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Shifting sowing of camelina from spring to autumn enhances the oil quality for bio-based applications in response to temperature and seed carbon stock

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ABSTRACT

Camelina [*Camelina sativa* (L.) Crantz] is an emerging oilseed crop that is attracting the interest of farmers in relation to its high environmental adaptability and low-input request. Camelina oil is an outstanding feedstock for the bio-based industry, since its unique composition allows multiple applications. Being spring camelina biotypes able to grow as both an autumn and spring crop in mild climates, and sowing date directly influences the temperature occurring during the seed filling stage, which likewise influences the final seed quality in terms of seed weight, oil and fatty acid (FA) content. A detailed study on the response of spring camelina to the effects of autumn and spring sowing is reported herein. The spring variety Midas was sown at six different sowing dates at the experimental farm of Bologna University (Italy) during two consecutive growing seasons (2015-16 and 2016-17). In parallel, three experiments were also carried out in a growth chamber with different ranges of temperatures during the seed filling period. Samplings of immature seeds over time, in both controlled environment and open field trials, allowed identification of a "time frame" in which the main variations in FA kinetics occurred. A "critical period", from 350 to 540 growing degree day after the start of flowering (GDD-AF) was identified as that in which the closest relation between the final camelina FA composition and temperature, during the seed filling stage, occurred. The adoption of this empirical model permitted early evaluation (about 10 d before harvest) of the final camelina oil composition with relevant implications for the bio-based industry. Autumn sowing dates were associated with increased plant aboveground biomass, seed yield, seed oil content, seed weight (TKW), and content of linolenic and eicosenoic acid. Since eicosenoic acid is a valuable feedstock for the bio-based industry, growing spring camelina, as an autumn crop, in the Mediterranean region allows significantly increase the quantity of this infrequent FA.

1. Introduction

Most widespread oilseed crops, i.e. rapeseed (*Brassica napus* L. var. *oleifera*), soybean (*Glycine max* L. Merr.), sunflower (*Helianthus annuus* L.), mainly retrieve the economic value of their oil in relation to its quality. Having stable, or at least predictable, oil quality would represent an added value for new emerging oilseed crops, such as camelina, which is currently entering the bio-based market in view of its unique fatty acid composition, as it allows a plethora of different applications (Berti et al., 2016). Considering all environmental factors, temperature during seed filling stage holds a primary role in determining the final FA composition in oilseed crops. In particular, low temperature promotes the activity of desaturase enzymes in the endoplasmic reticulum, increasing the production of linoleic (C18:2) and linolenic acids (C18:3) from oleic acid (C18:1) (Rodríguez-Rodríguez et al., 2016; Martínez-Force et al., 1998). Polyunsaturated FAs (PUFAs) are essential for maintaining membrane fluidity, allowing increased cell survival at low temperatures (Ohlrogge and Browse, 1995). According to Izquierdo et al. (2016), temperature not only affects desaturases activity but also the incorporation of FAs in triacylglycerol (TAG) molecules, leading to differences in the final oil composition. However, the effect of temperature on long chain FAs (C > 18), which are synthesized by elongase enzymes, using C18:1 as a starting point, still remains a controversial and poorly understood aspect. The relationship between temperature, oil composition, seed yield, and seed oil content in different oilseed crops has been thoroughly investigated in more than 100 studies between 1965 and 2017 (Fig. 1). More than one-half of the studies reviewed have investigated sunflower (30%), rapeseed (24%),

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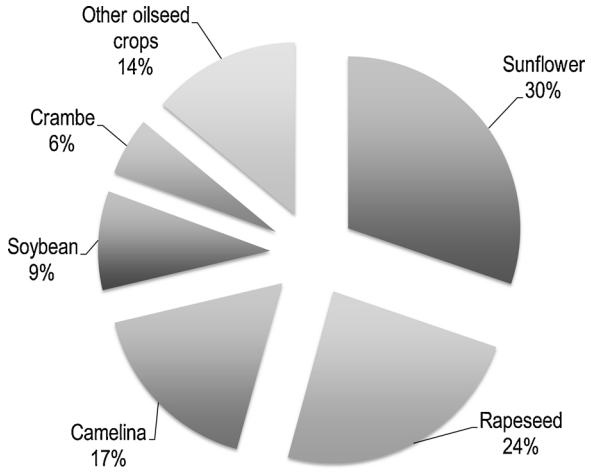

Fig. 1. Share (%) of reviewed literature, published between 1965 and 2017, reporting the effect of temperature on oil yield, oil content and fatty acid composition in different oilseed crops. (The full list of reviewed literature is reported in Annex I, of the supplementary materials).

and soybean (9%), which are staple oilseed crops, characterized by relevant contents of PUFAs, while 17% of the reviewed literature concerns camelina. In addition to the effect of temperature, a few published studies (i.e. Echarte et al., 2012; Ruiz and Maddonni, 2006) have also considered the effects of carbon availability in the seed (i.e. thousand kernel weight, TKW) as a determinant of final oil quality. Fatty acid biosynthesis is different among plant species and genotypes. In most seeds, carbon is delivered to FA metabolism via glycolysis into plastids (Bates et al., 2013), while a more efficient pathway takes place in plants with green seeds (e.g. *Brassicaceae)*, which can also use light as a starting point in *de-novo* FA synthesis, bypassing glycolysis (Bates et al., 2013).

Camelina, which is a "rediscovered" oilseed crop belonging to the *Brassicaceae* family, is characterized by significant seed oil content (26–43%, Righini et al., 2016) and a unique oil composition. The peculiarities of camelina seeds, compared to similar *Brassicaceae* oilseed crops, include: very high content of PUFAs (C18:2 + C18:3 > 50%), relevant amounts of C18:1 (−16%) and eicosenoic acid (C20:1 ~15%), and low natural presence of erucic acid (C22:1 < 4%). Furthermore, the availability of both spring and winter biotypes allows camelina to be grown under different environmental conditions (Berti et al., 2011, 2015; Gesch et al., 2014; Hunsaker et al., 2011; Masella et al., 2014; Zanetti et al., 2017) enlarging the potential effect of temperature on its final oil composition. Since sowing date directly effects the temperature during the seed filling stage and is often related to the final oil quality, shifting the sowing of camelina from autumn to spring would greatly modify its final oil composition and be of economic value. Understanding the factors and the mechanisms regulating final oil quality is fundamental for further development of this oilseed species on a commercial scale. In the Mediterranean, spring camelina can be sown in either autumn or spring, and this allows testing the effect of a wide range of different temperatures, occurring during the seed filling stage, on the productive performance and oil quality from camelina. Towards this end, a multi-year trial was established to compare the effect of autumn and spring sowing dates on productive performance of the spring camelina variety, Midas (designated throughout as Midas).

2. Materials and methods

Midas was developed at the Saskatoon Research and Development Center of Agriculture and Agri-Food Canada and is commercialized by Smart Earth Seeds (Saskatoon, Canada). Midas was used in all experiments with the aim to exclude a "genotype" effect and possibly test only a "temperature" effect. All field trials were established at the experimental farm of the University of Bologna, located at Cadriano, northern Italy (44°33′N, 11°23′E), from spring 2015 until summer 2017. The site is characterized by a silty clay loam soil, with a pH of 7.5 and an OM content of 1.6%. Bologna has a typical Northern Mediterranean climate (Metzger et al., 2005) with mean annual temperature of 13.4 °C and cumulative annual precipitation of 613 mm.

2.1. Preliminary study

A preliminary study was carried out both in open field (OF) as well as under controlled environmental conditions (CE) in order to identify the camelina-specific "time frame" in which the main variations in camelina FA composition occurs during the seed filling stage.

2.1.1. Open field (OF) trials

Midas was sown in 2015 at three different dates, as reported in Table 1: one in spring (April 04, OF1) and two in autumn (October 26 and 09, corresponding to OF2 and OF3, respectively). The same seeding rate (500 seeds m^{-2}), plot surface (10.5 m^2), and row distance (0.13 m) were adopted in all trials. Weed control and nitrogen fertilization (50 kg of N ha^{-1}, as urea, at stem elongation phase) were manually performed

Table 1
Sowing, flowering and harvest dates, and main meteorological characterization of the preliminary (open field OF trials) and sowing date (SD) studies set in Cadriano (Bologna) during.2015–2017.

GS	OF study ID	SD study ID	Sowing	Flowering Date	Harvest	Precipitation[a] (mm)	GDD-GS[b]	GDD-BF[b]	T_{min}-AF[c] (°C)	T_{max}-AF[c] (°C)
2015-16	OF1		4/1/15	5/15/15	6/26/15	190.5	1117	462	14.5	26.7
	OF3	SD1	10/9/15	3/14/16	5/24/16	491.2	1123	469	8.9	19.9
	OF2	SD2	10/26/15	3/24/16	5/26/16	426.2	1013	380	9.6	20.8
		SD3	2/12/16	4/29/16	6/20/16	383.2	1158	465	12.8	23.7
		SD4	3/15/16	5/7/16	6/28/16	226.6	1232	462	14.0	25.5
		SD5	3/30/16	5/14/16	7/7/16	247.4	1332	468	14.9	26.9
		SD6	4/12/16	5/24/16	7/13/16	237.4	1345	466	16.3	28.5
2016-17		SD1	10/13/16	3/20/17	5/22/17	327.2	1045	435	8.6	21.2
		SD2	10/25/16	3/24/17	5/24/17	271.8	982	372	8.8	21.6
		SD3	2/17/17	4/28/17	6/7/17	100.0	1023	484	12.2	24.8
		SD4	3/1/17	4/28/17	6/7/17	98.8	1000	461	12.3	24.9
		SD5	3/15/17	5/11/17	6/14/17	95.2	1056	506	14.4	28.1
		SD6	3/29/17	5/18/17	6/21/17	97.6	1095	506	15.4	29.4

[a] Cumulative precipitation during camelina growth cycle.
[b] GDD-GS = Growing degree days (GDD) accumulated during the whole growing season, GDD-BF = Growing degree days (GDD) accumulated until the start of flowering.
[c] Mean minimum or maximum temperatures occurred from the start of flowering until harvest.

in all trials, while irrigation and pesticide applications were never necessary. The experimental design was a randomized complete block with three replicates. Weather parameters (daily minimum, T_{min}, and maximum temperatures, T_{max}, and precipitation) were measured and recorded by automated weather stations located at the experimental farm. The moment in which more than 50% of camelina plants reached the BBCH phenological stage 60 (Martinelli and Galasso, 2011) was defined as the start of flowering. GDD after the start of flowering (GDD-AF) were then calculated as follows:

$$GDD\text{-}AF = \Sigma [(T_{max} + T_{min})/2\text{-}T_{base}]$$

Where T_{base} represents the base temperature for which a value of 5 °C was adopted, as reported by Gesch (2014). In all OF trials at specific growth stages after flowering, corresponding to 154, 210, 290, 350, 448, 540, 664 GDD-AF, the first six basal pods of the main stem of 15 camelina plants were sampled in each plot. Immature seeds were removed from the pods, weighed, and stored at −80 °C until laboratory analyses.

2.1.2. Controlled environment (CE) experiments

Three consecutive CE experiments were set up between August 2015 and June 2016 at the Department of Agricultural and Food Sciences, University of Bologna (Italy). Midas seeds were sown in 180 square-shaped pots of 0.11 x 0.11 m, filled with sandy soil (sand: 71%, loam: 19%, clay: 10%). Five seeds per pot were sown and then thinned to one individual plant per pot; in total, 180 pots were used in each experiment. The experimental design was completely randomized with three replicates. The plants were allowed to grow in a greenhouse set at constant temperature of about 18 °C, and day-length of 14 h. Fertilizer (20-20-20 NPK) was applied every two weeks to allow camelina growing under optimal conditions. When the first flowers on the main stem opened (start of flowering), corresponding to BBCH 60 (Martinelli and Galasso, 2011), plants were moved into a growth chamber. The day-night temperature ranges set in the growth chamber were different in the three CE experiments, corresponding to: 24-14 °C (CE1), 20-9 °C (CE2), and 14-4 °C (CE3). Temperature ranges were chosen to simulate the mean day-night temperatures registered in the last 10 years at the Cadriano experimental farm during the camelina seed filling stage of the corresponding OF trials (OF1, OF2 and OF3). The other environmental parameters: photoperiod (14-10 h day-night), humidity, and light intensity (60% RH, and 400 µmol m^{-2} s^{-1} of PAR) were maintained constant in all CE experiments. Pots were randomly moved inside the growth chamber to prevent a position effect, and irrigated three times a week to maintain plants under uniform, well-watered conditions. The environmental conditions (T_{min}, T_{max}, T_{mean}, and humidity) inside the growth chamber were monitored with a portable weather station T-monitor/ZT (µ-METOS, PESSL Instruments, Austria). As in the OF trials, all seeds contained in the first 8 basal pods of 30 plants (10 plants for each replicate) were sampled at fixed GDD-AF and stored at −80 °C until analyses.

2.1.3. Analytical methods

Seed moisture content was determined with the method reported by Rodríguez-Rodríguez et al. (2013). The lipid fraction of immature seeds was extracted with a modified version of the Hara and Radin method (Hara and Radin, 1978). One gram of camelina seeds were extracted by adding 20 ml of hexane/isopropanol (3:2 v/v), homogenized using an ULTRA-TURRAX (mod. T25, IKA, Germany), centrifuged, and the upper phase collected. This procedure was repeated three times by re-suspending seeds and collecting the suspension. Non-lipid fraction was removed by mixing the suspension with aqueous sodium sulfate (1 g of anhydrous salt and 15 ml of water) for 10 min in a separating funnel. Lipid phase contained in the upper layer, was then anhydrified by adding sodium sulfate anhydrous, left at 4 °C for three hours, and filtered. The extract obtained was first evaporated to dryness at 40 °C in a rotary evaporator (Rotavapor® R100, Switzerland) under vacuum, subsequently the residual solvent was removed by a weak flow of nitrogen and finally weighed to determine the total fat extracted. The extracted fat was stored in a mixture of hexane-isopropanol 4:1 v/v away from light at a temperature of −20 °C. Since preliminary analysis showed low amounts of free FAs in the samples (~1%), about 10 mg of lipid extract were dissolved in hexane and directly transmethylated with 20 µL of 2 N KOH in methanol. The composition of fatty acid methyl esters (FAMEs) was determined by injecting 1 µL of the supernatant in GC-FID (Thermo-Finnigan, 8000 series, Thermoquest, Italy) equipped with a capillary column in fused silica (Restek 2330, Restek Corporation, USA), (105 m long x0.25 mm I.D. x D.F. 0.20 µm), with a stationary phase 90% biscyanopropyl -10% cyanopropylphenyl polysiloxane, and data acquisition system (Chromcard, ThermoFinnigan, Italy) using the following instrumental conditions: mobile phase, helium; constant pressure 260 K Pa; injector temperature, 250 °C detector temperature, 250 °C oven temperature, 100 °C increase of 3 °C/min up to 180 °C maintained for 10 min, an increase of 3 °C/min up to 240 °C and maintained for 30 min; split ratio, 1:67. The reference standard 463 (NU-CHEK, USA), consisting in a blend of 52 FAMEs, was injected in order to identify the different peaks corresponding to each FA and calculate the correction factor (K), which allowed to align the response of the detector for each FAME.

2.2. Sowing date (SD) study

Midas was sown in open field in two autumn (SD1 and SD2) and four spring (SD3, SD4, SD5 and SD6) dates during two consecutive growing seasons 2015–2016 and 2016–2017 (Table 1); in total, data from 12 different trials were included in this study. The two growing seasons considered had different meteorological conditions, but camelina plants never showed any symptoms of stress. The agronomic management of the trials was the same as that reported in Section 2.1.1. The experimental design was a randomized complete block with four or three replicates (n = 4 or 3) in the autumn or spring SDs, respectively. At full maturity (BBCH 89 = nearly all pods are ripe, Martinelli and Galasso, 2011), all plants in the central 10 rows of each plot were manually cut and weighed to determine total biomass (TB) and then threshed using a plot combine harvester (Nursery Master, Wintersteiger, Austria). Residual moisture in total biomass, TB, and seed was determined by weighing (fresh weight) and oven drying representative sub-samples for each replicate at 105 °C until constant weight (dry weight). Later, small seed samples, representative of each replicate, were accurately cleaned with a lab blower and TKW (g) was determined with an automatic seed counter (DataCount S25, Data Technologies, Israel). Both post-harvest seed cleaning and TKW determination were performed at the Seed Research and Testing Laboratory (LaRAS) of the University of Bologna. The full characterization of seed quality (oil content and FA profile) was performed at the laboratories of Agriculture and Agri-Food Canada (AAFC), Saskatoon (Canada), according to the methods reported by Zanetti et al. (2017).

2.3. Statistical analysis

Prior to ANOVA, the homoscedasticity of variance was verified with Bartlett's Test for $P \leq 0.05$. In the preliminary study, a one-way ANOVA, separately for the OF and CE experiments, was adopted to test the effect of different temperatures for each sampling date. When ANOVA revealed statistically different means, the LSD's test was used to separate means ($P \leq 0.05$). In the SD study, a two-way ANOVA was carried out to test the effect of sowing date and growing season on the productive performance of Midas (total biomass, seed yield, TKW, seed oil content, and principal FA content) considering "year" as a random effect. When an ANOVA revealed statistically different means, the LSD test was used to separate means ($P \leq 0.05$). The correlation analyses among principal FAs (C18:1, C18:2, C18:3 and C20:1, %) and mean

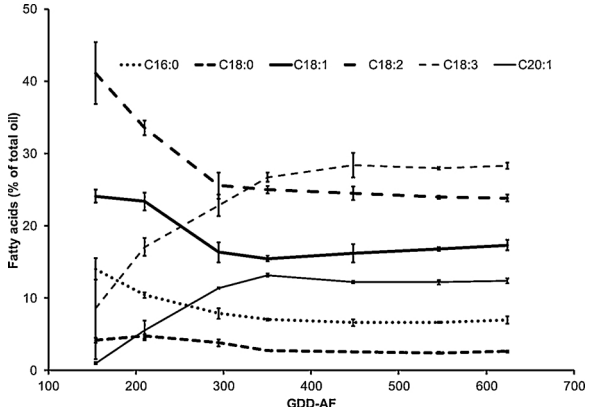

Fig. 2. Accumulation kinetics of the principal fatty acids (FAs) in developing seeds of Midas in the open field trial 1 (OF1) at different growing degree days after the start of flowering (GDD-AF): 154, 210, 290, 350, 450, 540, 620. Vertical bars: standard deviation.

T_{min}, T_{mean}, and T_{max} (°C), at different GDD-AF ranges, were performed to identify the critical period during seed filling stage. A further correlation analysis among principal FAs (C18:1, C18:2, C18:3 and C20:1, %) and TKW was performed. The significance level of the Pearson's correlation coefficient (r) was tested for $P \leq 0.05$. All statistical analyses were performed using COSTAT software version 6.204 (CoHort Software, USA).

3. Results

3.1. Preliminary study

The OF1 trial served to identify when the main variations in principal FAs occur in developing seeds of Midas (Fig. 2). From the first sampling, carried out at about 154 GDD-AF (earlier samplings were not feasible due to low content of lipids in immature camelina seeds), the percentages of C16:0, C18:1 and C18:2 started to decrease, while C18:3 and C20:1 increased ($P \leq 0.05$). Any significant variation in Midas oil composition was not surveyed after 540 GDD-AF, and thus the "time frame" from 154 until 540 GDD-AF was the one in which the majority of changes in camelina FA composition occurred.

The kinetics of principal FAs in developing camelina seeds was significantly affected by temperature in both OF and CE experiments (Fig. 3). Despite a similar trend for all the principal FAs, differences among temperatures were generally more evident in the OF trials than in the CE ones. Oleic acid content (C18:1) was quite stable in the CE experiments (Fig. 3) and only negligible differences were seen between the two sampling dates (350 and 450 GDD-AF). Otherwise, in the OF experiments, after 350 GDD-AF, differences in oleic acid content were always significant ($P \leq 0.05$) with higher values seen in the warmer trial (OF1). Again, for the linoleic acid content (C18:2), differences related to temperature were more pronounced in the OF trials than in the CE ones. In OF, C18:2 content in camelina developing seeds was significantly higher when plants were grown at higher temperature (OF1, Fig. 3). In the growth chamber, the same trend was found, but the differences were lower but still significant. Conversely, in both CE and OF experiments, for linolenic acid content (C18:3) highly significant differences in response to temperature were identified between 350 and 540 GDD-AF with higher values associated with lower temperature (CE3 and OF3, Fig. 3). Finally, for eicosenoic acid content (C20:1) the variation related to temperature was limited but significant in both OF and CE trials at the last sampling date (540 GDD-AF), with higher values associated with colder temperatures (OF3 and CE3, Fig. 3).

3.2. Sowing date (SD) study

The ANOVA results for TB, seed yield, TKW, oil content, and FAs in response to sowing date and growing season (GS), and their interaction, are reported in Table 2. Total biomass and seed yield were highly influenced by all tested factors as well as by the SD by GS interaction (Table 3), presumably in response to the large difference in the precipitation pattern of the two growing seasons studied (Table 1). Interestingly, in 2016/17 the highest seed yield values were reached at SD2, SD3, and SD4 (Table 3), while in 2015/16 the highest seed yields were achieved in the two autumn SDs (SD 1 and SD2). Even if 2016/17 spring SDs were characterized by low precipitation, Midas appeared to be well adapted to water-limited conditions, and to prefer this over wet conditions, such as those characterizing the spring SDs in 2015/16 (Table 1), in which TB and seed yields were decreased (Table 3). Seed oil content (%) and TKW (g) were significantly increased when sowing was anticipated in autumn or in early spring, independently of the GS (Table 3), probably in relation to the increased sensitivity of these two parameters to temperatures, during the seed filling stage, rather than to precipitation. The seeds of Midas plants sown in spring contained significant higher amounts of C18:1 and C18:2 (%), independently from GS, corroborating the fundamental role of temperature in FA biosynthesis. Differently, the highest contents of C18:3 and C20:1 (%) were found in plants sown in autumn (Table 3). Despite some detectable differences between the two growing seasons in T_{min} and T_{max}, after the start of flowering, in the considered set of trials (Table 2), the greatest diversity on the Midas FA profile was observed when shifting sowing from autumn to spring.

With the objective to identify the best fitting, in term of temperature and specific GDD-AF, that is able to predict final camelina oil quality, a set of linear regressions was tested to relate each of the principal camelina FAs (C18:1, C18:2, C18:3 and C20:1) with minimum, maximum, or mean temperatures occurring in the "time frame" between 154 and 540 GDD-AF, identified in the preliminary study. For all the considered FAs in all the evaluated "critical periods" the Pearson coefficients were found significant for $P \leq 0.01$, thus the highest the absolute value of the Pearson coefficient the closest was considered the relationship between temperature and final seed oil composition. As reported in Table 4, the "critical period" from 350 to 540 GDD-AF was identified as the one more closely related to the final contents of all the principal FAs contained in Midas seeds. Concerning temperature, mean T_{max} showed the highest relation with final content of C18:1, C18:2 and C18:3, while C20:1 showed the highest relation with mean T_{min}. Furthermore, all the principal FAs in Midas seeds were also highly correlated with TKW ($P \leq 0.05$, Fig. 4). In particular, higher TKW (g) corresponded to increased C18:3 and C20:1 contents. Conversely, TKW was negatively correlated with C18:1 and C18:2 (%).

4. Discussion

From the results obtained in the present study, the effects of temperature and sowing date on camelina oil quality were further corroborated (Obeng et al., 2019; Obour et al., 2017; Zanetti et al., 2017). The increase of C18:1 and C18:2 contents (%), together with the decrease of C18:3 (%) at elevated temperatures, corresponding to SD5 and SD6, are in agreement with the results of Gilbertson et al. (2014) and Berti et al. (2002) in borage (*Borago officinalis* L.). Recently, Obour et al. (2017) reported that temperatures above 25 °C, during the seed filling stage, caused a significant reduction in PUFAs in camelina oil, also showing negative correlation between C18:2 and C18:3 contents. These results are related to the consequential biosynthesis of these two FAs in the metabolic pathway of camelina oil formation, in which C18:2 is the precursor of C18:3 (Obeng et al., 2019; Obour et al., 2017). Interestingly, the increase of both C18:3 and C20:1 at lower temperatures, corresponding to autumn sowing (SD1 and SD2), is in line with the results reported by Zanetti et al. (2017) who reported a significant

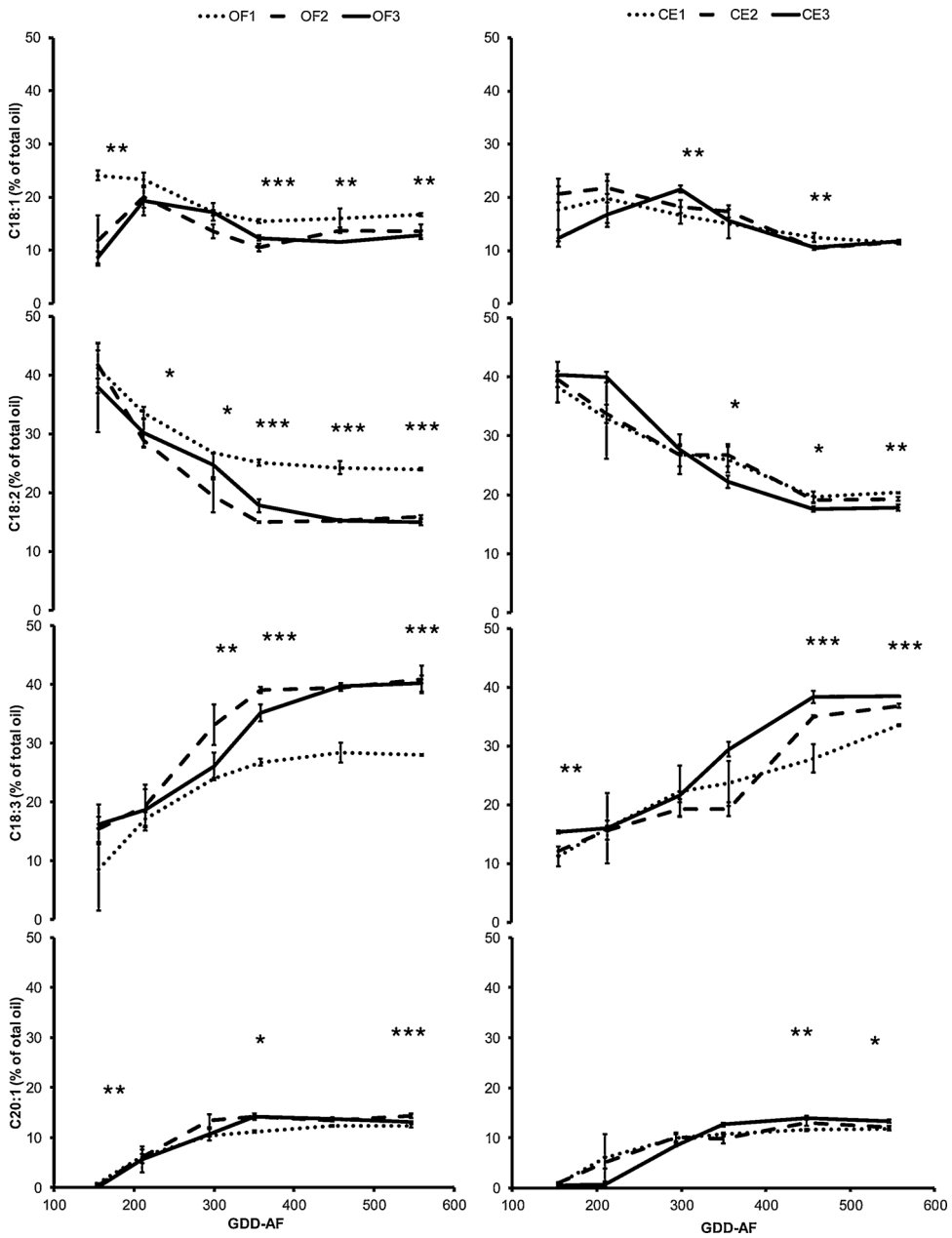

Fig. 3. Accumulation kinetics (% of total oil) of the principal fatty acids (C18:1 = oleic acid, C18:2 = linoleic acid, C18:3 = linolenic acid, C20:1 = eicosenoic acid) in developing seeds of Midas at different growing degree days after the start of flowering (GDD-AF) in open field (OF, left) and controlled environment (CE, right) trials, set in Bologna (Italy) in 2015-16. *, **, *** corresponds to $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ (LSD's test), respectively. Vertical bars: standard deviation.

Table 2
ANOVA results for the main effects: sowing date (SD), growing season (GS) and the interaction sowing date × growing season in the SD trial set in Cadriano (Bologna) during 2015–2017 for the surveyed parameters at harvest: total biomass (TB) seed yield, thousand kernel weight (TKW), seed oil content, oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and eicosenoic acid (C20:1) contents.

Factors	TB	Seed yield	TKW	Seed oil content	C18:1	C18:2	C18:3	C20:1
SD	***	**	***	***	***	***	***	***
GS	**	***	***	*ns*	***	***	***	**
SD × GS	**	***	***	*ns*	***	*	***	*

* = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001 (LSD's test); *ns* = not significant.

positive correlation between these FAs. The increased amount of C18:3, together with a consequent decrease in C18:1 and C18:2 contents, caused by earlier sowing dates (SD1 and SD2), agreed with the results by Obeng et al. (2019) and Pavlista et al. (2011), who had sown camelina in different spring dates during the seasons 2013–2015 in western Kansas (KS) and 2005–2006 at Scottsbluff (NE), respectively. These authors found that earlier sowing dates anticipated the camelina seed filling period with a consequent increase in PUFAs due to lower temperatures. Pecchia et al. (2014) documented a significantly higher amount of C20:1 in the oil of camelina sown in autumn compared to that sown in spring in a study in northern Italy, under similar conditions. The critical period identified for camelina principal FAs, occurring between 350 and 540 GDD-AF (Table 4), was very similar to that determined by Baux et al. (2013) in rapeseed, when considering the different T_{base} used to calculate GDD-AF (0 vs. 5 °C, in Baux et al. (2013) and in the present study, respectively). The same authors were able to

Table 3
Total biomass (TB), seed yield, oil content, thousand kernel weight (TKW), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and eicosenoic acid (C20:1) contents in the sowing date (SD) study set in Cadriano (Bologna) during 2015–2017. Mean value ± standard error. Different letters: significant different means for the interaction "SD × GS" within each parameter surveyed ($P \leq 0.05$, LSD test).

SD	GS[a]	TB	Seed yield (Mg DM ha^{-1})	TKW (g)	Oil content (% of total)	C18:1	C18:2	C18:3 (% of total oil)	C20:1
1	2015/16	12.10 ± 2.04ab	2.23 ± 0.37bc	1.00 ± 0.01 cd	41.33 ± 0.46	12.75 ± 0.66c	16.22 ± 0.42f	36.79 ± 0.50b	14.50 ± 0.24a
	2016/17	7.80 ± 1.05cde	1.59 ± 0.21de	1.15 ± 0.02a	41.44 ± 0.26	12.74 ± 0.22c	16.92 ± 0.16f	36.51 ± 0.34b	13.88 ± 0.12bc
2	2015/16	9.63 ± 1.71bc	2.30 ± 0.40abc	1.04 ± 0.01b	40.41 ± 0.33	13.07 ± 0.15c	14.74 ± 0.49 g	39.08 ± 0.51a	14.18 ± 0.10ab
	2016/17	13.08 ± 0.49a	2.58 ± 0.13abc	1.18 ± 0.01a	40.52 ± 0.74	12.88 ± 0.13c	16.79 ± 0.20f	36.51 ± 0.34b	13.89 ± 0.02bc
3	2015/16	5.47 ± 0.18de	1.24 ± 0.10ef	1.02 ± 0.02bc	39.43 ± 0.98	14.52 ± 0.52b	18.47 ± 0.26e	34.33 ± 0.39c	12.85 ± 0.08ef
	2016/17	11.45 ± 0.33ab	2.93 ± 0.06a	1.04 ± 0.01b	41.28 ± 0.38	17.94 ± 0.33a	20.60 ± 0.18d	30.49 ± 0.42d	13.02 ± 0.28def
4	2015/16	4.44 ± 0.21e	0.99 ± 0.02ef	1.04 ± 0.01bc	41.01 ± 0.21	14.53 ± 0.23b	19.35 ± 0.18e	32.92 ± 0.19c	13.02 ± 0.04def
	2016/17	10.09 ± 2.09abc	2.85 ± 0.10ab	0.97 ± 0.02de	39.99 ± 0.09	17.83 ± 0.12a	21.89 ± 0.56bc	28.36 ± 0.46ef	13.28 ± 0.14de
5	2015/16	4.67 ± 0.55e	0.87 ± 0.08f	0.94 ± 0.01ef	40.00 ± 1.27	15.27 ± 0.55b	20.90 ± 0.11 cd	30.66 ± 0.11d	13.47 ± 0.03 cd
	2016/17	8.75 ± 0.07bcd	2.08 ± 0.03 cd	0,91 ± 0.01f	38.06 ± 0.35	17.53 ± 0.40a	23.25 ± 0.39a	27.00 ± 0.71f	12.93 ± 0.06ef
6	2015/16	4.26 ± 0.60e	1.13 ± 0.10ef	0.86 ± 0.02 g	36.77 ± 0.21	15.28 ± 0.41b	22.83 ± 0.45ab	28.45 ± 0.30e	13.28 ± 0.14de
	2016/17	5.17 ± 0.12de	1.01 ± 0.04ef	0.84 ± 0.02 g	36.47 ± 0.16	16.88 ± 0.16a	23.10 ± 0.37a	27.40 ± 0.59ef	12.81 ± 0.13f

[a] GS = growing season.

Table 4
Pearson coefficient "*r*" of the correlations between the average T_{min}, T_{mean}, T_{max} and different times, expressed as GDD-AF, and the final oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and eicosenoic acid (C20:1) content of Midas seeds from different dates in Bologna (Italy) during the 2015–2017. In bold, the "critical periods" identified for the different FAs as that with the highest r values. All reported Pearson coefficients are significant for $P \leq 0.05$ (LSD's test).

FA	Temperature	Critical periods (GDD-AF[a])				
		100-300	150-350	170-490	218-305	350-540
C18:1	T_{min}	0.74	0.79	0.81	0.72	0.80
	T_{mean}	0.81	0.85	0.89	0.79	0.90
	T_{max}	0.80	0.85	0.90	0.78	**0.91**
C18:2	T_{min}	0.83	0.89	0.89	0.80	0.87
	T_{mean}	0.90	0.93	0.92	0.84	0.92
	T_{max}	0.91	0.93	0.93	0.84	**0.93**
C18:3	T_{min}	−0.83	−0.90	−0.89	−0.81	−0.86
	T_{mean}	−0.91	−0.94	−0.94	−0.86	−0.94
	T_{max}	−0.91	−0.94	−0.95	−0.86	**−0.95**
C20:1	T_{min}	−0.64	−0.69	−0.76	−0.56	**−0.82**
	T_{mean}	−0.72	−0.76	−0.79	−0.66	−0.81
	T_{max}	−0.73	−0.78	−0.80	−0.68	−0.80

[a] GDD-AF = growing degree days after flowering start.

build an empirical model to predict the final C18:3 content in 61 different rapeseed varieties in a 10-year study simply basing on the mean T_{min} in that critical period. In the present study, high and significant correlation coefficients ($r >$ 0.82, in absolute value) were found between both T_{max} and T_{min}, during the critical period (between 350 and 540 GDD-AF) and the principal FA amounts at harvest, demonstrating the relevant effect of temperature during seed filling stage on camelina oil composition. Schulte et al. (2013), reviewing the available literature, reported that camelina oil composition was almost independent of temperature compared to sunflower, soybean, and canola. Nevertheless, these authors suggested that the limited range of temperatures (19–28 °C) investigated in camelina studies, compared to those for the other oilseed crops (10–40 °C), might explain the low correlation found in camelina. In the present study, Midas, sown in both autumn and spring allowed investigation of a wide range of temperatures from 8.9 °C, for T_{min}, up to 28.5 °C, for T_{max}, and excluded a "genotype" effect, thus permitting thorough analysis of the climate effect alone.

Furthermore, in a multi-year and multi-location study across Europe and Canada, Righini et al. (2017) investigated empirical relationships between the final oil quality of Midas and the mean T_{min} during the "critical period" using the same methods adopted herein. Interestingly, for C20:1 a higher correlation coefficient and elevation of the regression lines were found in the present study than in multi-location study (Righini et al., 2017), thus demonstrating that it is effective to grow

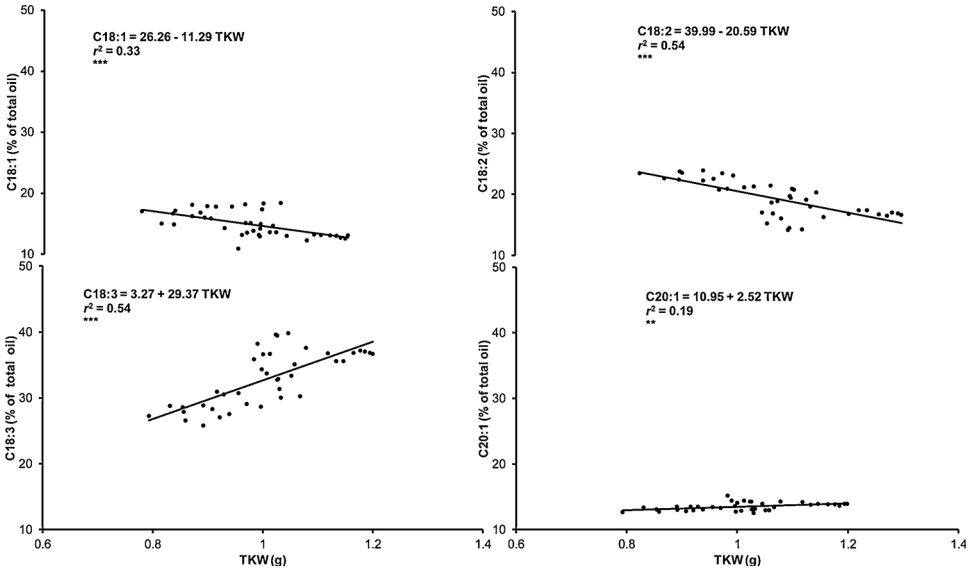

Fig. 4. Correlation between the principal fatty acid (C18:1 = oleic acid, C18:2 = linoleic acid, C18:3 = linolenic acid, C20:1 = eicosenoic acid) content (% of total oil) and thousand kernel weight (TKW, g) at harvest of Midas plants sown at different times in Bologna (Italy) during 2015–2017. **, *** corresponds to $P \leq 0.01$, $P \leq 0.001$ (LSD's test), respectively.

Midas as a winter crop in the Mediterranean region than by adopting spring sowing in colder environments (e.g., Canada) with the aim to increase the final C20:1 content. Presumably, the longer permanence of autumn sown plants in the soil (+130 d SD1 vs. SD6) allows to significantly increase the aboveground biomass and seed yield (Table 3), with a consequently higher carbon stock available. The effect of sowing date on biomass and seed yields have been reported by Berti et al. (2011), who found a significant increase of these parameters in early autumn sown camelina in the Mediterranean climate of Chile. The reported a significant decrease of Midas seed weight (TKW) associated with delayed sowing, in agreement with the results of Berti et al. (2011). In the present study, the effect of a higher amount of carbon stocked in Midas, sown in autumn, was tested by putting in correlation each of the principal FA content with the TKW, which was considered as the "carbon sink" at harvest. To the best of the authors' knowledge, only few published studies (i.e., Echarte et al., 2012; Ruiz and Maddonni, 2006) have considered the effects of C availability, at the seed level, on final FA composition, while none has ever considered the effects of both C stock and temperature on final oil quality of camelina. From the present results, it might be possible to reach the following interpretations of FA metabolism in camelina in response to different SDs:

- Under low assimilate availability for FA metabolism (low TKW) and high temperatures (i.e. spring sowing), membrane desaturase and elongase activities in plastids are presumably altered, thus resulting in increased contents of C18:1 and C18:2;
- Conversely, in conditions of high assimilate availability (high TKW) and low temperatures (i.e. autumn sowing), the desaturation of oleic acid is promoted, leading to the maximum accumulation of C18:2 and C18:3; at this point, the increased availability of C18:1, which is also the substrate for the elongase producing C20:1, promotes a further increase in C20:1.

This interpretation is supported by Echarte et al. (2012), who addressed the regulation of fatty acid biosynthesis in sunflower by assimilate supply to seeds (C stock). Echarte et al. (2012) found that when the oleic desaturation to linoleic is saturated and high assimilates are still allocated into sunflower seeds, there is a consequent increase in oleic content. In the case of the typical biosynthetic pathway of *Brassicaceae* species, which also includes long chain monounsaturated fatty acids (C > 20), different from *Compositae* (sunflower family), this might explain the final differences in oil composition in camelina (increased C20:1) compared to sunflower (increased C18:1) when substrate (C stock) availability is higher.

5. Conclusions

The growing interest of the European bio-based industry for camelina is driving the introduction of this new oilseed crop into different domestic environments, in relation its wide adaptability. The development of empirical relationships among principal FAs and temperature occurred during a specific phase of seed filling (i.e. "critical period") permitted early evaluations of the final oil composition of camelina almost 10 days before harvest. This will have important implications for the bio-based industry, determining in advance the "oleochemical value" of camelina oil. The acquired knowledge of response mechanisms of camelina to different temperatures and sowing dates will undoubtedly have an impact not only on the present understanding of the physiological processes and their regulation, but also in defining optimized agronomic crop management. In conclusion, in a northern Mediterranean climate, autumn sowing of spring camelina varieties appeared to be the best agronomic practice to increase both seed yield and oil quality for bio-based applications.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2019.05.009.

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