L.; floret yield; crop modelling; decision support system for agrotechnology transfer (DSSAT)

1. Introduction

Safflower (*Carthamus tinctorius* L.) is a member of the Compositae family, and has a long tradition of use by humans, mainly for its high quality oil, which includes highly polyunsaturated fatty acids [1–5]. It is also used as a medicinal plant, bird seed, cut flowers, livestock forage, and tea [1,3,6,7]. Additionally, it has traditionally been used as a colorant for textiles and food [1–3]. Today, China still cultivates safflower for its flowers, but on an international level, the importance of this sector is very small [3].

Different studies claim that there is a general negative influence of artificial food colorants on the behavior of children, regardless of whether they have attention-deficit/hyperactivity disorder (ADHD) or not [8,9]. Based on this concern and the general demand for healthier foods, interest in natural dyes is growing today, and the natural colorant business, especially in the food coloring sector, is continuing

to grow worldwide [10–13]. In many countries, the yellow colorants of safflower are being increasingly used in the food and beverage industry for coloring fruit juice, fruit syrup, candies, or pastries [7,14–16]. The yellow pigment of safflower is cheaper than saffron, highly soluble in water, can be used at different temperatures and pH values, and is stable to light in aqueous solutions [7,14–16].

Due to the increasing demands of the food industry, the florets for colorant production have mainly been imported from Asia, where most safflower is produced [3,17]. However, this leads to problems such as delivery difficulties or pesticide residues. Studies have shown that safflower cultivars can be grown in Central European conditions [18–22]. However, these studies have been carried out for the production of seeds for the oil industry. None of these cultivars has been tested for their production of florets. In order to guide farmers to adopt new production systems or new directions of use, field trials need to be carried out to develop the appropriate guidelines.

Field trials are complex, very specific to a given site and season, and cost- and labor-intensive [23]. Crop growth modeling is a useful tool in research to simulate growth and potential yield [23,24]. One plant simulation model that has been used by many scientists worldwide for many years is the Decision Support System for Agrotechnology Transfer (DSSAT) [23,25]. In DSSAT, information on the weather, soil, crop/cultivar traits, and experimental data are used to simulate the growth and yields of different crops [23,24]. The DSSAT software [23] includes models for many crops following two types of templates, CERES and CROPGRO, where the latter uses a single common computer code plus read-in text files for species, ecotype, and cultivar parameters, which allows it to be adapted to a number of crops such as fababean [26], and more recently, safflower [27] and quinoa [28]. The existing model for safflower was developed to predict the safflower seed yield of one cultivar grown under four different irrigation treatments in New Mexico [27]. Singh et al. [27] described how relationships and parameters in the species and cultivar files of the CROPGRO model were adapted to simulate safflower based on the literature and growth analysis data collected in the field. This existing CROPGRO safflower model was used in our study as a first step to test growth and yield simulations for safflower under conditions of southwestern Germany. Because this work aims to use safflower as a colorant for the food industry, a new approach to model floret yield was created.

Therefore, the objectives of the present study were to: (i) evaluate the existing model against data collected in southwestern Germany over two years; (ii) modify the species, ecotype, and cultivar files to improve model simulations of growth and yield; (iii) reproduce the observed growth and yield correctly for the two seasons in Germany (2017 and 2018), as well as the original New Mexico dataset [27]; and (iv) integrate a new subroutine to simulate the floret yield.

2. Materials and Methods

2.1. Plant Material

Two cultivars of safflower were used in this experiment. These two cultivars were selected because of their different origin (one German and one Chinese cultivar) and flower color characteristics. Both cultivars are mainly used for floret production. While the German cultivar, "C1", is spiny with mainly yellow flowers, the Chinese cultivar, "C2", is thornless, with mainly orange flowers (Figure 1).

Figure 1. The two cultivars of safflower used in the field experiment: (**a**) German cultivar; (**b**) Chinese cultivar.

2.2. Site Characteristics

The field experiments were carried out in 2017 and 2018 at the experimental station Ihinger Hof of the University of Hohenheim in southwestern Germany (48°44′ N, 8°55′ E, 478 m a.s.l). The average temperature is 9.6 °C, with an annual average rainfall of 683.4 mm. In comparison, the experimental years 2017 and 2018 had mean temperatures of 9.2 °C and 10.2 °C, and annual rainfall of 654 mm and 526 mm, respectively. The weather data was recorded by an automatic weather station close to the fields. In 2018, in almost all months (except June), the average temperature was higher than in 2017 (Table 1). In addition, maximum temperatures differed between the two years, with higher values for May and June in 2017 and for July and August in 2018. On average, rainfall was higher in 2017 compared to 2018, with most of the rainfall occurring during the flowering period (July). Solar radiation was higher in 2018 (except June) compared to 2017.

Table 1. Mean, maximum, and minimum temperature (°C), monthly rainfall (mm), and average solar radiation (MJ m^{-2} d^{-1}) at the experimental site "Ihinger Hof" during the field experiments in 2017 and 2018.

Year	Month	T_{mean} (°C)	T_{max} (°C)	T_{min} (°C)	Rainfall (mm)	Solar Radiation (MJ m^{-2} d^{-1})
2017	April	7.1	21.7	−4.9	29.0	15.6
	May	13.6	30.4	−0.1	47.0	18.9
	June	18.3	32.0	5.2	72.2	23.5
	July	18.2	31.7	9.0	109.9	18.1
	August	18.1	29.7	7.0	69.3	16.2
2018	April	12.4	26.6	−2.1	17.4	18.5
	May	14.9	26.9	2.6	75.1 [1]	18.9
	June	17.4	28.0	4.3	32.5	21.3
	July	19.9	32.9	9.0	32.0	21.6
	August	19.6	33.6	4.2	28.8	17.4

[1] 37.7 mm of the monthly rainfall was recorded during one hour on 31 May.

The soils were classified as vertic Luvisol in 2017 and vertic Cambisol in 2018 [29]. The soil texture was determined according to the method described by Köhn [30]. Most of the Luvisol and Cambisol soils are fertile, and are appropriate for the cultivation of many types of crops [29,31]. Inorganic mineral nitrogen contents in soil (N_{min}) were measured according to the method described by Thun and Hoffmann [32], by the use of a flow injection analyzer (FIAstar 5000 Analyzer, FOSS GmbH, Hamburg, Germany). Marked differences can be seen in the mineral nitrogen content in 0–90 cm depth, which totaled around 124 and 45 kg N ha^{-1} in 2017 and 2018, respectively (Table 2). The organic

carbon contents were determined with a vario Macro cube (Elementar Analysesysteme GmbH, Hanau, Germany) based on the method described by Dumas [33]. Organic carbon contents in the upper two soil layers were the same in both years, with 1.2% at 0–30 cm and 0.7% at 30–60 cm depth. At a depth of 60–90 cm, the organic carbon contents differed, with 0.6% in 2017 and 1.3% in 2018. In 2017, the upper two soil layers had a lower clay concentration but a higher silt concentration compared to 2018.

Table 2. Organic carbon contents, inorganic mineral nitrogen content, and soil texture of the soil in the field experiments in 2017 and 2018.

Year	Depth (cm)	C_{org} (%)	N_{min} (kg ha^{-1})	Clay (%)	Sand (%)	Silt (%)
2017	0–30	1.2	75.0	27.1	2.7	70.2
	30–60	0.7	37.0	27.2	2.4	70.4
	60–90	0.6	11.6	33.1	3.3	63.6
2018	0–30	1.2	24.7	34.4	2.7	62.8
	30–60	0.7	10.8	30.6	4.0	65.4
	60–90	1.3	9.7	17.4	13.3	69.2

2.3. Field Experiments

The field trial was designed as a randomized, complete block design with three replications for a total of 24 plots in each year with a plot size of 32 m^2 (8 m × 4 m). Two safflower cultivars (German (C1) and Chinese (C2)) were grown in two row spacing (12 (S1) and 33 cm (S2)) and two sowing densities (40 (D1) and 75 plants m^{-2} (D2)) to determine the cultivar and management effects on plant morphology, growth, and final yield. Row orientation was north–south in 2017 and east–west in 2018. The S1 plots contained 28 rows, the S2 plots 12 rows.

Before the field trial was established, previous crops were wheat and triticale in 2017 and 2018, respectively. After harvesting, the residues of those crops were incorporated with the cultivator "POM Meteor" (MEZGER Landtechnik GmbH and Co. KG, Ditzingen, Germany) to a depth of 5 cm. Five months before sowing, the fields were ploughed with a "Juwel 8 TCP V" (LEMKEN GmbH and Co. KG, Alpen, Germany) to a depth of around 25 cm. Soil samples were taken in April 2017 and 2018 to determine the mineral nitrogen content, organic carbon content, and soil texture at depths of 0–30, 30–60, and 60–90 cm (Table 2). Shortly before sowing, the seed bed was prepared by tilling with the rotary harrow "HRB 403" (Kuhn Maschinen-Vertrieb GmbH, Genthin, Germany) and the prism roller "Simplex" (Güttler GmbH, Kirchheim/Teck, Germany) to a depth of 6 cm in 2017 and 2018. Safflower was sown at a depth of 2 cm on 25 April 2017 and 19 April 2018 with a plot driller "Deppe D82" (Agrar-Markt DEPPE GmbH, Bad Lauterberg-Barbis, Germany). The target-value of soil mineral nitrogen content was 80 kg N ha^{-1}. Based on N_{min} before sowing (Table 2), in 2017, no nitrogen fertilization was required, while 40 kg N ha^{-1} was applied as calcium ammonium nitrate with the fertilizer broadcaster "UKS 230" (RAUCH Landmaschinenfabrik GmbH, Sinzheim, Germany) shortly after sowing in 2018. Weeding was done manually twice in 2017, 35 and 43 days after sowing (DAS). Due to the high weed pressure in 2018, weeds were manually removed eight times (6, 11, 14, 19, 22, 26, 33, and 41 DAS) until the beginning of the branching stage, at which time the plants are no longer susceptible to weeds [34–36].

2.4. Data Collection

2.4.1. Non-Destructive

Measurements were conducted in the center rows of the plots at a weekly interval starting at 35 DAS in 2017 and 26 DAS in 2018, when the plants had reached leaf development [34]. BBCH stage according to Flemmer et al. [34], plant height and width (perpendicular to the row orientation), number of green and senescent leaves, number of internodes and branches of main shoots, and the total number of inflorescences (capitula) were determined. At the beginning, these measurements were recorded on

10 plants per plot, which were randomly chosen in the center rows. Due to the large amount of work required, the measurements were done on only five plants per plot after the fourth sampling date.

2.4.2. Destructive

During the growing period, the biomass partitioning to different plant parts was determined at 14-day intervals until final grain harvest. The first samples were collected 55 DAS in 2017 and 39 DAS in 2018, when most plants had started branching. In the center rows of each plot, an area of 0.25 m^2 was cut at the soil surface, and the fresh weight of the whole sample was recorded. Two representative plants per sample, which were equal to the average in height and weight, were selected and separated into branches, leaves, senescent leaves, capitula, and florets. The leaf area was determined with a scanner. Due to the large number of leaves, only a subsample was scanned. According to Corre-Hellou et al. [37], the total leaf area was calculated using the specific leaf area (SLA) of the subsample and the dry weight of all leaves. The fresh weight of all plant parts was determined, dried at 40 °C (florets), 70 °C (samples for subsequent nitrogen analyses), or at 100 °C until a constant weight was achieved to determine dry matter. Using the ratio of dry to fresh weight of all plant parts of the two separated plants and the remaining dry weight, the total dry weight of all plant parts of the harvested area was calculated.

In addition, plants from an area of 0.25 m^2 were cut at the soil surface each week during the flowering period. The fresh weight of the whole sample was recorded and then separated into capitula, florets, and residual plant parts, and the fresh weight of these plant parts was determined. All plant parts and a subsample of residual plant parts were dried as described above, and the dry weight and dry matter concentrations were recorded. Using the ratio of the dry to fresh weight of the subsamples of residual plant parts, the dry matter of the residual plant parts of the harvested area was calculated.

Florets were harvested when most flowers bloomed on three consecutive days (97–99 DAS in 2017, 96–98 DAS in 2018), with one replication on each day. An area of 1 m^2 was cut at the soil surface and the fresh weight of the complete sample was determined. The sample was separated into capitula, florets, and residual plant parts, and the number of capitula was recorded. The fresh weight of all listed plant parts was recorded, and all plant parts and a subsample of residual plant parts were dried as described above, and dry weight and dry matter concentration were calculated.

In addition to florets, a harvest of grains was performed on two consecutive days (126 and 127 DAS in 2017, 124 and 125 DAS in 2018), as soon as the plants reached maturity. In each plot, an area of 1 m^2 was cut at the soil surface, and the fresh weight of the complete sample was determined. The whole sample was divided into capitula and residual plant parts. Due to the difficulty involved in separating grains from the capitula, only a subsample was divided into heads, grains, and residual plant parts. All these parts were weighed, dried until constant weight was achieved, and then weighed again to record the dry weight and dry matter concentrations of all the plant parts. The dried capitula of the whole sample were threshed with a laboratory thresher for single plants, "LD 180" (Wintersteiger AG, Ried/I., Austria), in order to obtain the grain yield of 1 m^2.

The total nitrogen concentrations of the capitula, florets, grains, and the remaining plants were analyzed using a vario Macro cube (Elementar Analysesysteme GmbH, Hanau, Germany) according to the method described by Dumas [33].

2.5. Model Input

The following minimum data were used as model inputs for the model simulation: site characteristics (latitude, longitude and slope), daily weather data (global solar radiation, maximum and minimum temperature, rainfall, wind speed, and relative humidity), soil data for different depth (Table 2), initial conditions (e.g., previous crop and its residues), and management data (e.g., cultivar, sowing date, sowing density, row spacing, and tillage) [38]. The model was simulated with water balance turned on. Due to the fact that the experimental station could only take soil samples to 90 cm and safflower is a deep-rooting plant, the soil was modelled to be deeper in DSSAT [1,3]. The soil water-holding traits of the 60–90 cm layer were replicated, and three more layers to 180 cm were created.

Destructive samplings were carried out every two weeks or weekly during flowering on an area of 0.25 m^2, but larger samples consisting of 1 m^2 land area were collected during the main flowering and at the final grain harvest. Due to their smaller statistical variability, sample cuts of larger land areas were more trustworthy than cuts of smaller areas [27]. Therefore, it was necessary to compensate for this difference in size with a bias-adjustment [27,39]. As a reference, the 1 m^2 cut at the main flowering time (fifth cut on 31 July in 2017 and sixth cut on 24 July 2018) was taken, because at this time, the plant growth had almost reached a plateau. To calculate the bias-adjustment, the tops weight of the various destructive cuts was taken. For example, in 2017, the computed land-area tops weight of the fifth (large area reference cut) cut was divided by the computed land-area tops weight of the fourth (small area) cut. The same was done in 2018. From these calculations, the mean bias value was calculated. This resulted in bias-adjustment coefficients of 0.93 for 2017 and 0.85 for 2018. In the next step, all numerical and weight-dependent variables of the smaller samples (0.25 m^2) were multiplied by the year-dependent bias-adjustment coefficient to compensate for the bias associated with the lower level of precision of the smaller sample size.

2.6. Model Evaluation and Adaptation Based on Literature and Data

The default model with input weather, soils, and management information was simulated, and the simulations were compared to the observed phenology and growth dynamics. Where simulations disagreed with observations, parameters (in species, ecotype, and cultivar files) were modified in a sequential approach, as used by Boote et al. [26], in the following order: (1) reproducing the crop life cycle (phenology, e.g., time to emergence, first leaf, reproductive stages, and maturity), (2) rate of leaf appearance, canopy height, and width, (3) specific leaf area, leaf area index, and partitioning among vegetative organs, including rate of total biomass accumulation, (4) onset, rate, and duration of pod addition and seed growth, and lastly, (5) new coding to mimic floret growth. See the following sections in Results and Discussion for the parameter modifications targeted against specific crop observations. Model improvement with modified parameters was judged by improvement in the simulated means, root mean square error (RMSE), and Willmot Agreement Index (*d*-index; for definition of statistical indices, see Section 2.7) of the various observed plant components being targeted. While the approach was sequential, there was some iteration (stepping back to improve a previously-modified parameter). Besides modifications based on comparisons with the observed data, some parameter modifications were made based on a literature review, while some shifts in cardinal temperatures were based on the contrast between the warm versus cool weather of the two seasons. There was no automated optimization. Rather, the DSSAT graphical program computes the statistics (mean, RMSE, and *d*-index) of the targeted time-series observations for each of the 16 treatments (2 cultivars × 2 row spacing × 2 sowing densities × 2 seasons). By observing the graphs and statistics, we could determine the parameters that needed modification, although some iteration and sensitivity analysis was involved. For a given observation, e.g., leaf weight, we computed the average of the simulated and observed time-series means, the RMSE, and the *d*-index, over the 16 treatments. The goal was to come close to the observed mean, to reduce RMSE, and to increase the *d*-index for the important targeted observations. Final harvest values were only given the same weighting as part of the time-series data.

2.7. Statistics

The Willmott Agreement Index (*d*-index, (1)) and the root mean square error (RMSE, (2)) were computed to evaluate the robustness of the simulated data with the measured data, and were calculated as follows [40,41]:

$$d\text{-index} = 1 - \left[\frac{\sum_{i=1}^{N} (S_i - M_i)^2}{\sum_{i=1}^{N} \left(\left| S_i - \overline{M} \right| + \left| M_i - \overline{M} \right| \right)^2} \right], \; 0 \le d \le 1 \quad (1)$$

$$\text{RMSE} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(S_i - M_i)^2} \quad (2)$$

where N is the number of sample data points, S_i are the simulated values, M_i are the measured values, and $\overline{M}$ is the mean of the measured values.

The *d*-index is an indicator of consistency between the tendencies in simulated and measured data, and is between 0 (no fit) and 1 (perfect fit). The RMSE shows the general difference between the simulated and measured data expressed in the unit of the variable, and should be as small as possible [40–43].

3. Results and Discussion

3.1. Model Modification

Testing the existing safflower model with the data collected in a two-year field experiment indicated that major changes in the species, ecotype, and cultivar files were needed to improve model simulations [24]. By comparison to good but slight under-estimations in the year 2018 (high yields, hot year), the simulation results indicated that the model overestimated the values of 2017 (lower yields due to suboptimal wet weather conditions after the beginning of flowering). Thereafter, the model parameters were modified considering data sets from both years with different environmental conditions, using 16 data sets each (2 years over 8 treatments: 2 cultivars, 2 row spacing, and 2 sowing densities) (see Figure 5a).

The measured observations showed relatively small differences for the cultivation methods (row spacing and sowing density) (see Figure 5a). Because the response of the crop model to cultivation methods was also relatively small, we decided to focus on the main differences between cultivars and years, and therefore, only four plotted lines (two cultivars in two years) are usually shown in the following graphs. From the recorded raw data, mean values for the treatments of the respective cultivar from the respective year were calculated. However, despite the focus on the four treatments (two cultivars in two years) for the graphs in this paper, all model modification and associated statistics of model fit were based on data of all 16 treatments. In the crop model, row spacing and sowing densities were entered for each treatment.

Model parameter modification was carried out in a sequential process as described in Section 2.6; only the final simulated outputs and parameterization values are given below.

3.2. Life Cycle and Canopy Development

With a systematic and sequential approach to model adaptation, one of the first tasks should be to properly simulate plant development and life cycle [44]. An essential part of the CROPGRO Crop Template is the phenology simulation which uses parameters of the species, cultivar, and ecotype files, which include, for example, information about the cardinal temperatures in the species file or phase durations for different life cycles in the cultivar file [23]. Because plant development, life cycle, and plant growth are influenced by temperature and day length, the correct cardinal temperatures and the different physiological durations are an important first approach [23,45,46].

Therefore, the first step was to evaluate the cardinal temperatures in the species file and the different durations for the respective development phases in the cultivar and ecotype files (Table 3).

Table 3. Cardinal temperatures (base (Tb), first optimum (Topt1), second optimum (Topt2), and maximum (Tmax)), and shape of function used for growth and development processes defined in the species file of default vs. modified safflower in the CROPGRO model.

CROPGRO Species Parameters	Shape	Default Safflower				Modified Safflower			
		Tb	Topt1	Topt2	Tmax	Tb	Topt1	Topt2	Tmax
Vegetative Development	Lin. [1]	3.0	28.0	30.0	38.0	3.0	22.0	30.0	38.0
Early reproductive development	Lin. [1]	3.0	28.0	32.0	43.0	3.0	28.0	32.0	43.0
Late reproductive development	Lin. [1]	3.0	28.0	38.0	45.0	3.0	28.0	38.0	45.0
Light-saturated leaf photosynthesis (vs. current temperature)	Lin. [1]	5.0	35.0	40.0 [3]	45.0	4.0	35.0	40.0 [3]	45.0
Light-saturated leaf photosynthesis (vs. minimum temperature)	Qdr. [2]	0.0	19.0	- [6]	- [6]	−2.0	14.0	- [6]	- [6]
Leaf relative expansion	Lin. [1]	12.0 [4]	22.0	- [6]	- [6]	8.0 [5]	21.0	- [6]	- [6]
Pod addition rate	Qdr. [2]	14.0	21.0	26.5	40.0	9.0	24.0	26.5	40.0
Seed growth rate	Qdr. [2]	6.0	21.0	23.5	41.0	6.0	21.0	23.5	41.0

[1] Lin. = linear, interpolation between cardinal temperatures. [2] Qdr. = quadratic function. [3] relative rate of 0.8. [4] relative rate of 0.25. [5] relative rate of 0.3. [6] relative rate remains high above Topt1.

The defined cardinal temperatures include the base temperature (Tb), the first optimum temperature (Topt1), the second optimum temperature (Topt2), and the maximum temperature (Tmax) in °C (Table 3). For the phenological parameters, it was necessary to set a lower Topt1 temperature for the rate of leaf appearance. The measured leaf appearance data were used to adjust the value for the Topt1 in the model from the default 28 °C (based on [47]) to the modified 22 °C. Based on the measured data, the resulting vegetative growth curves and literature references indicated that safflower can tolerate and grow well at lower temperatures during vegetative and reproductive phases [2,48,49]. Another important modification was related to the temperature effects on leaf photosynthesis. Initially, the cardinal temperatures of sunflower and soybean photosynthesis were used [44,50,51]. Based on the measured data of our two-year German field experiment, the temperatures were shifted towards those used in the fababean model, thereby achieving an overall better fit. This change is supported by the literature, suggesting that safflower can tolerate temperatures down to −7 °C, depending on the stage of development [26,44,52].

Further, physiological day durations are an essential model component [23,45]. Tables 4 and 5 show the phase duration changes made in the ecotype and cultivar files. The model was adapted to the two cultivars used in the field trials in 2017 and 2018. After the modifications of the species, ecotype and cultivar files were completed, Singh's original data of the cultivar "PI8311" was evaluated with the new model settings (Tables 4 and 5). The modified version of the model resulted in a better fit for Singh's experiments [27], indicated by statistical parameters Wilmott Agreement Index (*d*-index) and RMSE [40,41].

Safflower is generally regarded as a daylength-neutral, long-day plant, and the origin of the cultivar plays a decisive role [3,53]. In order to be able to correctly model the growth phases, such as the time to anthesis, safflower is defined here as day-neutral in the model [27], setting critical long daylength to 23.0 h and PPSEN = 0.001. Because of the definition as a day-neutral plant, the minimum rate of reproductive development under long days and optimal temperatures is irrelevant, and therefore, is not listed in the cultivar file table (Table 5).

In the following section, important parameters are described in more detail. All modifications made in the model are listed in Tables 3–8.

Table 4. Model parameter names and definitions in the ecotype file in the CROPGRO model for the default and modified safflower cultivar (PI8311) vs. German (C1) and Chinese (C2) cultivars grown in Germany.

CROPGRO Ecotype Parameters	Definition of Parameter	Cultivars			
		Default Safflower Cultivar	Modified Safflower Cultivar	Safflower Cultivars Cultivated in Germany	
		PI8311	PI8311	C1	C2
PL-EM	Time between planting and emergence (PL-EM)(TD) [1]	3.60	5.0	5.0	5.0
EM-V1	Required time from emergence to first true leaf (TD) [1]	6.0	6.0	3.0	3.0
FL-VS	Time from first flower to last leaf on main stem (PD) [2]	7.00	7.00	14.0	14.0
TRIFL	Rate appearance of leaves on the main stem (leaves TD^{-1}) [1]	0.36	1.00	1.00	1.00
RWDTH	Relative width in comparison to the standard width per node defined in the species file	0.85	0.75	0.75	1.00
RHGHT	Relative height in comparison to the standard height per node defined in the species file	0.85	0.75	0.80	1.00

[1] Thermal days. [2] Photothermal days.

Table 5. Model parameter names and definitions in the cultivar file in the CROPGRO model for the default and modified safflower cultivar (PI8311) vs. German (C1) and Chinese (C2) cultivars grown in Germany.

CROPGRO Cultivar Parameters	Definition of Parameter	Cultivars			
		Default Safflower Cultivar	Modified Safflower Cultivar	Safflower Cultivars Cultivated in Germany	
		PI8311	PI8311	C1	C2
EM-FL	Time from plant emergence to flower appearance (PD) [1]	17.0	16.5	10.3	10.3
FL-SH	Time between first flower and first pod (PD) [1]	3.0	3.2	4.5	4.5
FL-SD	Time between first flower and first seed (PD) [1]	15.0	14.7	13.0	13.0
SD-PM	Time between first seed and physiological maturity (PD) [1]	30.0	30.5	28.5	28.5
FL-LF	Time between first flower and end of leaf expansion (PD) [1]	20.25	20.25	18.00	18.00
LFMAX	Maximum leaf photosynthetic rate at 30 °C, 350 ppm CO_2, and high light (mg CO_2 m^2 s^{-1})	2.20	1.50	1.55	1.80
SLAVR	Specific leaf area of cultivar under standard growth conditions (cm^2 g^{-1})	200.0	250.0	250.0	260.0
SIZELF	Maximum size of full leaf (cm^2)	115.0	115.0	115.0	115.0
XFRT	Maximum fraction of daily growth partitioned to seed and shell	0.55	0.64	0.67	0.76
WTPSD	Maximum weight per seed (g)	0.040	0.054	0.064	0.052
SFDUR	Seed filling duration for pod cohort (PD) [1]	29.0	29.0	30.0	30.0
SDPDV	Seeds per pod at standard growth conditions (no. pod^{-1})	22.25	18.00	22.25	22.25
PODUR	Duration of pod adding (PD) [1]	17.0	18.0	19.0	17.0
THRESH	Weight percentage of seeds in pods (%)	51.3	47.0	55.0	61.0
SDPRO	Potential seed protein (g (protein) g $(seed)^{-1}$)	0.14	0.14	0.14	0.14
SDLIP	Potential seed lipid (g (oil) g $(seed)^{-1}$)	0.33	0.33	0.33	0.33

[1] Photothermal days.

Table 6. Model parameter names and definitions for photosynthesis and leaf growth parameters, leaf senescence factors, and evapotranspiration defined in the species file of default vs. modified safflower in the CROPGRO model.

CROPGRO Species Parameters	Definition of Parameter	Default Safflower	Modified Safflower
	Photosynthesis parameters		
SLWREF	Specific weight at which LFMAX is defined (g cm^{-2})	0.0035	0.0025
LNREF	Leaf N concentration at which LFMAX is defined (%N)	4.90	4.50
	Leaf growth parameters		
FINREF	Specific leaf area of leaves at plant emergence (cm^2 g^{-1})	150.0	200.0
SLAREF	Specific leaf area of the standard reference cultivar at peak early vegetative phase (cm^2 g^{-1})	270.0	260.0
SLAMAX	Upper and lower limit of specific leaf area (response to solar radiation)	650.0	650.0
SLAMIN	(cm^2 g^{-1})	110.0	260.0
	Leaf senescence factors		
SENRTE	Grams of leaf mass that are lost per gram of protein mobilized (g)	1.00	1.10
SENRT2	Rate of abscision after physiological maturity	0.20	0.10
ICMP	Light compensation point for senescence of lower leaves because of excessive self-shading by the crop canopy (mol PPFD m^{-2} d^{-1})	0.80	0.40
PORPT	A stand-in for petiole mass abscised per unit leaf mass abscised (petiole mass leaf $mass^{-1}$)	0.58	0.01
	Evapotranspiration		
KEP	Extinction coefficient for solar radiation for partitioning of potential ET to T	0.57	0.50

Table 7. Model parameters for vegetative partitioning, canopy height and width growth parameters, and leaf senescence factors as a function of the vegetative stage defined in the species file of default vs. modified safflower in the CROPGRO model.

CROPGRO Species Parameters	Partitioning-(Fraction), Potential Internode Length (m) and Leaf Senescence at a Given V-Stage									
	Default safflower values									
XLEAF	0.0	1.5	3.3	5.0	7.8	10.5	30.0	40.0	final	
YLEAF	0.43	0.44	0.45	0.44	0.39	0.35	0.34	0.34		
YSTEM	0.09	0.12	0.19	0.26	0.34	0.46	0.46	0.46		
FRLFF									0.25	
FRSTMF									0.54	
	Modified safflower values									
XLEAF	0.0	2.0	4.0	6.5	9.8	15.0	35.0	50.0	final	
YLEAF	0.43	0.44	0.45	0.44	0.43	0.40	0.37	0.34		
YSTEM	0.09	0.12	0.19	0.26	0.30	0.41	0.43	0.46		
FRLFF									0.20	
FRSTMF									0.63	
	Default safflower values									
XVSHT	0.0	1.0	4.0	6.0	8.0	10.0	14.0	16.0	20.0	40.0
YVSHT	0.030	0.053	0.063	0.066	0.069	0.066	0.062	0.051	0.034	0.006
YVSWH	0.030	0.051	0.062	0.064	0.066	0.063	0.059	0.046	0.025	0.001
	Modified safflower values									
XVSHT	0.0	1.0	4.0	6.0	8.0	10.0	14.0	18.0	26.0	40.0
YVSHT	0.020	0.023	0.034	0.038	0.042	0.045	0.049	0.052	0.052	0.045
YVSWH	0.020	0.021	0.030	0.030	0.030	0.028	0.023	0.021	0.014	0.001
	Default safflower values									
XSTAGE	0.0	5.0	14.0	30.0						
SENPOR	0.0	0.0	0.12	0.12						
XSENMX	3.0	5.0	10.0	30.0						
SENMAX	0.0	0.2	0.6	0.6						
	Modified safflower values									
XSTAGE	0.0	5.0	18.0	40.0						
SENPOR	0.0	0.0	0.04	0.06						
XSENMX	3.0	5.0	18.0	40.0						
SENMAX	0.0	0.2	0.5	0.5						

Table 8. Model parameter names and definitions for plant composition parameters (fractions of the total tissue dry weights) defined in the species file of default vs. modified safflower in the CROPGRO model.

CROPGRO Species Parameters	Definition of Parameter	Default Safflower	Modified Safflower
PROLF_	Protein concentrations of leaf tissue		
	I = "maximum"	0.356	0.306
	G = "normal growth"	0.140	0.140
	F = "final"	0.112	0.090
PROST_	Protein concentrations of stem tissue		
	I = "maximum"	0.150	0.150
	G = "normal growth"	0.100	0.100
	F = "final"	0.035	0.035
PRORT_	Protein concentrations of root tissue		
	I = "maximum"	0.092	0.092
	G = "normal growth"	0.064	0.064
	F = "final"	0.056	0.056
PROSH_	Protein concentrations of shell tissue		
	I = "maximum"	0.250	0.200
	G = "normal growth"	0.150	0.150
	F = "final"	0.050	0.050
PCAR_	Carbohydrate-cellulose concentrations of tissues		
	Leaf (LF)	0.405	0.455
	Stem (ST)	0.572	0.572
	Root (RT)	0.711	0.711
	Shell (SH)	0.500	0.550
	Seed (SD)	0.470	0.420
	Nodule (NO)	0.480	0.480

3.2.1. Leaf Number

In order to design the crop life cycle correctly, it is very important to calibrate the plant growth parameters in the species file correctly, which includes the effect of temperature on leaf appearance rate, leaf relative expansion, and other processes (Table 3) [45]. Based on the measured data, the first optimum temperature (Topt1) for the leaf appearance rate was reduced from 28.0 to 22.0 °C. Depending on the stage of development, safflower has different temperature requirements [48]. Since leaves are also formed during the rosette stage, and safflower tolerates very low temperatures during this stage, the lower temperatures can be justified [3,49,52]. The parameters affecting leaf appearance rate could not be calibrated for the original model of Singh et al. [27] because the leaf number was not recorded in their experiment. Given the lack of data, default parameters were assumed from the soybean model; this may explain the need for modifications in the present work [44]. It was important to set the leaf appearance rate correctly. The time between planting and emergence (PL-EM) was too short, and was increased to 5.0 for both the PI8311 cultivar and the new cultivars (Table 4). The model is designed to simulate the appearance of fully developed leaves. Because leaf tips were counted during this data collection for both non-destructive and destructive measurements, the time between emergence to first true leaf (EM-V1) had to be shortened by half. In addition, the rate of leaf appearance (TRIFOL) was increased significantly from 0.36 to 1.00, based on the observed rate of leaf appearance over time (Figure 2 and Table 4).

This was an essential step, because the rate of V-stage progression (given as leaf number on the main stem) affects the modeled shift in the partitioning of daily assimilates among leaf and stem and the leaf area index (LAI) as well. The maximum leaf photosynthetic rate at 30 °C, 350 ppm CO_2, and high light (LFMAX) was adjusted based on the correct prediction of biomass over time, and was set at 1.50 mg CO_2 m^{-2} s^{-1} for the PI8311 cultivar, 1.55 mg CO_2 m^{-2} s^{-1} for the German, and 1.80 mg CO_2 m^{-2} s^{-1} for Chinese cultivar (Table 5).

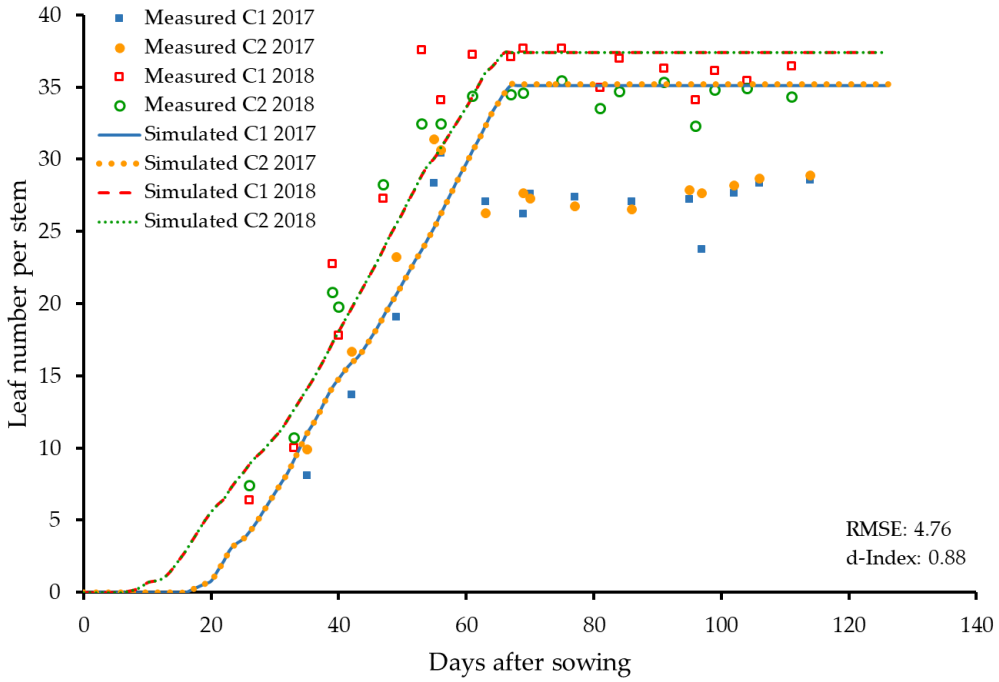

Figure 2. Simulated (lines) and measured (symbols) leaf number on the main stem as a function of days after sowing for the German (C1) and Chinese (C2) safflower cultivars in 2017 and 2018. Root mean square error (RMSE) and Wilmott Agreement Index (*d*-index).

The simulated and measured leaf number on the main stem for both cultivars in both years is shown in Figure 2. Comparing the simulated and measured leaf number on the main stem, the year 2018 could be simulated very well, but 2017 was overpredicted, at least after the 50th DAS. This was also reflected in the RMSE and *d*-index separately for both years. In 2017, the RMSE was 6.52, the *d*-index 0.77, while 2018 had a lower RMSE of 3.01 and a higher *d*-index of 0.97. A possible reason for the good plant growth in 2018 could be the warm and dry weather during flowering, which is very suitable for safflower because sunny, warm conditions accelerate growth, and moisture could promote diseases (Table 1) [2,54,55]. A comparison between the simulated and measured leaf number of the main stem of both cultivars and years resulted in a RMSE of 4.76 and a *d*-index of 0.88 (Figure 2).

3.2.2. Height and Width

The height and width of the plants were measured weekly. Parameters for both the "PI8311" cultivar and the newly-added two cultivars "C1" and "C2" were modified (Table 4). The relative width in comparison to the standard width per internode (RWDTH) was modified for the PI8311 cultivar from 0.85 to 0.75 and for the new cultivars "C1" and "C2" to 0.75 and to 1.00 (Table 4). Also, the canopy width increment (YVSWH) and internode length (YVSHT) as a function of plant vegetative node stage had to be modified (Table 7). A comparison between the simulated and measured canopy widths for all four treatments showed an underprediction of canopy width with a RMSE of 0.08 and a *d*-index of 0.66 (data not shown). The relative height parameter (RHGHT) that modifies the standard internode length per internode was changed for the PI8311 cultivar from 0.85 to 0.75 and for the newly-added cultivars "C1" and "C2" to 0.80 and to 1.00 (Table 4). There was an impact of year on canopy height (Figure 3). The canopy height in 2017 was overpredicted with a RMSE of 0.20 and a *d*-index of 0.83, but 2018 was simulated very well, with a RMSE of 0.07 and a *d*-index of 0.98. The reasons for this could be the temperature, which affects the plant height [48,56,57]. Figure 3 shows that plants in 2018 were taller than in 2017, which could be related to the warmer and drier weather conditions in 2018 (Table 1). Additionally, in both years, the Chinese cultivar (C2) grew taller than the German one (C1)

(Figure 3); a possible reason for this could be that the cultivar or its origin also plays an important role in how tall the plants grow [48,56,58]. Knowles [58] characterized many safflower cultivars, and showed that the Far Eastern cultivars grow taller, whereas the European ones tend to be medium-sized. After modifying the parameters based on the obtained dataset, the canopy height had a RMSE of 0.14 and a *d*-index of 0.90.

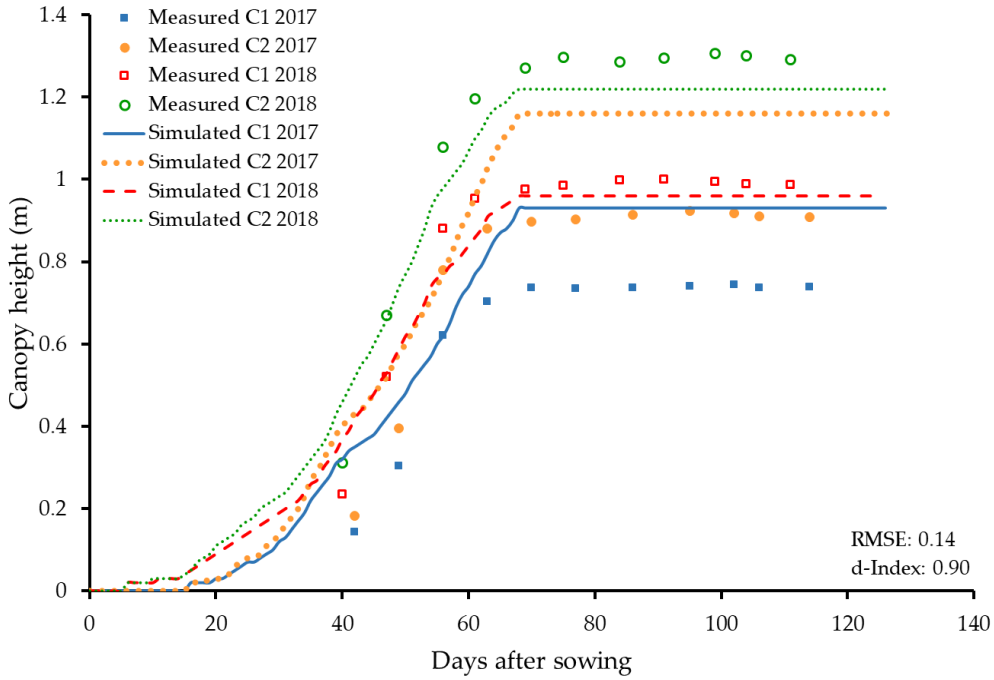

Figure 3. Simulated (lines) and measured (symbols) canopy height as a function of days after sowing for the German (C1) and Chinese (C2) safflower cultivars in 2017 and 2018. Root mean square error (RMSE) and Wilmott Agreement Index (*d*-index).

3.2.3. Time to Flowering

To model the growth of safflower, it is important to notice that the capitula (flower heads) are already present before flowering occurs, and that the seeds begin to grow shortly after flowering [27]. In order to represent this correctly in the model, the true anthesis is ignored, and the time of the first capitulum is entered as first pod date (PD1T). Hence, the time (in photothermal days, PD) between the "false" first flower and the first pod (capitulum) (FL-SH) was defined. The sum of the time from plant emergence to flower appearance (EM-FL), plus the time between the first flower and first seed (FL-SD), is entered to achieve "true" anthesis [27]. With the correct phase duration between first flower and first seed (FL-SD) and the correct first seed date (PDFT), as well as the time span between first seed and physiological maturity (SD-PM), the correct physiological maturity is achieved. In addition, the cardinal temperatures of vegetative growth were modified prior to this step in order to allow more rapid plant growth at lower temperatures to simulate vegetative growth correctly (Table 3). The time between plant emergence and flower appearance (EM-FL) was shortened, and the time between first flower and first pod (FL-SH) had to be extended (Table 5). Nevertheless, it was important to keep the time between EM-FL as long as possible and the time between FL-SH as short as possible in order to achieve a better transition of partitioning between leaf and stem. After flowering and the time of the first pod was set correctly, the time from first flower to the appearance of the last leaf on the main stem (FL-VS) could be modified. FL-VS affects the timing of the last leaf appearance as well as the endpoint of any further height increase, and was extended from 7.0 to 14.0 PD (Table 4) [34].

3.2.4. Specific Leaf Area and Leaf Area Index

In the default model, important parameters affecting the SLA were assumed to be similar to the soybean model. The model coding allows SLA to be influenced by solar radiation and cardinal temperatures; therefore, it was necessary to adapt the original model parameters affecting SLA to new environmental conditions in Europe and to the two different cultivars (Table 3) [24,59]. Simulated LAI indicated that leaf expansion started too slowly, and that SLA was too low; therefore the SLA-related parameters (especially SLAMIN) were increased. Based on the measured data, the base temperature (Tb) for leaf expansion was reduced from 12.0 to 8.0 °C and the first optimum temperature (Topt1) from 22.0 to 21.0 °C. The lower Tb and Topt1 for leaf expansion also acted to increase SLA and LAI.

As the SLA of a cultivar under standard growth conditions (SLAVR) and the specific area of the standard reference cultivar at the peak early vegetative phase (SLAREF) should be close to the same value, the SLAVR for the cultivars "PI8311" and "C1" were set to 250 cm^2 g^{-1}, and for the cultivar "C2" to 260 cm^2 g^{-1} (Table 5). In the species file, the value SLAREF was set to 260 cm^2 g^{-1} (Table 6). Closely linked to these values are the upper and the lower limits of SLA with regard to the response to solar radiation (SLAMIN, SLAMAX). The increase in SLAMIN from 110 to 260 cm^2 g^{-1} had the largest effect on SLA, while SLAVR and SLAREF were set to 260 cm^2 g^{-1} to be close to SLAMIN (Table 6). Modification of SLAMAX was not necessary.

All these modifications resulted in significant improvements in the simulated SLA by increasing the *d*-index and reducing the RMSE. After all the modifications were carried out, a comparison of the simulated and the measured data showed an underprediction of the values of 2018, with a RMSE of 27.46 and a *d*-index of 0.83, and an overprediction of the values of 2017, with a RMSE of 20.83 and a *d*-index of 0.72. Comparing the simulated and measured SLA of all treatments over both years and both cultivars, a RMSE of 24.14 and a *d*-index of 0.78 was obtained (Figure 4).

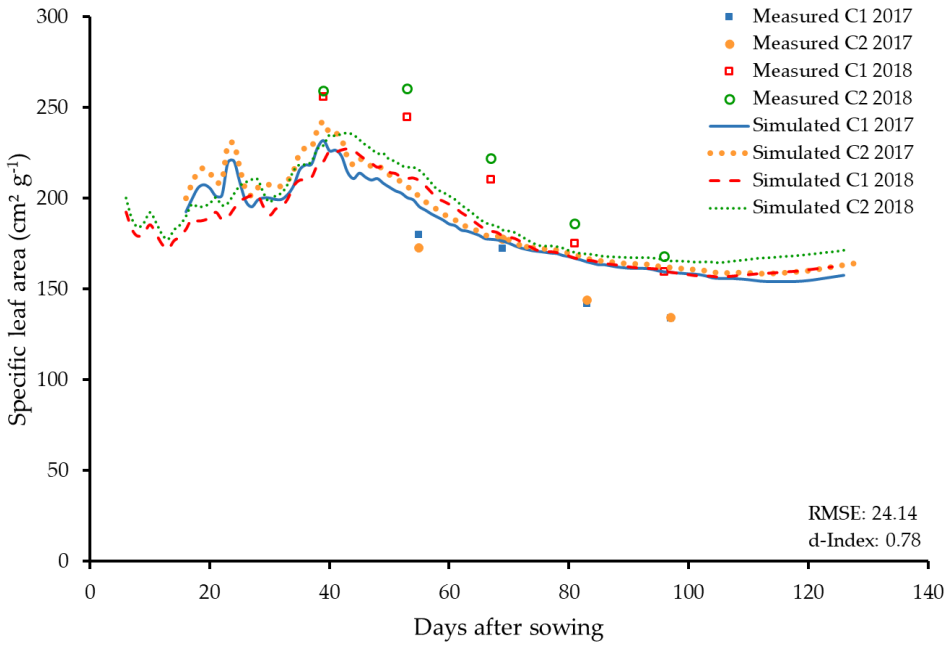

Figure 4. Simulated (lines) and measured (symbols) specific leaf areas as a function of days after sowing for the German (C1) and Chinese (C2) safflower cultivars in 2017 and 2018. Root mean square error (RMSE) and Wilmott Agreement Index (*d*-index).

All the modified parameters related to SLA led to an improvement of LAI in both the RMSE and the *d*-index. Figure 5 shows the LAI. Figure 5a shows simulations of all 16 treatments, while Figure 5b has only four curves (two cultivars in two years), and neglects the small effects of row spacing and sowing density.

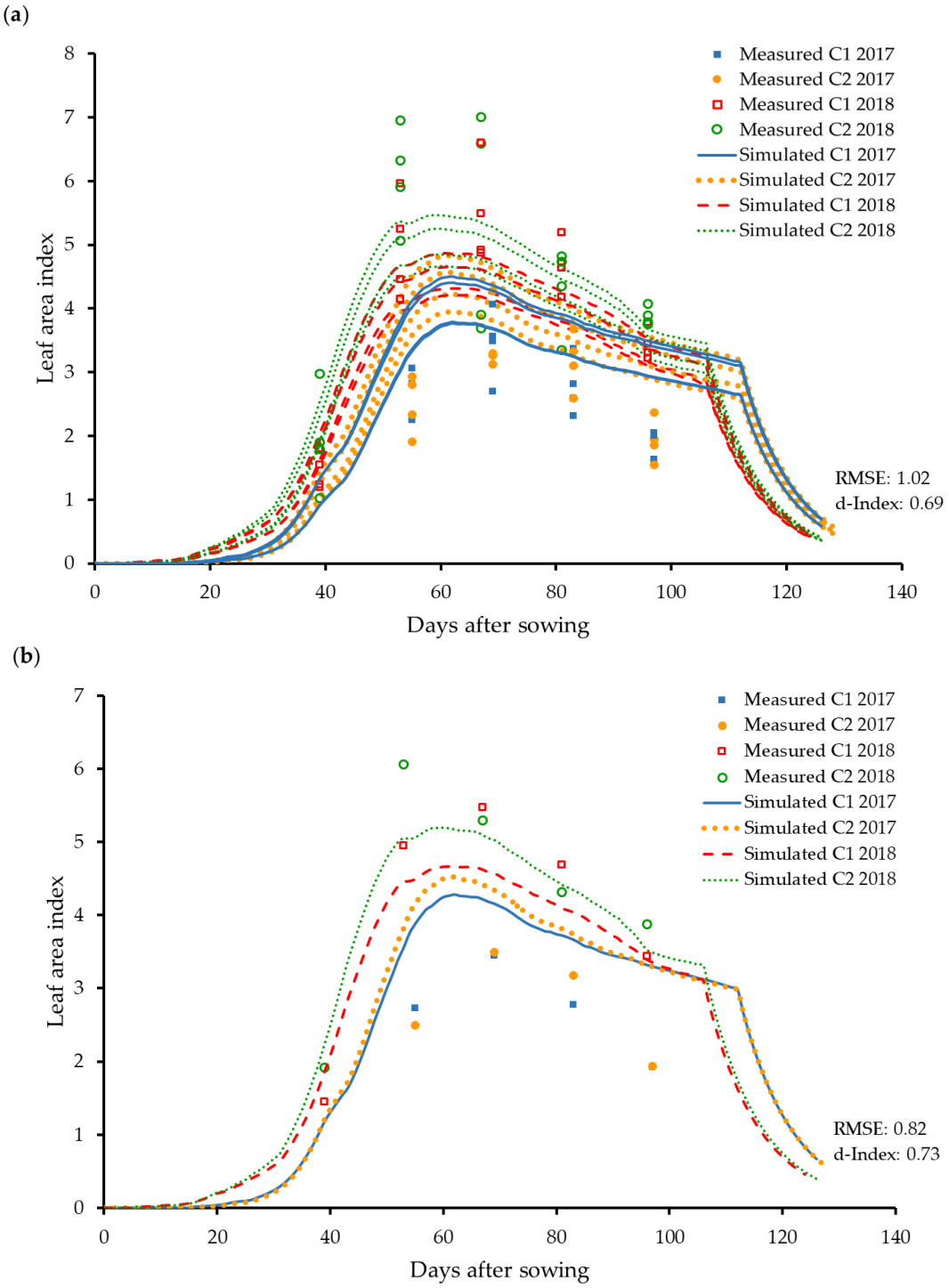

Figure 5. Simulated (lines) and measured (symbols) leaf area index as a function of days after sowing (**a**) for all treatments where the model was simulated for two cultivars each at two row spacing and two sowing densities in 2017 and 2018 = 16 treatments in total, and for (**b**) German (C1) and Chinese (C2) safflower cultivars in 2017 and 2018 (simulated and observed data per cultivar were averaged over row spacing and sowing density). Root mean square error (RMSE) and Wilmott Agreement Index (*d*-index).

The correct termination of leaf area expansion and the correct peak of LAI was achieved by reducing the time between first flower and end of leaf expansion (FL-LF) from 20.25 to 18.00 PD (Table 5). In Figure 5, the slow decline of the simulated LAI at the end of life cycle is a result of protein mobilization and the value of SENRTE (Table 6). The following steep decline is set by maturity (SD-PM) and the SENRT2 (Table 6). This has already been noted by Stern and Beech [60], who reported a rapid rise in LAI to 4–5, and then a drop to almost zero at harvest.

After modification, simulated LAI for 2017 was overpredicted compared to the measured LAI, with a RMSE of 1.12 and a *d*-index of 0.51 (Figure 5b). In 2018, the opposite was observed, where the simulated values slightly underestimated the measured values for LAI, with a RMSE of 0.53 and a *d*-index of 0.95. One reason for the higher LAI in 2018 could be the warm and dry weather conditions, which are very appropriate for safflower growth [2,54]. The adjustment of the parameters to best simulate both years and both cultivars resulted in a RMSE of 0.82 with a *d*-index of 0.73.

3.3. Dry Matter Accumulation and Partitioning

The dry matter growth of new plant organs depends on various factors, such as the availability of carbohydrates and the partitioning to different plant parts [23]. Important parameters influencing photosynthesis and dry matter accumulation include the partitioning to the root. If considerable assimilates are allocated to the root very early in the growth cycle, this occurs at the expense of shoot growth and leaf area formation, which then develop more slowly [45]. Because partitioning depends on the growth stages of the plant, the following table shows the partitioning-fractions, internode length, and leaf senescence in relation to the respective V-stage (Table 7) [23].

The partitioning functions are used to determine the daily distribution of assimilates to tissues at a given V-stage. It is important to distribute this correctly, as distribution to leaf tissues defines the LAI. LAI, in turn, affects the amount of assimilates for the formation of reproductive organs during the reproductive phase. Based on the measured data, the partitioning for leaf and stem was changed, and more assimilates were shifted into the leaves. Due to the faster rate of leaf appearance based on the observed leaf number (Figure 2), the shift of the partitioning towards the stem was initially too fast (in the default model), with increasing V-stage leading to less leaf and more stem mass in the end. As the leaf appearance rate on the main stem (TRIFL) was very high, the corresponding V-stages had to be renumbered and extended (Tables 4 and 7). Since total partitioning altogether sums up to 100%, the missing part in the partitioning after YLEAF and YSTEM always represents the partitioning to the roots. Since roots were neither harvested nor weighed in the field trials, this proportion of roots could not be evaluated. Since it was desirable that more of the partitioning go into the leaves, the fraction to stem was reduced while keeping the allocation to root unchanged, in order to retain the same total (Table 7). Another important change was the fraction of the vegetative dry matter growth allocated to leaf and stem at the end (FRLFF and FRSTMF) by decreasing the leaf and increasing the stem percentage. After the partitioning to the leaves was correctly defined as a function of V-stages, LAI was increased, allowing more photosynthesis to take place, which, in turn, increased biomass formation and, later, also reproductive organ formation. Despite these changes, the overall biomass formation was still a little too low, and SWREF (specific weight at which LFMAX is defined) was reduced to achieve a greater slope to increase the amount of biomass (Table 6).

After all the modifications had been made, the model indicated a good fit for leaf weight and stem weight, with RMSE of 327 and 1449 and d-indices of 0.78 and 0.81, respectively (data not shown).

Figure 6 shows the development of the aboveground biomass (tops) during the vegetative period. The model indicated a good fit at the beginning when the weather conditions were still relatively warm and dry (Table 1 and Figure 6). After 80 DAS, data of year 2017 were overpredicted with a RMSE of 3208 and a *d*-index of 0.75, whereas the year 2018 was underpredicted, with a RMSE of 3432 and a *d*-index of 0.91. The over- and under- predictions are mainly the result of forcing the model to best simulate the data across two different years, with 2018 being an ideal year for safflower growth, while the humid conditions in 2017 led to diseases and an overall lower yield. Comparing the simulated

and measured tops weights of both years and cultivars, a RMSE of 3320 and a *d*-index of 0.83 were obtained (Figure 6). The decrease of the biomass after peak vegetative growth and towards 110–120 DAS is typical for safflower, and can be explained by the seed formation, leaf senescence, and maturity of the plant [60].

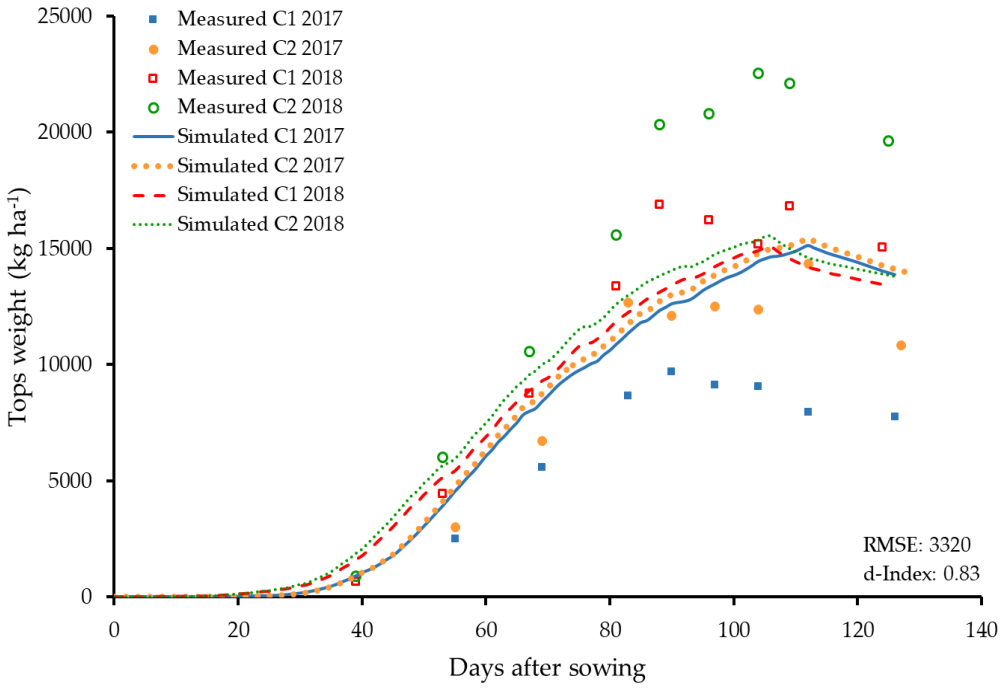

Figure 6. Simulated (lines) and measured (symbols) tops weight as a function of days after sowing for the German (C1) and Chinese (C2) safflower cultivars in 2017 and 2018. Root mean square error (RMSE) and Wilmott Agreement Index (*d*-index).

Because the default safflower model was adapted from the soybean model, it is possible to orientate oneself on the structures of the soybean model, in which the plant consists of roots, leaf, stem, shell, and seed [27,44,61]. These vegetative organs are defined as having constant compositions, i.e., proportions of compounds for the different organs, except that protein (N) is allowed to vary under N-stress [61]. Based on this fact, the sum of lipids (PLIPLF), protein (PROLFI), carbohydrates (PCARLF), lignin (PLIGLF), minerals (PMINLF), and organic acid (POALF) for a particular new plant organ under non-limiting nitrogen should always sum to 1.00 (Table 8) [27]. Under N stress, the actual protein can be less than the potential (PROLFI), in which case the PCARLF increases as the complement. In Table 8, only protein and carbohydrate-cellulose concentrations are shown, because other compositions of components such as lipids were not changed from the default model. Based on the nitrogen concentration data of leaves (data not shown), the target PROLFI was reduced. Since the sum of the individual components of a plant organ must always be equal to 1.00, the protein concentration was reduced from 0.356 to 0.306, while the proportion of carbohydrate–cellulose in the leaf was increased by the same amount, from 0.405 to 0.455.

3.4. Reproductive Organs and Yield Influencing Parameters

Cardinal temperatures in the species file were changed for the pod addition rate (Table 3). In the original model by Singh et al. [27], the cardinal temperatures of the pod addition rate of soybean were used [44]. A reduction of Tb was necessary to achieve sufficiently high pod addition rate at the relatively cool temperatures observed in Germany, as compared to those in New Mexico (Table 3).

Temperature for Tb was reduced from 14.0 to 9.0 °C, which also resembles the Tb of sunflower seed-set, which is 7.5 °C (Table 3) [44].

In order to set the correct onset of increase of pod dry weight and also later onset of seed dry weight, FL-SH was set to 4.5 PD, while FL-SD was set to 13.0 PD [45]. As the duration of the flowering period is influenced by genetics as well as environmental conditions such as temperature, an adaptation of the model for other cultivars was necessary [62,63]. The duration of pod addition (PODUR) also has an impact on the increase in pod and seed mass at the beginning of their formation [45]. PODUR for the default cultivar "PI8311" and for the cultivar "C1" was increased slightly, from 17.0 to 18.0 and 19.0 PD, respectively, to add pods and seeds more slowly [45]. For the cultivar "C2", a value of 17.0 PD was used to shorten the lag phase and to add pods and seeds more rapidly (Table 5) [45]. The model code allows for indeterminate species by reserving some assimilates available for leaves and stem, even after pod and seed formation. While this maximum fraction of daily growth partitioned to seed and shell (XFRT) can be 1.0 for totally determinate plants, it is lower than that for safflower. With an XFRT value of 0.55 for the original model, not enough seeds were formed. Therefore, XFRT was increased for both the PI8311 cultivar and the two new cultivars (Table 5). By comparison, the XFRT of highly-bred soybean is 1.00 [44]. All these modifications had positive effects on the modelling of the pod weight and pod harvest index, as the RMSE was reduced for both simulated variables and the *d*-index was increased.

A comparison between the simulated and measured pod weights showed a slight overprediction in 2017, with a RMSE of 901 and a *d*-index of 0.93. In contrast, a comparison of the simulated and measured values for 2018 showed a slight underprediction, with a RMSE of 1287 and a *d*-index of 0.95. After the parameters were modified with data from both years and both cultivars, the comparison of the simulated and measured pod weight resulted in a RMSE of 1094 and a *d*-index of 0.94 (Figure 7a).

Simulated pod harvest indices for 2017 and 2018 were similar to the measured values, with RMSE of 0.026 and 0.039 and *d*-indices of 0.98 and 0.99, respectively. Measured pod harvest index with data from both years and both cultivars was well simulated by the model, with a RMSE of 0.03 and a *d*-index of 0.98 (Figure 7b).

The time between first seed and physiological maturity (SD-PM), maximum weight per seed (WTPSD), seed filling duration for pod cohort (SFDUR), and weight percentage of seeds in pods (THRSH) in the model were set on the basis of the measured data set. To define the beginning of seed maturity and physiological maturity correctly, time between first seed and physiological maturity (SD-PM) had to be extended for the cultivar "PI8311", while the SD-PM for the other two cultivars had to be shortened (Table 5). Because genetic potential weight per seed (WTPSD) was too small for the PI8311 cultivar, the final seed size was too low, and WTPSD was increased for the PI8311 cultivar and for the two newly-added cultivars (Table 5). Because single seed growth rate depends on the maximum shelling percentage of cohorts, the shelling percentage should be a little below the measured one [45]. As a result, the weight percentage of seeds in pods (THRESH) for cultivar "PI8311" had to be reduced and THRESH for cultivars "C1" and "C2" had to be raised to 55.0 and 61.0% (Table 5). Due to the lack of measurements of seed size over time, the seed filling duration for pod cohort (SFDUR) could not simply be set. Because the simulated shelling percentage of the new dataset was a little high, SFDUR was increased from 29.0 to 30.0 PD (Table 5) [45].

Because the grain yield was only recorded at maturity, a statement about the *d*-index for grain yield is not possible. A comparison between the simulated and measured harvest indexes, shelling percentages, grain weights, and unit grain weights indicated a small underprediction, with RMSE of 0.06, 9.53, 1715, and 6.27, respectively, over all treatments, cultivars, and years.

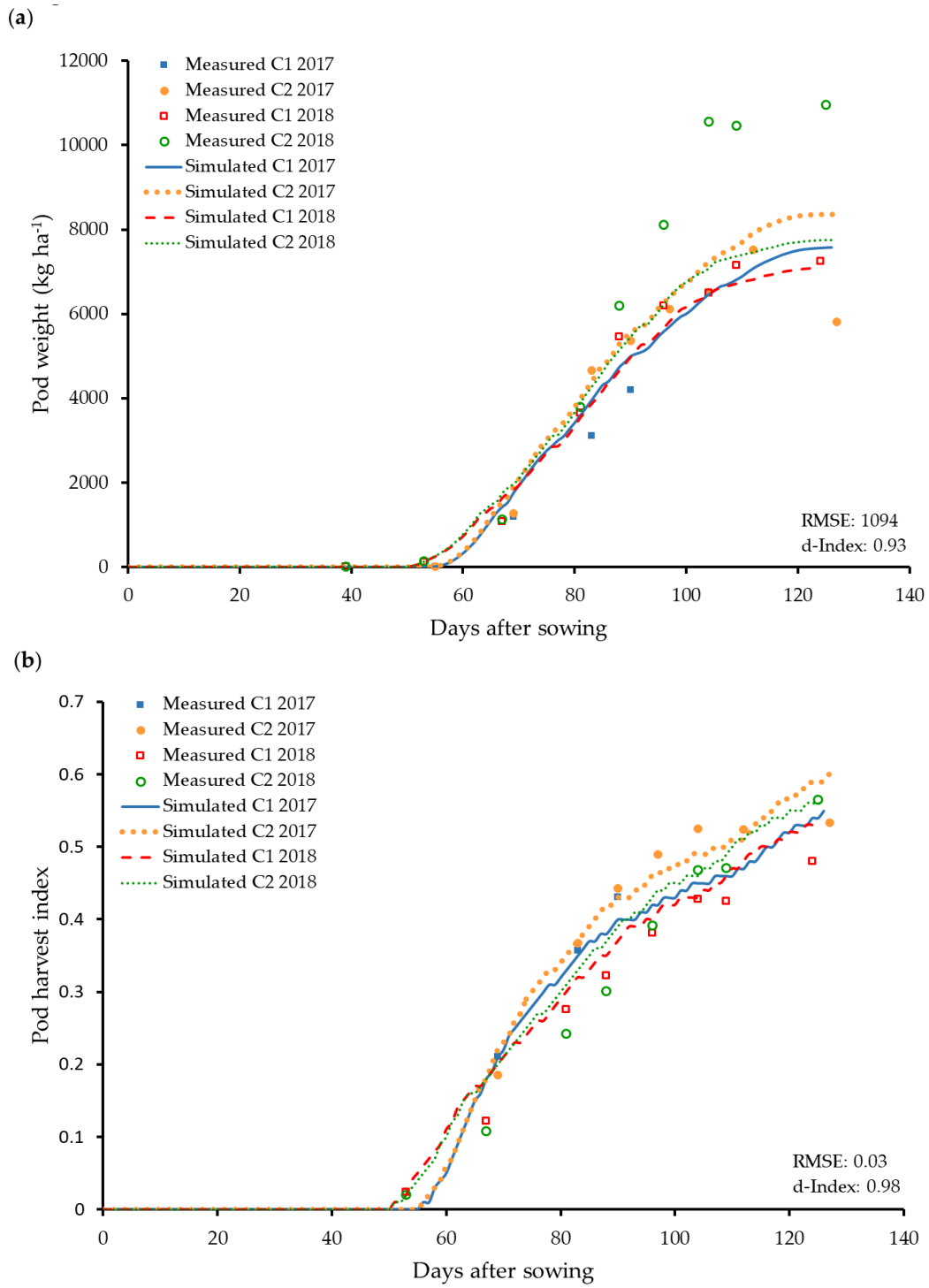

Figure 7. Simulated (lines) and measured (symbols) (**a**) pod weight and (**b**) pod harvest index as a function of days after sowing for the German (C1) and Chinese (C2) safflower cultivars in 2017 and 2018. Root mean square error (RMSE) and Wilmott Agreement Index (*d*-index).

3.5. Modelling of Floret Yield and the Relationship to the Flower Capitulum

After the modification of parameters affecting pod and seed growth, a modeling approach was developed to predict the yield of the florets. In order to estimate the size, and thus, possibly the

productivity of the flower pod (capitula), the ratio of floret weight to capitulum weight was calculated. The derived relationship indicated a stable but slowly-increasing ratio over time, reaching a peak ratio at the time when the highest floret yield was achieved (Figure 8a). A distinction had to be made between the two cultivars due to the different size of capitula and the mass of florets per capitulum. The developed relationship of floret fraction over time (Figure 8a) has an analogy in the model to the pod harvest index over time. A linear relationship was obtained for the ratio of floret yield to capitulum versus the pod harvest index for each cultivar. The equation of the linear relation was normalized and recorded on the basis of the maximum ratio of floret weight to capitulum weight. To predict the floret weight ratio, the values of the initial (HIPIN) and maximum (HIPMX) pod harvest index defining the onset and maximum of the floret fraction relative to the pod harvest index, the ratio of floret weight to capitulum weight (PETALX) were used for each cultivar.

Based on the lowest possible RMSE and highest possible *d*-index, the parameters were set to HIPIN = 0.2320 and 0.2970, HIPMX = 0.3950, and 0.4800, and PETALX = 0.04690 and 0.07080 for cultivar C1 and C2, respectively.

These parameters were used in the following Equation (3) and the result used according to Equation (4):

$$\text{FPETAL} = \text{PETALX} \times \text{MIN}(1.0,\text{MAX}(0.0,(\text{HIP} - \text{HIPIN})) / (\text{HIPMX} - \text{HIPIN}))) \quad (3)$$

$$\text{IF (FPETAL.LT. PETALX) PETAL} = \text{PODWT} \times \text{FPETAL} \quad (4)$$

where FPETAL is the floret weight ratio, PETALX is the maximum ratio of floret weight to capitulum weight, HIP is the pod harvest index, HIPIN is the initial pod harvest index at which floret begins, HIPMX is the maximum pod harvest index at which floret dry matter increase ceases, and PODWT is capitulum (pod-plus-seed) weight.

The fraction of florets to pod weight averaged over four treatments of two cultivars and two years were slight overpredicted by the model, with a RMSE of 0.01 and a *d*-index of 0.76 (Figure 8a).

Figure 8b indicates the floret weight of the two cultivars in both years after the adaptation process. In general, cultivar C1 had a lower RMSE, i.e., 66.49, compared to that of cultivar C2, i.e., 128. However, cultivar C2 had a higher *d*-index, 0.87, compared to that of cultivar C1, i.e., 0.71. The reasons for the higher RMSE despite the higher, and therefore, better *d*-index of cultivar C2 are the generally higher floret yields of cultivar C2, which explain the higher RMSE. Floret weights in 2018 could be predicted better, with a RMSE of 96.33 and a *d*-index of 0.91, in comparison to 2017, with a RMSE of 98.15 and a *d*-index of 0.67. The better weather conditions in 2018 resulted in higher and more stable yields. A comparison between simulated and measured floret weights over both cultivars and both years showed an overprediction, with a RMSE of 97.24 and a *d*-index of 0.79 (Figure 8b).

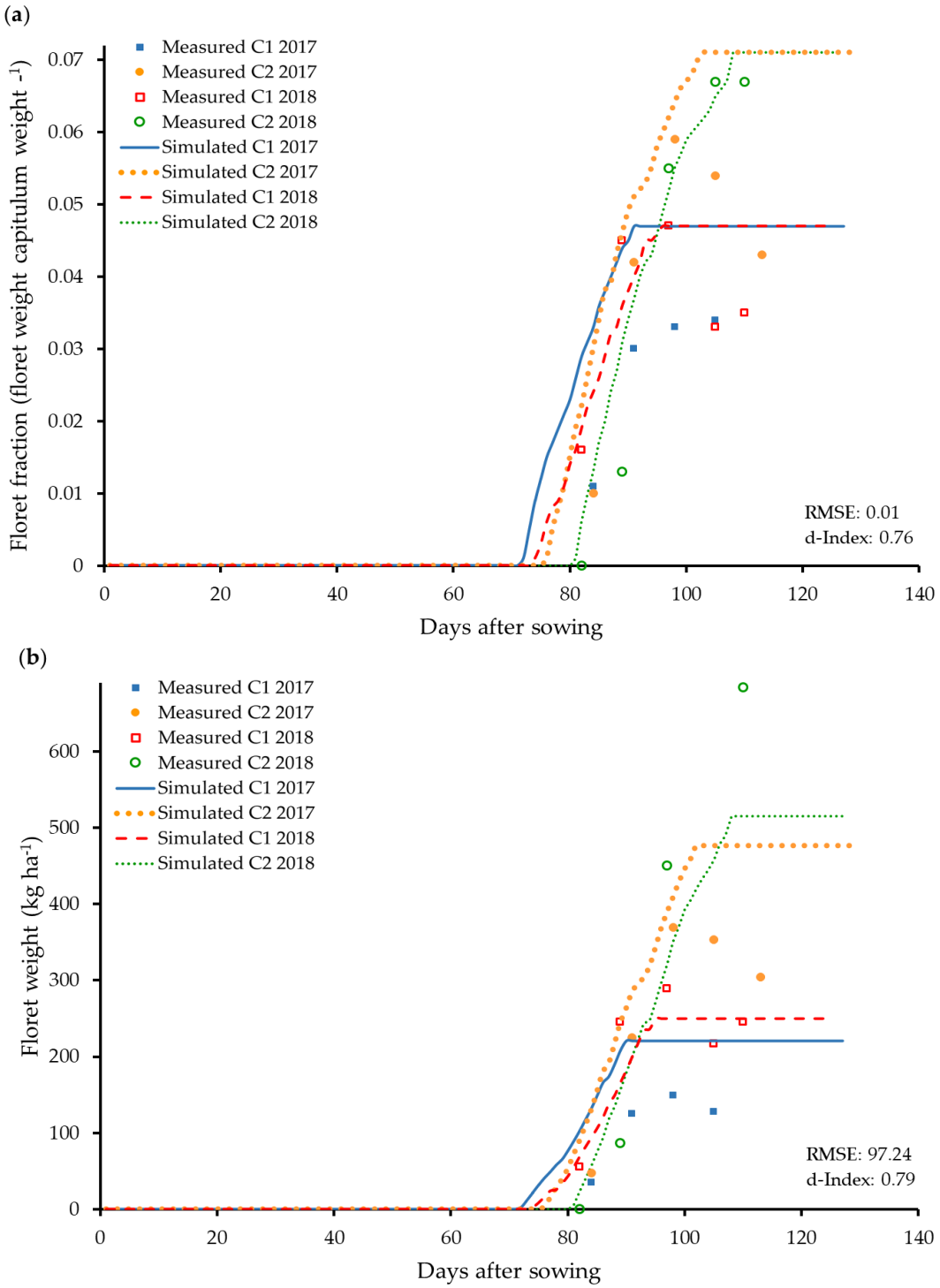

Figure 8. Simulated (lines) and measured (symbols) (**a**) floret fraction and (**b**) floret weight as a function of days after sowing for the German (C1) and Chinese (C2) safflower cultivars in 2017 and 2018. Root mean square error (RMSE) and Wilmott Agreement Index (*d*-index).

4. Conclusions

A successful simulation of growth, yield, and floret yield of two safflower cultivars under field conditions in southwestern Germany with a modified DSSAT CROPGRO safflower model was achieved

in this study. Model parameters were modified using field data of two cultivars grown in Germany in two years. Importantly, the model with new parameters continued to simulate well the original safflower experiments in the DSSAT, on which the prior default model parameters had been set. The newly-modified species, ecotype, and cultivar parameter files should be included in the next DSSAT model release for safflower. The introduction of the new variables PETALX, HIPIN, and HIPMX into the model made it possible to predict the floret yield at different harvest times over the flowering time separately for two cultivars.

However, further research is needed to validate the modified model, and above all, the new tool for predicting the floret yield. The performance of the model to predict floret yield should be tested with other independent data, because it has worked well under good weather conditions so far (2018), but only fairly under suboptimal conditions (2017, rain during flowering). The differentiation of the model between the cultivation systems (sowing density and row spacing) and cultivars should be further evaluated with additional experiments, as the number of branches and, thereby, also the number of capitula are influenced by plant spacing [3,64–66]. Water balance was turned on and a light water stress occurred in 2018, three weeks prior to physiological maturity, which could have reduced the final biomass and pod mass in 2018. Therefore, further evaluation under water deficit situations would be appropriate. The N balance was not simulated because of the low number of data values of tissue nitrogen concentration, lack of nitrogen treatments in the field trials, insecurity associated with soil N mineralization, and because the default safflower model had been simulated with N balance turned off [27]. In future studies, attention should be paid to the N balance, and the model should be tested with N fertility treatments, including zero N treatments, as well as careful initial conditions and soil N measurements. Emphasis should be placed in future experiments on recording the number of seeds, seed weight, and the development of seeds in general, to be able to improve this part of the model even further.

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6. General discussion

The main focus of the present study was to establish cultivation guidelines and to develop a harvesting system for florets of safflower in Europe. It was important to adapt various parameters of the cultivation system to the local conditions, which are different from the existing cultivation areas. In addition, a mechanical harvesting system, which would be essential for economic cultivation in Europe, was investigated. By evaluating and modifying an existing crop growth model for safflower, and adding a new approach to predict floret yields, the simulation of growth, floret yield and yield of safflower in a new cultivation region, could be tested.

These topics have each been covered in detail in different publications. **Publication I** focused on the cultivation system and the important parameters such as different cultivars, row spacing, sowing densities and harvesting dates in order to find the most productive combination of these parameters. **Publication II** covered the topic of mechanical harvest by comparing three different threshing parameter settings, their suitability and influence on yield. In order to reduce the uncertainties associated with the cultivation of safflower for floret production under different climatic conditions, **Publication III** dealt with the topic of modeling and its evaluation, modification and addition of a new subroutine of floret yield. These topics were covered and discussed in detail in the respective publications. The general discussion will therefore focus on using the information gained from the publications to point out further aspects like climate change, the possible future cultivation of safflower in Europe and opportunities that could result from breeding, both in terms of yield and mechanical harvest. In addition, it will be discussed, if the origin of cultivars has an influence on yield and quality parameters and if modeling can be used as suitable tool to evaluate growth and yield of different cultivars in various regions or under different climatic conditions. The approach of modeling and the new subroutine for predicting the floret yield and the transferability to other crops or other compounds is also covered in the general discussion. Also the potential of other food colorings and further research fields are discussed.

6.1 Climate Change

The effects of climate change on agriculture, its productivity and yields in Europe can already be seen today (EEA, 2019). Yield data for soft wheat, winter barley and grain maize already showed on average -16, -14 and -29% lower yields for e.g. Germany in 2018 compared to the yields in 2013–2017 (Bussay et al., 2018). Also in other European countries such as Poland or the Czech Republic soft wheat, winter barley and grain maize showed lower yields

of -9 to -14, -7 to -12 and -6 to -17% on average in 2018 compared to the 2013–2017 yield (Bussay et al., 2018). On the other hand, there are also countries such as Bulgaria or Croatia which benefited from the warm year 2018 by average higher yields of soft wheat, winter barley and grain maize of around 6, 9 and 18% compared to the yields in 2013–2017 (Bussay et al., 2018). The observed and predicted impacts of climate change indicate an increase in heat extremes and a decrease in summer precipitation for continental regions such as Germany, Poland, Hungary and Serbia (EEA, 2017). For Mediterranean regions such as Spain, Portugal, South of France and Italy, additional increasing risks of drought and biodiversity loss are predicted with a simultaneous decrease in crop yields (EEA, 2017). Nevertheless, there are also regions, such as the boreal region with countries like Finland, Estonia and Lithuania, which could benefit from the predicted effects of climate change like decrease in snow and ice cover, increase of precipitation with rising crop yields (EEA, 2017). A look into the future also shows that crop yields in Spain, for example, will fall by 15–30% in 2080 compared to 1961–1990, depending on the scenario, while they will increase to the same extent in Sweden, Finland and Norway, for example (Kelemen et al., 2009).

In general, in most regions European summers will get drier and the average annual temperature will rise (Figure 2) (EEA, 2014; EEA, 2015; EURO-CORDEX, 2014; Jacob et al., 2014).

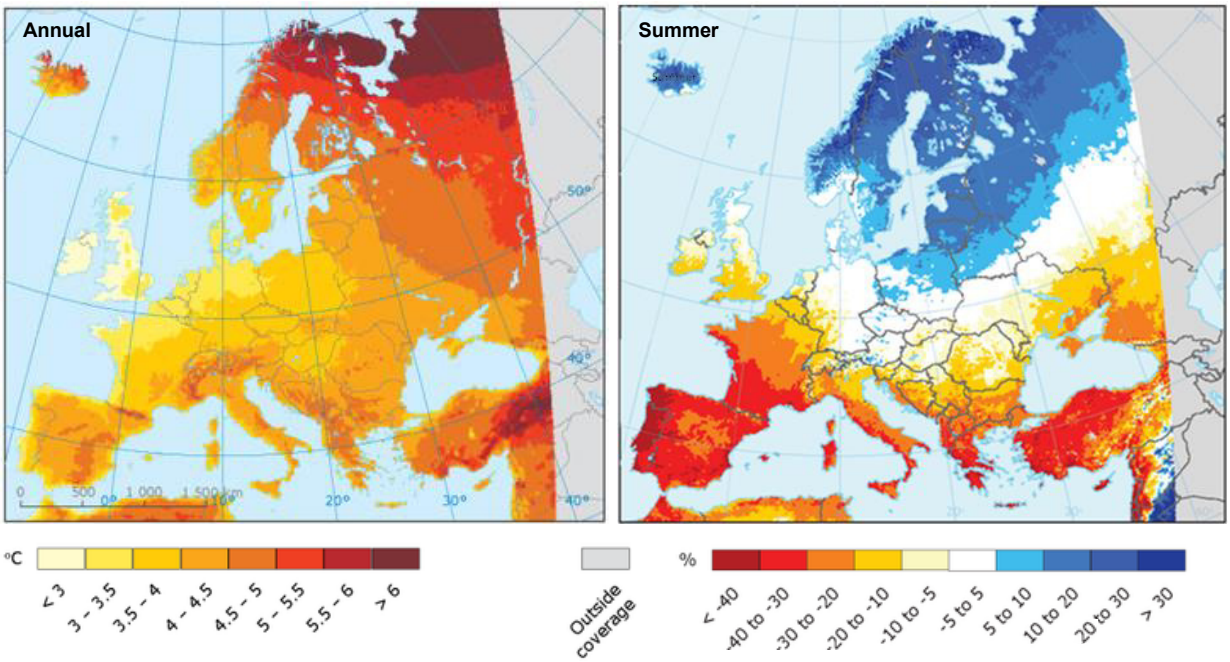

Figure 2: Predicted changes in annual temperature (°C) (left) and summer precipitation (%) (right) in 2071–2100 compared to 1971–2000. Reference: (EEA, 2014; EEA, 2015; EURO-CORDEX, 2014; Jacob et al., 2014)

This could have a positive effect on yields of heat- and drought-loving plants in particular, as already shown by the average increase in sunflower yields of ~ 16 % in 2018 compared to the years 2013–2017 in Europe (Bussay et al., 2018). For safflower the same growing areas

are recommended as for sunflowers, namely regions with warmer temperatures and less rainfall during flowering in June–July as safflower is mainly grown in climates which are hot and dry (Biertümpfel et al., 2013; Emongor, 2010). The positive development of the sunflower yield in 2018 could also indicate that the location requirements for plants such as sunflower and safflower will be improved in the future and that the yields of the safflower in Europe could also increase in the future. Safflower could be a good alternative under the conditions predicted for the future because it prefers warmer temperatures and is considered drought, heat and salt tolerant (Biertümpfel et al., 2013; Drangmeister, 2011; Emongor and Oagile, 2017).

Another problem that has occurred so far in the cultivation of safflower in some areas of Europe is precipitation during and after flowering, which can lead to diseases (Armah-Agyeman et al., 2002; Dajue and Mündel, 1996; Drangmeister, 2011). Due to climate change, decreasing summer precipitation is predicted in some regions of Europe, e.g. in Italy or France (Figure 2). This could lead to a better suitability of safflower for cultivation. Up to now, the sowing of safflower is not recommended too early in e.g. Germany (beginning to end of April) (Drangmeister, 2011). Reasons for this are that in Germany frosts occur frequently until the Ice Saints in mid-May. Safflower tolerates temperatures down to -7°C at the beginning of growth, but only until stem elongation (Emongor, 2010). From then on, safflower is sensitive to frost (Berglund et al., 2007), and if sowing is done earlier than April, the plant could already have passed the development stage up to which it tolerates low temperatures. Therefore, higher average temperatures (Figure 2), could allow earlier sowing dates, which would have some advantages. Several studies showed that earlier sowing dates can lead to a higher number of branches and capitula, floret yields and pigment contents (Ibrahim et al., 2016; Patanè et al., 2020). Also the higher temperatures during sowing could positively influence the germination of safflower (Balashahri et al., 2013). With regard to mechanical harvesting, higher temperatures would have advantages, which could result in an earlier maturity of the plant, thus in higher dry matter contents and better threshing efficiency (Hatfield and Prueger, 2015; Naveen Kumar et al., 2013; Peiretti, 2009; Wang et al., 2009).

In general, safflower thus shows potential for cultivation in many European regions. In addition, the declining yields of other crops in recent years have shown that alternatives are needed for the future (Bussay et al., 2018). The harvest of the florets takes place earlier than the harvest of the seeds, so safflower would be well integrated into the crop rotation for this purpose. The predicted higher temperatures and lower summer rainfall open up the possibility of growing cultivars from other climates, which are specialized for the purpose of florets, in many regions of Europe in the future.

6.2 Cultivars and their origin

In order to make the cultivation of safflower for floret yields in Europe attractive and economic, cultivars with high carthamidin contents and yields would be a decisive factor. The fact that cultivars from different origins differ in their characteristics has been known for many years (Ashri et al., 1974; Knowles, 1969). As safflower is, depending on the cultivar, generally regarded as a day-length neutral, long-day plant, this would not be a limiting factor for cultivation in Europa (Dajue and Mündel, 1996; Johnston et al., 2002). Therefore, 61 accessions from different origins were examined regarding their carthamidin content in an additional experiment. In a climate chamber pot experiment 61 accessions originating from five continents were tested for their morphological (e.g. number of branches), phenological (e.g. flowering period) and yield and quality traits such as floret yield and carthamidin content. Of the 61 accessions, 17 were from Europe, 24 from Asia, 11 from Africa, 8 from North America and 2 from Australia. For the pot experiment, 6 seeds of an accession were sown with a depth of 2 cm in each of 3 pots per accession. After their germination it was thinned out to 4 plants per pot. The three pots per accession were arranged in a completely randomized design in two climate chambers. The plants were illuminated with a 400-watt lamp from 6 a.m. to 10 p.m. and the temperature was 20 °C throughout with a constant humidity of 58%. Capitula were harvested when the outer florets started to get weak and the florets were removed from the capitula with tweezers. Fresh weight was recorded for both the capitula and the florets. After drying the capitula at 60 °C and the florets at 40 °C for 48 hours, dry weight was recorded. This procedure of harvesting was carried out during the flowering of the plants from the end of May to the beginning of July every 2–3 days for all capitula in the described stage. At the end of the flowering period, when all florets were harvested, their carthamidin content was determined according to a method of the FAO and Mohammadi and Tavakoli (FAO, 1998; Mohammadi and Tavakoli, 2015), with minor adjustments.

These unpublished results of a climate chamber pot experiment with 61 accessions of five continents (Figure 3), showed that accessions of different origins have significantly different carthamidin contents (Figure 4). No significant differences in carthamidin contents were found between the accessions derived from Europe, Africa and Australia (Figure 4). The latter achieved the highest carthamidin contents (Figure 4). The highest carthamidin contents in general were obtained from the African accessions from Morocco, Ethiopia and Egypt PI_393498 (5.18%), PI_273875 (4.64%) and PI_306595 (4.40%). Reasons for the high levels of carthamidin could be that safflower was originally cultivated in these regions as a natural colorant (El Bassam, 2010; Emongor, 2010; Singh and Nimbkar, 2016; Weiss, 1971).

This indicated that in these regions, where the use of coloring was particularly important, these accessions were also strongly selected in this direction. However, Asian accessions showed the significantly lowest average carthamidin contents of 3.17% in this experiment.

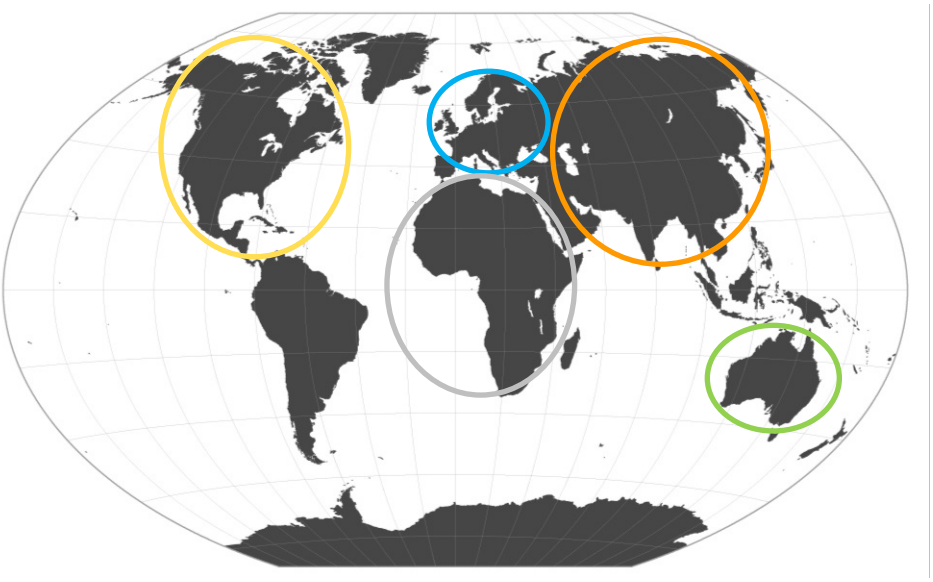

Figure 3: Representation of the continents from which the accessions originate. Reference: Modified according to (Mygeo.info, 2008).

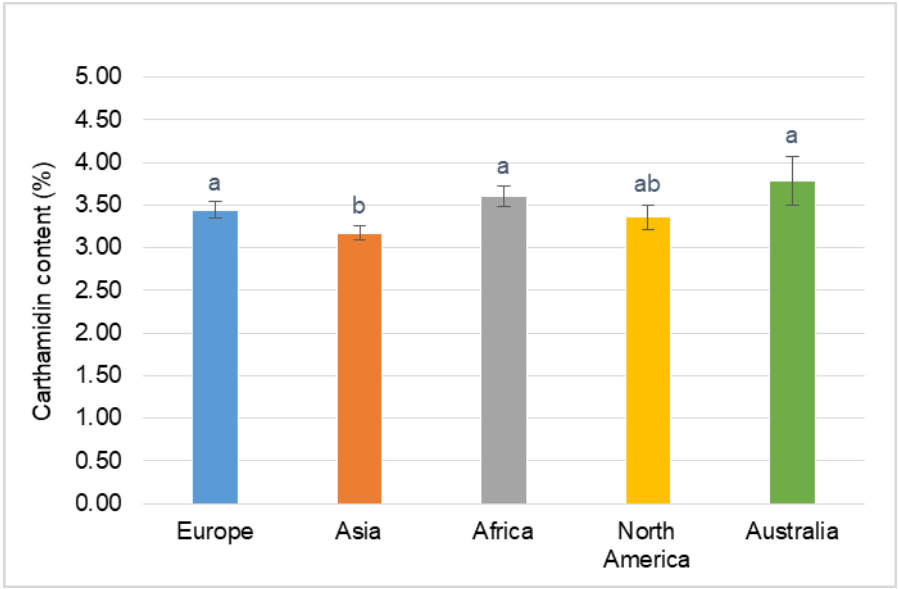

Figure 4: Mean values with standard error (bars) and significant differences for carthamidin contents from the florets of the accessions (%). Mean values with same lowercase letters are not significantly different between the continents with a *p*-value of 0.05 (continent: 0.0237; accession (continent): 0.0018).

In this preliminary test, the florets were harvested when the outer florets started to get weak. Various studies indicate that the proportion of carthamidin or flavonoid is at its maximum at the onset of flowering or during flower formation, an earlier harvest date might have shown higher carthamidin contents (Mohammadi and Tavakoli, 2015; Salem et al., 2011).

Therefore, in order to evaluate the optimum harvest dates with the maximum carthamidin contents, further tests with different harvest dates should be performed with the appropriate accessions of this study.

The Chinese cultivar C2, which was tested both in the field and in the climatic chamber, was able to achieve higher carthamidin contents under field conditions than in the preliminary climatic chamber test (carthamidin content: 2.66%) (Steberl et al., 2020a). One reason for this could be the higher temperatures during flowering in the field (maximum in July 2017 and 2018 with over 30°C), whereas these were constant at 20°C in the climate chamber. The Chinese cultivar is adapted from its origin to hot weather conditions, which enables an optimal development during flowering and which could lead to the higher carthamidin levels maintained at higher temperatures. Another reason for the differences between the carthamidin contents in the field and in the climatic chamber test could be the higher radiation in the field. Due to higher UV-B radiation, which can now be observed due to a declining ozone layer, an increase in flavonoid accumulation has already been observed in some plant species as a protective mechanism (Julkunen-Tiitto et al., 2015; Ryan et al., 2001; Sisa et al., 2010). Therefore, further field studies with accessions of different origins and their harvest on different harvest dates would be recommended.

The results of the climate chamber test showing different carthamidin contents of accessions of different origins is consistent with another study by Cao et al. in which dried florets of safflower of different geographical origins were examined (Cao et al., 2019). In the study 32 metabolites, including neocarthamin, which is also a colorant, were examined and it was shown that it was possible to distinguish the samples according to their relative content of different metabolites into different pools of origin (Cao et al., 2019). Reasons could be that genes, which are similar from samples of the same origin, determine similar metabolites (Cao et al., 2019). The study also found that climatic factors, such as sunshine hours, relative humidity and temperatures during cultivation showed significant correlation with most metabolites (Cao et al., 2019).

In the climate chamber investigation of this study, differences between the accessions from different origins and e.g. their number of capitula per plant or their floret yield per capitulum were shown. For example, the accessions from Europe had the highest number of capitula and floret yield per capitula, while the accessions from Australia had the lowest. Other studies also showed that characteristics such as the number of branches or capitula can differentiate depending on the origins of the accessions. For example, safflower plants from Ethiopia showed many branches, while safflower from Sudan showed rather a medium number of branches (Knowles, 1969). Also other studies indicated differences in yield

components, e.g. in the number of capitula, depending on their origin (Ashri et al., 1974; Chapman et al., 2010). Since the number of branches influences the number of capitula and thus the yield of florets (Singh et al., 2008; Zheng et al., 1993b), the number of branches or capitula would also be an interesting selection criterion besides the carthamidin contents with regard to suitable safflower cultivars for floret cultivation.

Overall, the origin of an accession and the selection of traits, such as a high number of capitula per plant or a high carthamidin content of the florets offer possibilities to make the cultivation of safflower florets in Europe more attractive. Previous limitations of safflower cultivation in Europe, such as for example the yield stability, which is mainly negatively influenced by precipitation during flowering, could be solved in the future with the help of different breeding objectives for safflower. Breeding other ideotypes could also help to solve the problematic mechanical harvesting due to the branched plants and the resulting different maturation of the capitula.

6.3 Breeding

In the field of safflower breeding are many possibilities that could be used to make cultivation, harvesting, yield and quality criteria such as floret yield or carthamidin content more attractive for floret cultivation in future in Europe.

In general, the breeding of hybrids is a key factor in the development of high-performance safflower plants (Singh et al., 2008; Singh and Nimbkar, 2016). The breeding of male sterile lines already allows an increase of the seed and oil yield of 20–25%, which could already be shown with the hybrid NARI-H-23 (Singh and Nimbkar, 2016). Due to the high standard heterosis, i.e. the performance of hybrids, for both floret yield (188%) and number of capitula per plant (173%) (Singh et al., 2008), hybrids could also play a decisive role with regard to high yields in the floret production. In addition, both correlation and path analysis have shown, that the floret yield was significantly affected in a positive way by e.g. the number of branches, the capitula diameter and the number of florets per capitula (Alba et al., 2007; Singh et al., 2008). Therefore, breeding towards hybrids with a high number of capitula or branches could result in high floret yields. Furthermore, it has already been shown that hybrids could perform better than other cultivars under different agricultural and climatic conditions (Singh and Nimbkar, 2016). This could argue for a breeding of hybrids with the aim of high floret yield, because of their superiority in terms of higher yields and better adaptation to different regions. This could increase the floret and the monetary yields (Singh et al., 2008; Singh and Nimbkar, 2016), and the applicability to different regions. However, disadvantages would be that, if farmers want to produce their own seeds, these positive yield

effects could be lost in the second generation (F2). As a result, farmers depend on the annual purchase of seeds, which would lead to costs, but which might still be worthwhile due to the higher yields of the hybrids.

A previous problem, which limited the cultivation of safflower in many European countries due to rainfall during flowering, could be solved by breeding resistant cultivars. Diseases like the fungal infection by *Botrytis* or foliar diseases like *Alternaria* due to precipitation during and after flowering (Drangmeister, 2011; Singh and Nimbkar, 2016) are at present a large problem in e.g. Germany and could lead to total failures (Biertümpfel et al., 2013). There are resistant cultivars already approved in Germany, such as the Botrytis-resistant cultivar ‘Sabina’ (Biertümpfel et al., 2013), which were bred for seed and oil yield and not for floret yield or carthamidin content. Therefore, research into breeding for resistant cultivars with the breeding goal of higher floret and carthamidin yields might enable the so far limited cultivation of safflower in regions with higher rainfall during the flowering period of safflower.

Due to the discovery of synthetic colorants in 1856 (Garfield, 2002), the coloring with plant colors and their cultivation came to a complete standstill within 50 years in e.g. Germany (Biertümpfel and Wurl, 2009; Biertümpfel et al., 2013). This also led to the fact that the cultivation in this area and also in the area of breeding was not further investigated. The fact that safflower is generally a minor crop also contributes to the fact, that the genetics of safflower were little researched (Leus, 2016). Today, the use of safflower for seed and oil production is a priority, which is why breeding is mainly focused on increasing seed and oil yields (Singh and Nimbkar, 2016). For the breeding of safflower for seed and oil use, the color of the flowers is not considered to be important (Golkar et al., 2010; Pahlavani et al., 2004). This is why most of the cultivars available on the market are not optimized for floret cultivation and colorant production. When safflower is grown for flowers, the color as well as the spinelessness plays a crucial role (Golkar et al., 2010; Pahlavani et al., 2004). Research on spines has different results. Narkhede and Deokar reported four genes that determine the dominant inheritance of spines (Narkhede and Deokar, 1986). However, Pahlavani's research with Iranian genotypes revealed that a single, dominant gene determines the inheritance of spines (Pahlavani et al., 2004).
Research into the inheritance of flower color, which can range from white, yellow, orange and red, has also resulted in various findings (Leus, 2016). Already in 1986, five genes were identified, which are supposed to be responsible for the color of the flowers (Narkhede and Deokar, 1986), whereas Golkar et al. mentioned only four genes, which are supposed to be responsible for the inheritance (Golkar et al., 2010). Recent research especially concerning the yellow color showed different inheritance types (Leus, 2016).

Crossbreeding of yellow-flowering plants with plants of other flower color, led to yellow flowers in the first generation (F1), whereas the subsequent F2 generation show again all color aspects, but with the majority of yellow flowers (Leus, 2016). However, if the original flower colors are not yellow, there are still plants with yellow flowers in the following generation, but these are in the minority (Leus, 2016). Thus, it can be concluded, that there is one dominant gene responsible for the inheritance of yellow, but that the recessive allele allows the formation of other colors in the following generation (Leus, 2016).
Another approach could be to find out, which floret color contains the most carthamidin. The Chinese cultivar C2, which has mainly orange florets, showed higher carthamidin contents than the yellow German cultivar C1 (Steberl et al., 2020a; Steberl et al., 2020b). Other studies also suggest that not necessarily the yellow florets contain the most yellow dye carthamidin. Also in a study by Mohammadi and Tavakoli, cultivars with slightly red and red florets showed the highest carthamidin contents and in a study by Salem et al., orange florets had the highest flavonoid contents, which also includes carthamidin (Mohammadi and Tavakoli, 2015; Salem et al., 2011).
Due to the rather low level of research in the field of spinelessness and flower color so far, and the many different approaches that could lead to a higher carthamidin contents, there is still a need for further research in order to promote the increasing demand for natural colorants in the future through specially bred cultivars.

Safflower has mainly been grown as a minor crop on marginal soils that were unsuitable for growing more profitable crops such as cereals or cotton (Singh and Nimbkar, 2016). Therefore, the previous ideotype of safflower is adapted to marginal sites, characterized by up to tertiary branching and 30–40 capitula per plant (Singh and Nimbkar, 2016). The aim of the latest safflower breeding is to breed different ideotypes, which are adapted to different growing areas or to different uses (Singh and Nimbkar, 2016). One approach, for example, is the breeding of safflower plants, which only produce primary branches. Various studies have shown that plants with only primary branches could lead to a more uniform and earlier maturation with higher productivity (Karve et al., 1976; Ramachandram and Ranga Rao, 1989; Singh and Nimbkar, 2016). The genetics and breeding of such genotypes should be further researched, because above all the more uniform maturing of the capitula could bring a crucial advantage for one of the previous limitations in the safflower florets cultivation. In comparison to manual harvesting, where the florets are harvested daily during the flowering period at optimal maturity, it is important for mechanical harvesting that the capitula ripen as simultaneously as possible. Therefore, these primary branched genotypes could bring a decisive advantage, especially for floret cultivation. Also the approach of unbranched, one capitula genotype, could improve the mechanical harvest (Singh and Nimbkar, 2016). Both uniform maturity and better floret harvest due to single capitula at a uniform height, could

make production and harvesting more economical and therefore more attractive for farmers in the future.

However, breeding of these other ideotypes is still in its beginning and needs much research. It is not enough to focus only on the origin or the breeding of the cultivar, because the cultivar must also fit the requirements of the location. Various studies have indicated that changes in environmental conditions can affect traits such as number of capitula, floret yield or carthamidin content (Kizil et al., 2008; Salem et al., 2014). Publication I and II showed significant differences in the number of capitula and branches, carthamidin contents, floret and carthamidin yields between the two years (Steberl et al., 2020a; Steberl et al., 2020b). The significant differences in yield between year x cultivar and environment x cultivar also play a decisive role in determining whether a cultivar is suitable for cultivation under certain growing conditions (Hamza, 2015; Koutroubas et al., 2009; Mahashi et al., 2006). Also the testing of these ideotypes in different growing regions of Europe, their yields and also their yields of the next generations, still bring many uncertainties with them, which have to be researched in order to promote the cultivation of safflower for florets in the future. Years of field trials would be needed to test cultivars from other regions or new genotypes under local conditions in different areas of Europe. This is very time consuming and cost intensive. A first step towards an easier evaluation of cultivars worthwhile to be cultivated for florets, could be done by crop modeling.

6.4 Modeling as a tool

The Decision Support System for Agrotechnology Transfer (DSSAT) is a software solution for simulating crop growth and yield based on weather data, management practices and field-specific soil information (profile, texture etc.) (Hoogenboom et al., 2017). Currently crop growth and yield for over 42 crops can be simulated with DSSAT (Boote, 2020; Hoogenboom et al., 2017). The CROPGRO model, which is integrated into DSSAT, uses a crop template approach, including information about species, ecotype and cultivar parameters (Hoogenboom et al., 2017). The species file contains general crop information, such as cardinal temperatures, photosynthesis or partitioning parameters (Hoogenboom et al., 2017). The ecotype file can be used to enter characteristics that are common for several cultivars and vary less, such as time between planting and emergence, or time from emergence to first true leaf (Boote et al., 1998; Hoogenboom et al., 2017; Jones et al., 2003). Cultivar specific characteristics, such as time from plant emergence to flower appearance or between first flower and first pod can be entered into the cultivar file (Boote et al., 1998; Hoogenboom et al., 2017; Jones et al., 2003). The template approach has the advantage that values in

species and cultivar files can be changed without modifying the source code (Hoogenboom et al., 2010; Jones et al., 2003). This has the advantage, that it is less susceptible to errors and does not require programming skills. The disadvantage, however, is that if a plant does not correspond to the typical life cycle defined by the given specific plant growth functions, the simulation runs might show some errors (Hoogenboom et al., 2010).
Safflower was already integrated into DSSAT based on data from one cultivar with four irrigation treatments in New Mexico for predicting safflower seeds (Singh et al., 2016). This existing model was evaluated and modified in our study with two new cultivars under different environmental conditions from Southwestern Germany (Steberl et al., 2020c). In addition, with the newly integrated subroutine the floret yield was simulated for the first time as a new yield variable. With this modification, the updated safflower model could additionally be tested and improved with new cultivars under different environmental conditions. In a similar way the developed method can be adapted to the simulation of other crop yield components. Therefore, these aspects are discussed in this section.

The development and yield of safflower is influenced by many factors such as the environment, different management strategies and the cultivar (Bitarafan et al., 2011; Eryiğit et al., 2015; Kizil et al., 2008). As safflower is not yet cultivated for the production of florets in Germany, for example, crop models can be used for investigating under what conditions cultivation would be profitable. A sensitivity analysis could be conducted to simulate the potential yield that could be achieved for different cultivars under certain climatic conditions. With the help of modeling, many different scenarios can be tested, which arise with site-specific weather changes and it can help to assess the corresponding economic risks (Jones et al., 2003).
Also different management practices could be modeled. For example CROPGRO uses both leaf photosynthesis parameters and light interception, so that the calculated canopy photosynthesis could be used to simulate row spacing and plant density influences (Boote et al., 1998). As both row spacing and sowing density have been shown to have an influence on both floret yield and yield relevant parameters in safflower (Steberl et al., 2020a), it would be important to model the yield and growth of other cultivars from other regions under European conditions with different planting distances.
Nevertheless, further experiments are needed for the sensitivity analyses, e.g. data sets (leaf weight, pod weight etc.) from different regions with different temperatures or data sets of different cultivars to verify the crop model growth functions and yield estimations.
The first version of the safflower model was designed to simulate seed yield (Singh et al., 2016). With the help of the ratio of floret weight to capitulum weight and its analogy to the pod harvest index already implied in the model, the new subroutine in the safflower model can predict the floret yield (Steberl et al., 2020c). The model could even be used to

distinguish between different cultivars, which in turn could allow the simulation and suitability/yield estimation of cultivars from other origins in terms of floret yield (see section 6.2). This could help to increase the acceptance and cultivation of safflower by farmers through crop model based feasibility studies. Finding out what cultivars have higher carthamidin contents and yields under which kind of management would make safflower more attractive and more economically appealing for cultivation in Europe.

Being, that the decisive factor for the cultivation of safflower for florets is the carthamidin yield, which results from the floret yield and the carthamidin content, it is necessary to include the carthamidin content into the model as model output parameter for the purpose of optimising yield based on the simulated flower yield and carthamidin content. One uncertainty that exists is the optimal harvesting time, which has been investigated in publications I and II (Steberl et al., 2020a; Steberl et al., 2020b). When harvesting, a compromise has to be found between the initially high carthamidin content, which decreases with the maturity of the flowers, and the floret yield, which tends to reach its maximum at the end of flowering (Steberl et al., 2020a; Steberl et al., 2020b). As the carthamidin content is high at the beginning but then decreases, a variable should be determined in the model which shows a similar developmental process, so that it can be integrated into the model. If the pigment content, e.g. for other crops, is constant over the development, this could possibly be entered in the cultivar file for individual cultivars, as it is already the case for the proportion of protein or oil to seed weight (SDPRO and SDLIP) (Hoogenboom et al., 2017).

The most obvious approach to apply this to other crops would be, to use crops that are also grown for their colorants and have like safflower heads, capitula or pods, so that the approach could be used to predict the growth and yield by analogy with the pod harvest index. For example, modeling could be used to simulate growth and yield of other flower plants like dyer´s chamomile or marigold, which have been tested for their suitability for cultivation and coloring in Central Europe (Biertümpfel et al., 2013). Also yields of plants like hollyhock, calendula, blue pea or hibiscus could be predicted with the new approach. The advantage is the template approach of CROPGRO. New crops, which are similar to safflower by analogy of growth, could be integrated into the model by changing the species, ecotype and cultivar parameters without changing the CROPGRO code. To obtain and verify these data, experiments would be needed.

Modeling of other ‘Coloring Foods’, where the flowers or florets are not harvested, would be interesting in order to improve their yield estimation and therefore make the cultivation more attractive. For example, tomato is also a ‘Coloring Food’ and is already integrated in DSSAT (Scholberg et al., 1997). As stated for safflower, the decisive factor for modeling the color

yield, as a key variable for the color industry, would be the integration of the pigment content in the cultivar file of the model. If, for example, the pigment lycopene of tomato develops in a similar way to the yield of the tomato, this pigment could be integrated into the model by analogy with the pod harvest index as an index.

However, flavonoids are not only known for their coloring properties, but also for their pharmaceutical, nutraceutical and medicinal importance (Panche et al., 2016; Yusuf et al., 2017). They are known for their anti-carcinogenic, antioxidant properties and their regulatory function regarding enzymes (Panche et al., 2016). Many flowers in the plant world contain flavonoids. Examples of this are the Japanese medlar, calendula, Japanese pagoda tree or lisianthus (Balbaa et al., 1974; Davies et al., 1993; Honório et al., 2016; Zhou et al., 2011). Both the quality and the production of medicinal plants and their flavonoid contents are influenced by various management factors, such as the correct planting or harvesting period (Honório et al., 2016; Zhou et al., 2011). Also for these plants, the new subroutine and the analogy of the ratio of pod harvest index could be used to predict their flower yields. By integrating the flavonoid content into the cultivar file, for example, the model could then be used to model the optimal harvest time window for each cultivar and region. For example, in calendula with the dependence of the days after anthesis (onset of flowering), there is a decrease in the average yield, the weight of the fresh flower and the total flavonoid content (Honório et al., 2016). The relationship between average yield, weight of fresh flowers as well as the flavonoid content could be used in modeling to predict the optimal harvest time for calendula under different environmental and cultivation conditions. However, the transferability to other compounds such as bitter substances and essential oils or other medicinal plants such as absinthium, mountain arnica or Sankt John's wort could also function with the help of such relationships and should therefore be further investigated.

6.5 Potential of ‘Coloring Foods’

Overall, this thesis showed, that both the cultivation of safflower for florets in a region outside the main growing area and the mechanical harvesting with a combine harvester could work. The integration of a new subroutine for floret yield estimation in a plant growth model also opens up new possibilities for other food colorings to simulate their growth and yield potential in order to be able to make first estimates regarding their suitability in different growing regions with different cultivars.

The demand for natural colorants is increasing worldwide, especially in Europe the natural food colorant market is expected to play an important role in the future (Grand View Research, 2017). According to forecasts, the market for natural colorants is expected to grow

to sales of USD 1.5 billion in 2024 (Research and Markets, 2020; vegconomist, 2019). Both the appearance of a product, which according to surveys is used as one of the first selection criteria (Derndorfer and Gruber, 2017; Singh, 2006), and the increasing demands that food should be safer, lead to the fact that consumers, producers and the government are tending towards natural food (Shahid et al., 2013; Viera et al., 2019; Yusuf et al., 2017). As a result, the percentage of artificial colorants in the food sector in Europe will continue to fall (currently around 4–16%) (Mintel Group Ltd., 2016; Simon et al., 2017; Witham, 2016), also due to regulations in force in Europe (European Commission, 2013; GNT Group B.V., 2013). Especially in Europe, the limited cultivation of ‘Coloring Foods’ will not be sufficient to meet the demand, and the demand for regionally produced products will increase. Other 'Coloring Foods' that may become even more important in the future could be black and orange carrots, tomatoes, pumpkins, sweet potatoes, grapes or blueberries (Stich, 2016).

Previous limitations are their more expensive prices compared to artificial colors (Adeel et al., 2017; Sigurdson et al., 2017; Stich, 2016). Compared to artificial colorants, large quantities of raw material are needed and must be available, and usually higher quantities are needed to achieve the desired color intensity (Adeel et al., 2017; Rodriguez-Amaya, 2016; Sigurdson et al., 2017; Wrolstad and Culver, 2012). Also plants, which react sensitively to certain soil conditions such as pH-values, could limit the cultivation (Sigurdson et al., 2017). In field trials with other ‘Coloring Foods’, cultivation recommendations, which are especially, like in this study, designed for the production of pigments, for example suitable cultivation regions, cultivars, row spacing, crop densities and harvest dates could be evaluated to find optimal combinations of traits. This would be necessary in order to obtain the highest possible quantities of raw materials with the highest possible colorant content. With safflower, for example, there are studies which show that the floret yield increases with the height of the plants (Alba et al., 2007; Zheng et al., 1993a; Zheng et al., 1993b). The height of the plant could therefore also be another factor, that could be studied to increase the florets yield in safflower and thus the profitability of safflower for floret cultivation. The nitrogen supply has an influence on the height as well as on the number of capitula and the dry weight of the florets per plant (Abbadi et al., 2008; Seadh et al., 2012; Shahrokhnia and Sepaskhah, 2017; Soliman et al., 2012). The highest numbers of capitula per plant and florets per capitulum were obtained in a pot experiment with 2 g ammonium nitrate per pot (Abbadi et al., 2008), and also in field trials the highest number of capitula was found at about 190–200 kg nitrogen per ha (Seadh et al., 2012; Shahrokhnia and Sepaskhah, 2017). Therefore, further experiments are necessary to investigate this under the respective cultivation conditions and especially regarding its effects on the colorant yield. In this study, for example, some correlations for safflower regarding the floret yield could be examined, as e.g. that with

decreasing sowing density the number of capitula increases and thus also the floret yield (Steberl et al., 2020a; Steberl et al., 2020b).
Further correlations and the factors influencing them should be researched for safflower as well as for other ‘Coloring Foods’. Especially for ‘Coloring Foods’, which are used in this sector, but have not been bred especially for this purpose, like pumpkins, this should be done. Therefore, special cultivation recommendations for the production of food colorings should be researched for other ‘Coloring Foods’, in order to increase the attractiveness of these crops and thus meet the growing demand in the future.

But also the lower stability of natural pigments compared to artificial pigments is often a limiting factor (Delgado-Vargas and Paredes-Lopez, 2003). Therefore, research in the field of pre-treatment and extraction methods is necessary to increase the extraction yield and stability of the pigment in order to produce more natural colorants of higher quality (Ngamwonglumlert et al., 2017). Different pre-treatments such as thermal treatment with steam or chemical soaking have already been tested with different pigments and are used to incapacitate the enzymes that degrade the pigments (Ngamwonglumlert et al., 2017). Even unusual new extraction methods such as enzyme-assisted extraction or microwave-assisted extraction could be advantageous in terms of lower solvent consumption, higher eco-friendliness and higher speed compared to conventional extraction methods such as Soxhlet extraction (Dahmoune et al., 2014; Ngamwonglumlert et al., 2017). Depending on which pigment has to be extracted, the optimal combination of pre-treatment and extraction method should be researched. This could help to increase both the stability and the extraction quantity, thus decreasing the price and reducing the limitations that currently exist for natural colorants. The stability of the pigment also depends on other traits such as temperature, light and pH-value (Lemos et al., 2012; Martins et al., 2016; Ravichandran et al., 2013). These factors must be researched for each new 'Coloring Food', in order to define, for example, their applications (e.g. whether they can be used in low pH-value of yoghurts) and their packaging (do they change when exposed to light in the store). If, as in this study, one does not harvest the pure product of the florets, but the threshed material, i.e. also parts such as stem or leaves, which may contain interfering/bitter substances, these must be examined.. This would also have to be investigated, if the combine harvester will be used for safflower or other ‘Coloring Foods’ in the future.

A further limitation in the field of natural colorants is the mechanical harvest. As shown in publication II, harvesting the florets with a combine harvester seemed to be a feasible option, which could possibly also be transferred to other crops. The most widely cultivated flowering drug in Germany in terms of area under cultivation is chamomile, for which special harvesting machines have been available since the 1970s and research in this area is still ongoing

(Ehlert and Beier, 2014; Zimmer and Müller, 2003). Nevertheless, there are still flower drugs which are harvested manually, such as calendula or dill (Röhricht et al., 2003). The reasons for manual harvesting are often that it is only used in small-scale cultivation (Röhricht et al., 2003). Here, harvesting technology with the combine harvester could open up new possibilities. Especially for plants where the colorant is not only contained in the flowers, but also in the whole plant or in other parts of the plant, such as in the leaves of safflower (Biertümpfel and Wurl, 2009) this harvesting technique could be an advantage in terms of color yield. Another problem is that, when flowers are harvested during flowering, they have not yet matured. Therefore, mechanical harvesting, which is clogged by this still moist material, may not always be suitable for all plants. Therefore, research into other harvesting techniques, other combine harvester attachments could also make important contributions reducing the current limitations.

7. Summary

Natural colorants have been used for dyeing or drawing for a very long time. There is no clear legal definition of ‘natural colorants’. In most cases, these are pigments that are obtained from renewable raw materials such as plants, minerals, fungi, animals or microorganisms. The use of natural colorants is versatile and ranges from the coloration of cosmetics, clothing, pharmaceutical products to the coloration of food. After the invention of the first synthetic colorant ‘Mauveine’ in the 19th century, the demand for natural colorants decreased more and more. However, some studies in the cosmetic, textile or food sector have shown the negative sides of artificial colorants in recent decades. The studies report both on environmentally harmful effects due to the lack of degradability of the substances, and on their toxic or carcinogenic effects. This led, among other things, to the fact that some synthetic colorants, such as azo dyes, were banned in the EU. The increasing demand for natural colorants in recent years is due on the one hand to the prohibition of certain artificial colorants and on the other hand to the growing interest of the colorant industry and consumers in healthy and biodegradable products.
The increased demand, which particularly also applies to the area of food colorants, was further strengthened by a 'Guidance notes on the classification of food extracts with coloring properties' adopted by the EU in 2013. This guideline distinguishes between ‘food with coloring properties’ (‘Coloring Foods’) and ‘colorants’. Since then, the latter have been classified as additives, which must be legally approved and listed as colorants in the list of ingredients. This is not necessary for ‘Coloring Foods’ and therefore, they have been of crucial importance for the producers of food colorants ever since.

One of these plants, which is considered as ‘Coloring Food’, is safflower (*Carthamus tinctorius* L.), which today is mainly cultivated for seed and oil production. However, it is also known as safflower, which indicates its original use as a plant that provides color. Safflower forms many branches with capitula at their ends. The florets inside contain both red (carthamin) and yellow colorants (carthamidin). The latter is an interesting colorant for the food industry due to its water-soluble property. As the florets look very similar to those of the expensive saffron, they have been used for a very long time as a substitute for it to color e.g. soups or rice. The previous cultivation areas of safflower for florets-/colorant extraction are mainly in Turkey, India or China. Due to the increasing demand for ‘Coloring Foods’ the already existing area of safflower will not be sufficient in the future. Since safflower was already cultivated in Germany in the 16th century and current studies also show that its cultivation is possible in Germany or Europe, the cultivation of safflower in Germany or Europe could make a decisive contribution to meeting this increasing demand in the future.

There are still many opened questions regarding the cultivation of safflower in Germany or Europe. Among other things, recommendations for the cultivation of safflower to obtain the florets are still missing. Many parameters, such as cultivar, sowing density and the number of branches and capitula, as well as the harvesting date are decisive for a high floret yield with high carthamidin content. Another unanswered question, when cultivating safflower to obtain the florets in Germany or Europe, is the harvest. In the currently existing cultivation areas, the florets are harvested manually at optimal maturity. However, this manual harvest would not be economically viable in Europe due to higher wages. With the cultivation of new crops or new directions of use, which the cultivation of safflower for the production of florets in Germany or Europe would represent, there are also many uncertainties regarding the potential yield. The given challenges for the cultivation of safflower as a food colorant were dealt with in the present dissertation and resulted in the following objectives:

- to examine the effect of different cultivars, row spacing, sowing densities and harvest dates on yield parameters for safflower floret production under European conditions,
- to investigate a mechanical harvest with a combine harvester with regard to quality parameters, threshing and carthamidin yield,
- to evaluate and modify the DSSAT CROPGRO safflower model to simulate floret yield under European conditions.

For this purpose, field trials were conducted in 2017 and 2018. Based on these experiments, three scientific publications have been produced, which form the main part of this dissertation. **Publication I** investigated different cultivation parameters, such as two different cultivars, row spacing and sowing densities and their effect on the number of branches, capitula, floret yield, carthamidin content and yield at five different harvest dates. In this respect, it was shown that a lower sowing density of 40 plants m^{-2} produced a higher number of branches, capitula and higher yields of florets and carthamidin. The Chinese cultivar performed well in almost all parameters and the highest yields were achieved at harvest dates two to three weeks after flowering. The Chinese cultivar achieved the highest carthamidin yields on the third harvest date in 2018 with 34.14 kg ha^{-1}.

Publication II evaluated the differences of two cultivars, three threshing parameter settings at five harvest dates for their threshing yield, dry matter content and carthamidin content and yield. It was shown that the maximum threshing yield was achieved on the last harvest date with 748.78–1141.76 kg ha^{-1}, which could be attributed to the highest dry matter content obtained on this date. The carthamidin contents, however, reached their maximum (0.53–3.14%) on the first two harvest dates. The highest carthamidin yields (19 kg ha^{-1}) were

achieved on the last two harvest dates with the Chinese cultivar and the threshing parameter setting P3.

Publication III focused on the modification of the plant growth model CROPGRO for safflower to simulate seed yield and the integration of a new subroutine to simulate the floret yield of two safflower cultivars in two years. It was shown that key variables, such as specific leaf area simulation, could be improved by modifying the model (RMSE: 0.82–24.14 cm^{-2} g^{-1} and *d*-index: 0.73– 0.78). By analogy with a variable already integrated into the model (pod harvest index) and the introduction of new variables (PETALX, HIPIN, HIPMX), the floret yield could be simulated for the first time (RMSE: 97.24 kg ha^{-1} and *d*-index: 0.79). Both the modification and the new approach have improved the simulation of growth as well as seed and floret yield.

The cultivation of ‘Coloring Foods’ could offer farmers an interesting alternative to conventional crops. In addition to the expansion of crop rotation, the biodiversity and the currently much discussed image of agriculture could be improved. The present dissertation could show that the cultivation of safflower is possible in Germany or Europe. Furthermore, a suitable cultivation and harvesting system could be determined. This could help to reduce the current limitations for the production of florets in Germany and Europe, not only for safflower, but also for other ‘Coloring Foods’. As a result, cultivation could be made more attractive and more economical in order to be able to meet the increasing demand in the future on a regional basis.

8. Zusammenfassung

Natürliche Farbstoffe werden schon sehr lange zum Färben oder Zeichnen verwendet. Dabei gibt es keine eindeutige, rechtliche Definition für „natürliche Farbstoffe“. Meist werden darunter Pigmente verstanden, welche z.B. aus erneuerbaren Rohstoffen, wie Pflanzen, Mineralien, Pilzen, Tieren oder Mikroorganismen, gewonnen werden. Der Einsatz natürlicher Farbstoffe ist vielseitig und reicht von der Färbung von Kosmetik, Kleidung, pharmazeutischen Erzeugnissen bis zur Färbung von Lebensmitteln. Nach der Erfindung des ersten synthetischen Farbstoffes „Mauveine“ im 19. Jahrhundert sank die Nachfrage nach natürlichen Farbstoffen immer weiter. Einige Studien im Kosmetik-, Textil- oder Lebensmittelbereich zeigten in den letzten Jahrzehnten jedoch die negativen Seiten der künstlichen Farbstoffe auf. Die Studien berichten sowohl über umweltschädliche Wirkungen aufgrund der fehlenden Abbaubarkeit der Stoffe, als auch über deren toxische oder krebserregende Wirkung. Dies führte unter anderem dazu, dass in der EU einige synthetische Farbstoffe, wie z.B. Azofarbstoffe, verboten wurden. Die in den letzten Jahren steigende Nachfrage nach natürlichen Farbstoffen geht einerseits auf das Verbot gewisser künstlicher Farbstoffe zurück, andererseits auch auf das zunehmende Interesse der Farbstoffindustrie und der Verbraucher an gesunden und biologisch abbaubaren Produkten.
Die vermehrte Nachfrage, die sich insbesondere auch auf den Bereich der Lebensmittelfarben bezieht, wurde durch eine in der EU 2013 verabschiedete „Leitlinie zur Klassifikation von Lebensmittelextrakten mit färbenden Eigenschaften“ zusätzlich verstärkt. In dieser wird zwischen „Lebensmitteln mit färbenden Eigenschaften“ und „Farbstoffen“ unterschieden. Letztere werden seither als Zusatzstoff eingeordnet, welche sowohl rechtlich zugelassen, als auch in der Zutatenliste als Farbstoff aufgeführt werden müssen. Für „Lebensmittel mit färbenden Eigenschaften“ ist dies nicht notwendig und somit sind sie seither für die Produzenten von Lebensmittelfarbstoffen von entscheidender Bedeutung.

Eine dieser Pflanzen, die als „Lebensmittel mit färbenden Eigenschaften“ betrachtet wird, ist Saflor (*Carthamus tinctorius* L.), welcher heute hauptsächlich für die Samen- und Ölgewinnung angebaut wird. Jedoch ist er auch bekannt als Färberdistel, was auf seine ursprüngliche Nutzung als farbstoffliefernde Pflanze hindeutet. Saflor bildet viele Verzweigungen mit Blütenköpfchen an deren Ende. Die darin enthaltenen Blütenfäden enthalten sowohl rote (Carthamin) als auch gelbe Farbstoffe (Carthamidin). Letzterer stellt aufgrund seiner wasserlöslichen Eigenschaft einen interessanten Farbstoff für die Lebensmittelindustrie dar. Da die Blütenfäden denen des teuren Safrans sehr ähnlich sehen, werden diese schon sehr lange als dessen Ersatz zum Färben von z.B. Suppen oder Reis verwendet. Die bisherigen Anbaugebiete des Saflors zur Blütenfäden-/ Farbstoffgewinnung

befinden sich hauptsächlich in der Türkei, Indien oder China. Durch die steigende Nachfrage nach „Lebensmitteln mit färbenden Eigenschaften“ wird die bereits existierende Fläche von Saflor in Zukunft nicht ausreichen. Da Saflor bereits im 16. Jahrhundert in Deutschland angebaut wurde und auch aktuelle Studien zeigen, dass dessen Anbau in Deutschland bzw. Europa möglich ist, könnte der Anbau von Saflor in Deutschland oder Europa einen entscheidenden Beitrag leisten, diese steigende Nachfrage in Zukunft zu decken.
Für den Anbau von Saflor in Deutschland bzw. Europa ergeben sich noch viele offene Fragen. Unter anderem fehlen bisher Empfehlungen für den Anbau von Saflor zur Gewinnung der Blütenfäden. Für möglichst hohe Blütenfädenerträge mit hohem Carthamidingehalt sind viele Parameter, wie z.B. die Sorte, die Bestandesdichte und die davon abhängigen Anzahl an Verzweigungen und Blütenköpfchen als auch der Erntetermin entscheidend. Eine weitere unbeantwortete Frage beim Anbau von Saflor zur Gewinnung der Blütenfäden in Deutschland bzw. Europa stellt die Ernte dar. In den derzeit vorhandenen Anbaugebieten werden die Blütenfäden zur optimalen Reife manuell geerntet. Diese manuelle Ernte wäre jedoch in Europa aufgrund der höheren Löhne nicht wirtschaftlich rentabel. Mit dem Anbau neuer Kulturen bzw. neuer Nutzungsrichtungen, welches der Anbau von Saflor für die Produktion von Blütenfäden in Deutschland bzw. Europa darstellen würde, gibt es auch viele Unsicherheiten hinsichtlich des potentiellen Ertrages. Die gegebenen Herausforderungen für den Anbau von Saflor als Lebensmittelfarbstoff wurden in der vorliegenden Dissertation bearbeitet und ergaben folgende Zielsetzungen:

- Untersuchung des Einflusses verschiedener Sorten, Reihenabstände, Bestandesdichten und Erntetermine auf die Ertragsparameter für die Produktion von Saflorblütenfäden unter europäischen Bedingungen,
- Untersuchung der mechanischen Ernte mithilfe eines Mähdreschers hinsichtlich Qualitätsparametern, des Drusch- und Carthamidin-Ertrages,
- Evaluierung und Modifizierung des DSSAT CROPGRO Saflormodells, um den Blütenertrag unter europäischen Bedingungen zu simulieren.

Dafür wurden in den Jahren 2017 und 2018 Feldversuche durchgeführt. Basierend darauf sind drei wissenschaftliche Publikationen entstanden, die den Hauptteil dieser Dissertation bilden. **Publikation I** untersuchte verschiedene Anbauparameter, wie zwei verschiedene Sorten, Reihenweiten und Bestandesdichten und deren Auswirkung auf die Anzahl der Verzweigungen, Blütenköpfchen, Blütenfädenertrag, Carthamidingehalt und -ertrag bei fünf verschiedenen Ernteterminen. Diesbezüglich zeigte sich, dass eine geringere Bestandesdichte von 40 Pflanzen m^{-2} eine höhere Anzahl an Verzweigungen, Blütenköpfchen und höhere Erträge von Blütenfäden und Carthamidin erzielte. Die chinesische Sorte konnte dabei in fast allen erfassten Parametern überzeugen, und die

höchsten Erträge wurden bei den Ernteterminen zwei bis drei Wochen nach dem Blütebeginn erzielt. Die chinesische Sorte erreichte die höchsten Carthamidinerträge am dritten Erntetermin im Jahr 2018 mit 34.14 kg ha^{-1}.

Publikation II evaluierte die Unterschiede von zwei Sorten, drei Druscheinstellungen an fünf Ernteterminen auf deren Druschertrag, Trockensubstanzgehalt und deren Gehalt sowie Ertrag an Carthamidin. Es zeigte sich, dass die maximalen Druscherträge am letzten Erntetermin mit 748,78–1141,76 kg ha^{-1} erzielt werden konnten, welche auch auf die an diesem Termin höchsten erreichten Trockensubstanzgehalte zurückgeführt werden könnten. Die Carthamidingehalte erreichten hingegen an den ersten beiden Ernteterminen ihr Maximum (0,53–3,14 %). Mit der Druscheinstellung P3 wurden an den letzten beiden Ernteterminen mit der chinesischen Sorte die höchsten Carthamidinerträge erzielt (19 kg ha^{-1}).

Publikation III befasste sich mit der Modifikation des Pflanzenwachstumsmodells CROPGRO für Saflor zur Simulation des Samenertrages und der Integration eines neuen Algorithmus zur Simulation des Blütenfädenertrags von zwei Saflorsorten in zwei Jahren. Es zeigte sich, dass entscheidende Variablen, wie z.B. die Simulation der spezifischen Blattfläche, durch die Modifikation des Modells verbessert werden konnten (RMSE: 0,82–24,14 cm^{-2} g^{-1} and *d*-index: 0.73–0,78). Durch die Analogie zu einer bereits in das Modell integrierten Variable (pod harvest index) und der Einführung neuer Variablen (PETALX, HIPIN, HIPMX), konnte der Blütenfädenertrag erstmals simuliert werden (RMSE: 97,24 kg ha^{-1} and *d*-index: 0,79). Sowohl durch die Modifikation als auch durch den neuen Ansatz, konnte die Simulation des Wachstums sowie des Samen- und Blütenfädenertrags verbessert werden.

Der Anbau von "Lebensmitteln mit färbenden Eigenschaften" könnte für Landwirte eine interessante Alternative zu herkömmlichen Kulturarten bieten. Neben der Erweiterung der Fruchtfolge können die Biodiversität und das derzeit stark diskutierte Image der Landwirtschaft verbessert werden. Die vorliegende Dissertation konnte einerseits zeigen, dass der Anbau von Saflor in Deutschland bzw. Europa möglich ist. Darüber hinaus konnte ein geeignetes Anbau- als auch Erntesystem ermittelt werden. Dies könnte dazu beitragen, dass die bisherigen Limitierungen, welche für die Gewinnung von Blütenfäden in Deutschland bzw. Europa herrschen, nicht nur für Saflor sondern auch für andere „Lebensmittel mit färbenden Eigenschaften“ reduziert werden. Infolgedessen könnte der Anbau attraktiver und wirtschaftlicher gestaltet werden, um die steigende Nachfrage in Zukunft regional decken zu können.

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Eidesstattliche Versicherung

gemäß § 18 Absatz 3 Satz 5 der Promotionsordnung der Universität Hohenheim für die Fakultäten Agrar-, Natur- sowie Wirtschafts- und Sozialwissenschaften

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'Coloring Foods' - development of a suitable cultivation and harvesting system for florets of safflower (*Carthamus tinctorius L.)*

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