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ARTICLE

Cultivation of Saffron (*Crocus sativus* L.) in cold climates

Mohamed Amine Ayari, Marie-Pier Denis, Guy-Anne Landry, and Line Lapointe

Abstract: Saffron, an autumn crocus that produces a highly valuable spice, is grown mainly in Mediterranean climates. Nevertheless, saffron farms have been established recently in the province of Quebec. This led us to test cultivation practices that could influence plant phenology, saffron yield, and corm growth, including planting depth, planting period, and the application of fertilizers, mycorrhizal fungi, and biostimulants at planting. Soil temperature was monitored at the different planting depths throughout the year. Floral initiation was also monitored during spring and summer. Shoot emergence was delayed and final emergence reduced as planting depth increased; however, more shoots were produced by shallow-planted corms, which could lead to the production of corms too small to flower. The best time for planting saffron corm is between the end of July and the third week of August. Mineral fertilization hastened leaf emergence and improved corm production and their nutrient content. Neither the addition of mycorrhizal fungi or of biostimulants had any significant impact on saffron growth or flowering. Floral induction likely took place in July as flower bud appeared in early August. In most years, flower and saffron production was low in this location. It appears that soil temperature did not remain high for long enough during the summer to promote floral induction and autumn temperatures decreased too fast, limiting shoot and flower emergence most years. However, these climatic conditions did not affect corm production; corms could thus be sold to secure revenues for producers.

Key words: planting depth, planting date, floral induction, mineral fertilization, shoot emergence.

Résumé : Le safran est une épice de grande valeur produite par un crocus d'automne cultivé surtout sous climat méditerranéen. Pourtant, des fermes en produisant ont récemment vu le jour au Québec, ce qui a incité les auteurs à tester des pratiques agricoles qui pourraient modifier la phénologie de la plante, son rendement en safran et la croissance du corme, comme par exemple en modulant la profondeur et la période de plantation, de même que la fertilisation par application d'engrais, de mycorhizes ou de biostimulants. La température du sol aux différentes profondeurs de plantation a été suivie toute l'année. Les auteurs ont également suivi la mise à fleurs au printemps et à l'été. Quand elle augmente, la profondeur de plantation retarde l'émergence du feuillage et diminue le taux d'émergence final. Néanmoins, planter les cormes à faible profondeur accroît le nombre de pousses, ce qui pourrait aboutir à des cormes trop petits pour qu'il y ait floraison. Le moment idéal pour planter les cormes de crocus se situe entre la fin de juillet et la troisième semaine d'août. L'usage d'un engrais minéral accélère l'émergence des feuilles et accroît la production de cormes ainsi que leur teneur nutritive. L'ajout de mycorhizes ou de biostimulant n'a aucun effet significatif sur la croissance ou la floraison du crocus. L'initiation florale semble se produire en juillet, car les bourgeons floraux apparaissent au début d'août. La plupart des années, peu de fleurs et de safran ont été récoltés sur ce site. Il semble que le sol ne reste pas chaud suffisamment longtemps en été pour favoriser la mise à fleurs tandis qu'en automne, la température diminue trop rapidement, ce qui réduit l'émergence du feuillage et des fleurs la majorité des années. Ces conditions climatiques n'affectent toutefois pas la production de cormes, que les producteurs pourraient vendre afin de stabiliser leur revenu. [Traduit par la Rédaction]

Mots-clés : profondeur de plantation, date de plantation, initiation florale, engrais minéral, émergence.

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Introduction

Saffron has been traditionally cultivated across the Mediterranean basin, and in Iran and India for thousands of years. Its production had expanded across a broader longitudinal swath of Eurasia, under conditions similar to those in the Eastern Mediterranean and Asia Minor, where it likely originated (Gresta et al. 2008*b*). Cultivation of saffron corms (Iridaceae: *Crocus sativus* L.) in eastern North America began three hundred years ago with Pennsylvania Amish communities (Willard 2002). Although there are indications that saffron yield is higher in climate with warm summers, rain in autumn and cool winters, typical of the Mediterranean basin (Douglas et al. 2014), saffron production in the colder climate of Quebec, Canada, and adjacent New England has been ongoing for the past 5 to 10 years (The North American Center for Saffron Research and Development 2020). Given that these environments are much different from the Mediterranean basin, both in terms of climate and soil properties, many cultivation practices must be revisited to adapt saffron culture to these more northerly climates.

Saffron is the most expensive spice worldwide, consisting of the stigmas of the crocus flowers. Its vegetative organ, a corm, produces a limited number of flowers. Each stigma must be harvested individually as soon as the flower opens. The species is triploid and sterile, and thus propagate only by corms. Most producers cultivate it as a perennial, although it is possible to uplift corms every summer, sort them, then store them under dry conditions until replanting, as you do for annual production (Gresta et al. 2008*b*). Whereas most producers cultivate saffron for its flowers, some also commercialize corms. Most publications focus on flower and saffron yield; however, as only large corms flower (size 8 – 10 (circumference) or 8 g or more of fresh mass (Mollafilabi 2004; Douglas et al. 2014)), conditions that favor flowering also favor the production of large corms (Khorramdel et al. 2015; Koocheki and Seyyedi 2015; Ghanbari et al. 2019; Cardone et al. 2020).

Saffron's life cycle is characterized by a summer dormancy period, the emergence of the foliage and flowers in autumn, and long-lasting leaves that senesce by late spring or early summer. Soil and air temperatures play a very important role in the life cycle, controlling the entry and exit of dormancy along with floral induction during the dormancy period (Molina et al. 2005). Corms require exposure to temperatures between 23 and 27 °C for more than 50 days during the summer to optimize flower production in the following autumn (Molina et al. 2005). Exposure to high temperature (30 °C) during the summer also favour the production of larger corms the following spring (Siracusa et al. 2010). Many cultivation practices can influence the temperature to which the corm is exposed, such as depth and date of planting. Saffron corms are typically planted 10 to 30 cm deep, but most studies recommend a depth of 10 to 20 cm (Galavi et al. 2008; Gresta et al. 2008*b*; Vafabakhsh et al. 2010). Soil temperature decreases with depth during the summer (Ping 1987; Lubbe and Henry 2019), which would suggest planting closer to the surface in more northern climates compared with warmer climates. Shallow planting, however, could expose the corm to freezing temperatures (Ping 1987). Furthermore, it has been demonstrated that corm or bulb production in many geophytes, including saffron, increases as depth decreases (Chipman and Thorpe 1977; Hagiladi et al. 1992; Kumar et al. 2009; Verdaguer et al. 2010). This response could lead to the formation of numerous corms that are too small to flower (Kumar et al. 2009).

Date of planting can influence the temperature to which the corm is exposed during the summer; if planted in late summer, it can also delay shoot emergence. Planting time varies greatly among producer countries, from early June to mid-September (Kumar et al. 2009). Yet, only a few studies have tested different planting times within a single site and most have concluded that early summer was the best planting period to optimize saffron yield (Rehman and Lodhi 1977; Gresta et al. 2008*a*; Tookalloo et al. 2010; Amirnia et al. 2013). Imported corms only become available on the North American market in August, whereas local plants do not senesce before early July (G.-A. Landry, personal observation, Le ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec). Thus, corms from local sources may not become available for sale on the market until mid- to late-July at the earliest.

Saffron is a slow-growing crop, yet mineral fertilization improve saffron yield (Hosseini et al. 2004; Amiri 2008; Koocheki and Seyyedi 2015; Ghanbari et al. 2019) and corm growth (Khorramdel et al. 2015; Fallahi and Mahmoodi 2018; Seyyedi et al 2018; Ghanbari et al 2019). The best time to apply these amendments is at planting to avoid favouring weed growth through surface application. Manure is frequently applied to saffron fields to improve both soil organic matter and nutrient content (Gresta et al. 2008*b*). Chemical fertilizers, especially those providing nitrogen, have also been used (Kumar et al. 2009), as well as foliar applications of a complete solution that also contain micronutrients to compensate for their low availability in soils with high pH (Hosseini et al. 2004). Acidic soils that are more typical of eastern North America would definitely require an adjustment of the fertilizer composition. Like most geophytes, saffron produces a coarse root system; these root systems have usually undergone extensive mycorrhization and benefit from this symbiosis (Smith and Read 1997). Indeed, field-grown saffron is extensively mycorrhized (Kianmehr 1981; Lone et al. 2016). The addition of inoculum of *Glomus* spp. or *Pseudomonas fluorescens* Migula have been demonstrated to improve saffron growth and yield (Aimo et al. 2010).

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The objectives of this study were to identify the depth and date of corm planting that optimize saffron yield and corm production. Soil temperature was recorded at the different depths throughout the year, and the timing of flower initiation was monitored through sequential harvesting of corms during spring and summer. Organic fertilization, in combination with mycorrhizal fungal spores and two different bio-stimulants, was also tested for its effects on saffron growth and yield. Consumers' interest for local food production is growing (Adams and Salois 2010) which includes developing cultivation practices for exotic plants to fulfil some of these needs. Furthermore, although autumn crocus have traditionally been ignored as ornamentals (Dole and Wilkins 1999), saffron corms are readily available to consumers from both plant retailers and from saffron producers, often through online stores. There is thus an interest to produce saffron locally to satisfy the domestic demand for both specialty food products and for exotic ornamentals. This study constitutes a first step in the optimization process regarding saffron cultivation practices under northern climate conditions.

Materials and Methods

Experimental design and plant material

The study was conducted in 2016–2019, on a field site located near Baie-St-Paul, Quebec (lat 47.43, long 70.57, elevation 308 m). The climate of the region is humid continental (Kottek et al. 2006). Over the past 30 years (1981–2010), annual average temperature was 4.0 °C and annual precipitation reached 996 mm, of which 74% falls as rain. Snow is omnipresent during the winter months with annual snowfall averaging 257 cm. It can occur as early as October, but increases up to 60 cm per month for December and January than to 40 cm per month in February and March, before ending in April or May (Environment Canada 2010). Monthly climatic conditions from 2016–2020 are presented in Table A1 (Historique-Météo.net 2021).

Saffron corms of Grade 8–9 or 9–10 (corresponding to a diameter of 2.5 cm to 3.2 cm) were either purchased from Pur Safran (dug up from their growing site at Notre-Dame-de-Montauban, QC, Canada; mean fresh mass of 9.7 g in 2017 and of 11.5 g in 2018) or imported from the Netherlands by Emporium Safran Québec (mean fresh mass of 16.5 g). The purchase of corms from a local supplier (Pur Safran) earlier in the season allowed us to initiate first planting around July 20th. These locally grown corms were used only in the planting period experiment (for more details, see planting period sub-section).

The field was a hay meadow, located on a sloping hillside. The land has been uncultivated for at least 2 years, with a cover of grasses, perennials, and some shrubs. The soil is a glacial till with a sandy loam texture (66% sand, 24% silt, 10% clay; Dequen Soil Series in the Humo-Ferric Podzol Group). At the beginning of the summer, the vegetation was removed, after which the top 20 cm of soil was set aside. At the time of planting, the soil was loosened using a rake. Experiments were conducted using a randomized complete block design. Each plot consisted of four rows of 10 corms each with a spacing of 15 cm within rows and between rows, which represented a density of 32 corms m^{-2}. Plots were positioned at a distance of 30 cm from each other.

After the corms were planted, the topsoil was put back to form raised beds 20 cm high with a flat top at least 1.65 cm wide. The raised-bed cultivation system was selected to improve soil drainage at corm depth. Raised beds ran parallel to the slope and were set up at a distance of 30 cm from each other. For the planting depth experiment, raised beds were installed prior to planting, after which corms were dibble-planted at the desired depth.

For all experiments, weekly weeding was done manually as required or with a flame-weeder during the saffron dormancy period. None of the plots were irrigated.

Nutrient analyses

Soil samples were collected a few days before planting for each experiment. A sample was collected in the middle of each plot at planting depth and all samples from one block were mixed to form a composite block sample per experiment. Macro- and micronutrients, organic matter, pH, cation exchange capacity, and base saturation were determined by an independent laboratory (Agri Quanta Laboratory, Saint-Ours, QC). Macronutrients and trace elements were extracted using Mehlich III method. Phosphorus, potassium, calcium, magnesium, aluminum, manganese, copper, zinc, and boron concentrations were determined using optical plasma emission spectrometry. Organic matter was measured by loss-on-ignition at 375 °C, while nitrogen was estimated by the Kjeldahl method. Data are presented in Table A2. The same techniques were used to analyze fertilizer composition. The quantity of fertilizer to be added to each plot was then calculated.

Planting depth

A first trial was set up in mid-August 2016. Corms were planted 20 cm, 25 cm, or 30 cm deep. Four blocks along the slope gradient were established, with two repetitions of each treatment per block, for a total of 24 plots (960 corms).

Due to low emergence in spring 2017 and 2018, new plots were put in place in August 2018. We suspected that the plots were too close to the forest edge and were shaded at the beginning of the day in the autumn, reducing not only the amount of light received daily, but also the temperature of the soil. We chose a location where plots received full sun throughout the day. In addition to this modification, we tested shallower depths of 10, 15, 20, and 25 cm, given that snow settles early in the autumn, thereby limiting the risk of corm freezing

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during winter. Each treatment was repeated once per block in six blocks for a total of 24 plots (960 corms).

Floral induction phenology

Corms were randomly sampled from plots that did not receive any specific treatment. Three corms large enough to flower were collected every 2 weeks from mid-May 2018 until all leaves had reached complete senescence. Thereafter, corms were sampled weekly until early September (for a total of 12 weeks). Corms were halved vertically, then stained with methylene blue and observed under a binocular microscope.

Planting period

Corms were planted at four different dates during the summers of 2017 and 2018. In 2017, the corms were planted on 25 July, 7 and 21 August, and 5 September. During 2018, planting occurred on 25 July, 8 and 21 August, and 7 September. The two planting years constituted the blocks. There were four repetitions per block (year) and per treatment, for a total of 32 plots over 2 years (1280 corms). All corms were received at the same time within a single year. The corms were stored at 25 °C, in the dark, until the first planting date in 2017 and in 2018. Thereafter, the temperature was reduced to 20 °C to simulate soil temperature in the field during the same period.

Fertilization, mycorrhizal fungi, and biostimulants

A factorial design in complete random blocks was set up to test the following three factors: (*i*) organic fertilization at planting; (*ii*) addition of mycorrhizal fungi spores; and (*iii*) addition of either Turitek or the soil activator EarthAlive at planting. The fertilizer contained a mixture of feather meal 13-0-0 (462 kg ha^{-1}), bone powder 0-13-0 (1538 kg ha^{-1}), and Sul-Po-Mag 0-0-22 (1136 kg ha^{-1}). The mycorrhizal fungi (MykePro Gazon, Premier Tech containing 15 viable spores of *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler per g) were applied at a rate of 20 g (300 spores) per corm; the granules containing the spores were placed directly underneath the corm at the time of planting. Turitek Liquid Flowering and maturation (Ferme Eugénia, Rimouski, QC) is a combination of algae and liquid extracts from earthworm manure. Turitek was applied by soaking the corms in a solution of 1.5% *v*/*v* (Turitek/water) for 4 h, followed by twice watering with 5000 L ha^{-1} of 0.06% solution at 2-week intervals following planting. The EarthAlive soil activator (Earth Alive Clean Technologies Inc., Lasalle, QC) is a biofertilizer that contains *Pseudomonas* and two *Bacillus* soil species. It was applied at a rate of 26 mL per corm (5200 L ha^{-1}) at planting from a solution of 2.5 g L^{-1}. The 12 combinations of treatments, which include controls with no fertilization, no mycorrhizal fungi, or no biostimulants, were repeated in four blocks, for a total of 48 plots (1920 corms).

Data collection and laboratory analyses

Each autumn (2016, 2017, 2018, and 2019), emergence of the foliage from individual corms was noted once or twice a week. Counting and harvesting of flowers was performed every other day (2016 and 2018) or daily (2017) during the flowering season (autumn). The stigmas were removed from the harvested flowers and dried immediately (20 min at 50 °C) in a food dehydrator (Excalibur, Sacramento, CA) until the water content had decreased by 80%–90% (Carmona and Alonso 2004). Saffron was then weighed.

Following complete snowmelt in 2017, 2018, and 2019, final emergence was noted, together with the number of shoots per corm. Final emergence included both the shoots that had emerged during autumn and those that had emerged during the winter under the snow, minus those that did not survive the winter. At the end of May 2018, two to three roots from three corms per plot were collected in the fertilization trials to monitor mycorrhizal colonization. The roots were cleared with 10% potassium hydroxide, then with 1% hydrogen peroxide, followed by 1% hydrochloric acid. The roots were stained with Trypan blue, followed by acidified glycerol to preserve the roots until observations took place. A six-class mycorrhizal colonization scoring was used based on the percentage of mycorrhizal fungi structures in the roots: 0%; 1% (traces of colonization); less than 10%; 11%–50%; 51%–90%; and more than 90% (Trouvelot et al. 1986). Each root was observed and evaluated separately.

Leaf senescence was estimated in each plot weekly between 6 July and 30 July in 2018, using 7 classes: 0 %; 1% to 25%; 26% to 50%; 51% to 75%; 76% to 90%; 91% to 99%; and 100% leaf senescence. All estimates were made on a per plot basis.

Following complete leaf senescence (in late July), six corms per plot were dug up in the planting depth (2017), planting period (2018) and fertilization (2018) trials. These corms were chosen randomly. Fresh mass of the main corm and daughter corms was recorded, along with their number. The six corms per plot that were collected from the fertilization trials were freeze-dried, then ground using a high-speed rotary mill (ZM-200, Retsch, Haan, Germany). A sub-sample of ground material was digested in an H_2O_2-H_2SO_4 mixture (Lowther 1980). The nitrogen and phosphorus content were subsequently quantified by colorimetry, while potassium, calcium, and magnesium were determined by atomic absorption spectrometry.

Soil temperature was monitored in the two planting depth trials. In each plot, a probe (Thermochron 4 K, iButtonLink, LLC, Whitewater, WI, USA) recorded temperature continuously at the planting depth of the corms. In the first trial, temperature was recorded at 20, 25, or 30 cm depth, from autumn 2017 until autumn 2018. In the second trial, temperature was recorded at 10, 15, 20, and 25 cm depth in the summer of 2018 and

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recording continued up to autumn 2019. Data reported are mean daily temperature.

Statistical analysis

Each of the three experiments was analyzed independently, using a general linear model. A series of analyses of variance (ANOVAs), followed by Tukey multiple range tests were performed, using a 5% probability level. For both planting depth trials, blocks were assigned as a random factor whereas planting depths were a fixed factor. In the first trial (20 to 30 cm depths), emergence at the end of autumn and in spring were recorded over two seasons and data from season 1 (2016–2017) and from season 2 (2017–2018) were analysed separately. All other variables were recorded at the end of the first season. For the planting period experiment, year is the random factor. Two-way ANOVAs were therefore run on the emergence data collected at the end of autumn and in spring. Corms were only harvested at the end of the first season, and were compared among planting periods using one-way ANOVAs. The fertilization trial was analyzed as a factorial design with three fixed factors (fertilization, mycorrhizal fungi, and biostimulants) and one random factor (block). When there were enough flowers to run statistical tests, i.e., in autumn 2017, the appropriate analyses were performed for the planting period and the fertilization experiments.

Phenology of leaf (2017 and 2018) and flower emergence (in 2017 for the planting period and the fertilization experiments) data in the autumn, as well as phenology of leaf senescence in late spring 2018, were analyzed in all three experiments using a repeated measure model with treatments, dates of measurements, and their interaction as fixed factors. The mean daily soil temperatures were compared between the different depths using repeated measure ANOVAs. Data were analyzed separately for spring (mid-May to the end of June), summer (1 July to 13 August), autumn (1 September to 31 October) and winter (November to early May) to avoid masking differences among depths due to temperature inversions that take place between seasons. For the winter, monthly means were used, given that temperature did not fluctuate much between days. All statistical analyzes were performed using Statistix version 10 (Tallahassee, FL, USA).

Labelling

For all experiments, we used the following abbreviations to discriminate the planting year (P) from the year (S) in which the observations took place. For example, corms that were planted in 2016 and observed during the 2016–2017 season (autumn 2016 up to summer 2017) are identified as P16 – S16/17. As the growing season of saffron starts in autumn, the year of observation starts in the autumn and ends the following summer.

Results and Discussion

### Planting depth

Soil temperature

As expected, soil temperature significantly increased ($F_{2,21}$ = 4.12, P = 0.031) with increasing closeness to the soil surface during the summer (Fig. 1a). Yet, differences were small, 0.6 °C on average for July and August, between 20 and 30 cm depths. When the soil warmed the differences were close to 1 °C, but as soil cooled temperature temporarily became uniform across depths. An inversion occurred in the autumn as soil cooled faster close to the surface than at depth (Figs. 1a–1c). This inversion remained until January; afterwards, temperatures no longer differed among depths (Fig. 1c; month × depth interaction: $F_{12,126}$ = 3.3, P < 0.001). The same behaviour was observed the following year for the 10 to 25 cm depth plots, where soil temperature was slightly but significantly cooler closer to the surface from November to January (Fig. 1d; month × depth interaction: $F_{18,108}$ = 2.9, P < 0.001). Soil temperature differed by about 0.5 °C between 10 and 25 cm ($F_{3,18}$ = 3.0, P = 0.057) throughout the winter and remained generally around 0 °C, even close to the soil surface (10 cm), whereas minimum air temperature recorded from December to February was −33 °C to −36 °C (Environment Canada 2010).

The summer 2017 was cooler than the summer 2018 and 2019 (Fig. 1b; $F_{2,11}$ = 59.8, P < 0.001). Thereafter, temperatures dropped faster in 2018 and 2019 than in 2017 ($F_{2,112}$ = 97.1, P < 0.001). The number of days that the soil was at 23 °C or higher — the minimum temperature required to induce flower bud production (Molina et al. 2004) — was low at 20 cm in all three years where we recorded soil temperature (1 day in 2017 up to 12 days in 2018). This temperature was sustained for only 1 to 5 days before cooling again. Soil temperature did not differ among the shallower depths (10 to 25 cm) in July and August 2019 ($F_{3,20}$ = 2.2, P = 0.126) but reached 23°C or higher (mean daily values) during 12 to 16 days at 10 and 15 cm depths, including 5 to 6 days in a row at the end of July.

Depths of 20, 25, and 30 cm

During autumn 2016 (P16 – S16/17), planting depth had a significant effect on saffron shoot emergence (Table A3). Shoots started to emerge earlier from corms that were planted closer to the surface than from those that were planted deeper (Fig. 2a); significant differences were maintained until the last survey (23 November 2016; Table 1). Final emergence (which includes winter emergence and plant survival) at the end of the first growing season in spring 2017 was much higher than that recorded in the autumn; yet there was still a slight but significant difference between 30 cm (93%) and 20 or 25 cm (99%). During the following autumn (P16 – S17/18), percent shoot emergence prior to the arrival of permanent snow cover were very much lower

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Fig. 1. Mean daily soil temperature as a function of soil depth. (*a*) Mean daily soil temperature measured at 20, 25, and 30 cm depths between 1 July and 31 August 2017. (*b*) Mean daily soil temperatures measured at 20 cm depth from May to October in 2017, 2018, and 2019. (*c*) Mean daily soil temperatures measured at 20, 25, and 30 cm depths from November 2017 to April 2018. (*d*) Mean daily soil temperature measured at 10, 15, 20, and 25 cm depth from November 2018 to April 2019 and (*e*) from May 2019 to September 2019. N = 8 in 2017 and 2018, and N = 6 in 2019.
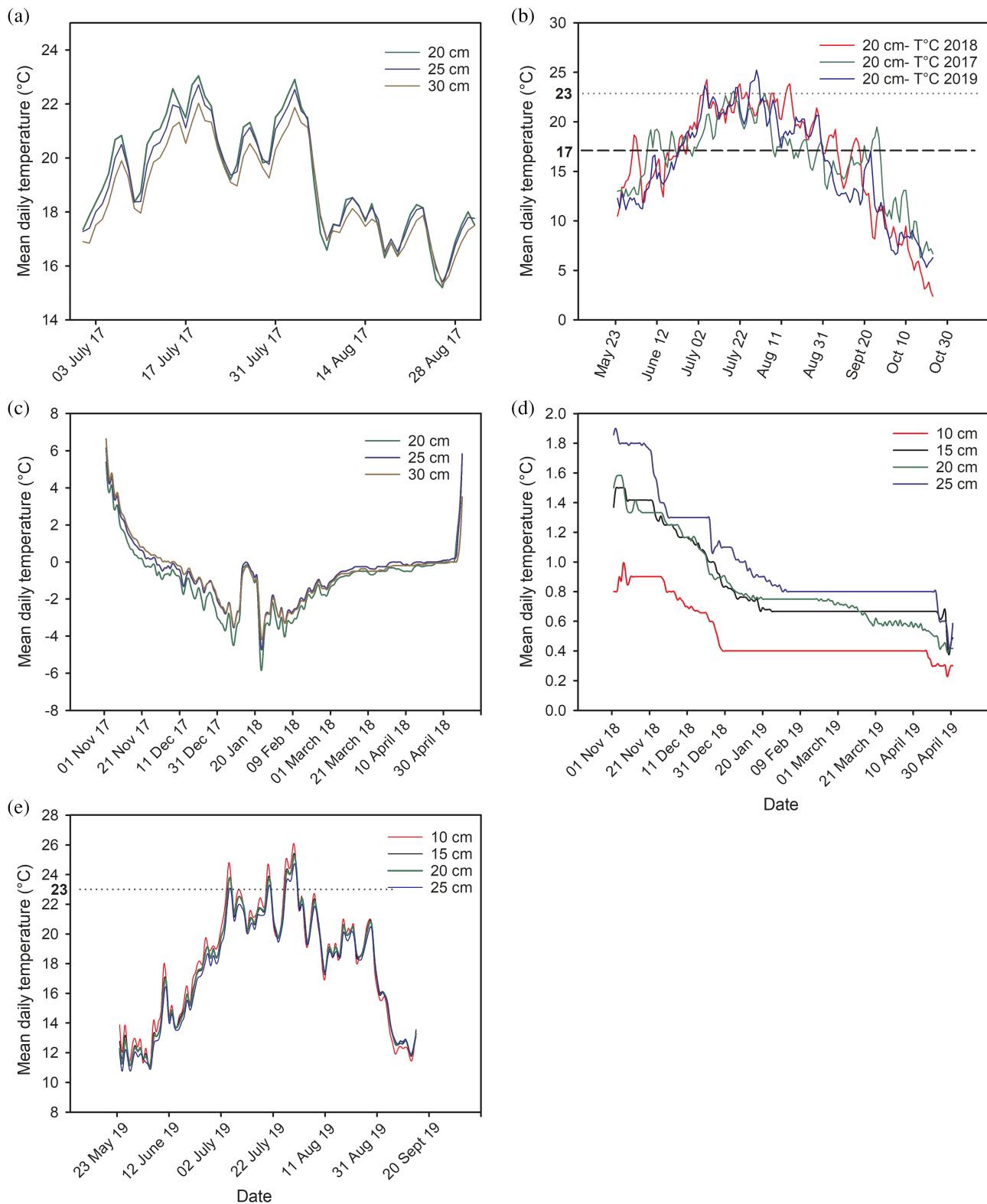
Fig. 2. Autumn per cent shoot emergence (mean ± standard error) (*a*) in 2016 (P16 – S16/17) and (*b*) in 2017 (P16 – S17/18) at 20, 25, and 30 cm depths. N = 8. A posteriori analyses of treatment effects following repeated measure ANOVA are presented as lowercase letters. P, planting year; S, the year data were collected.
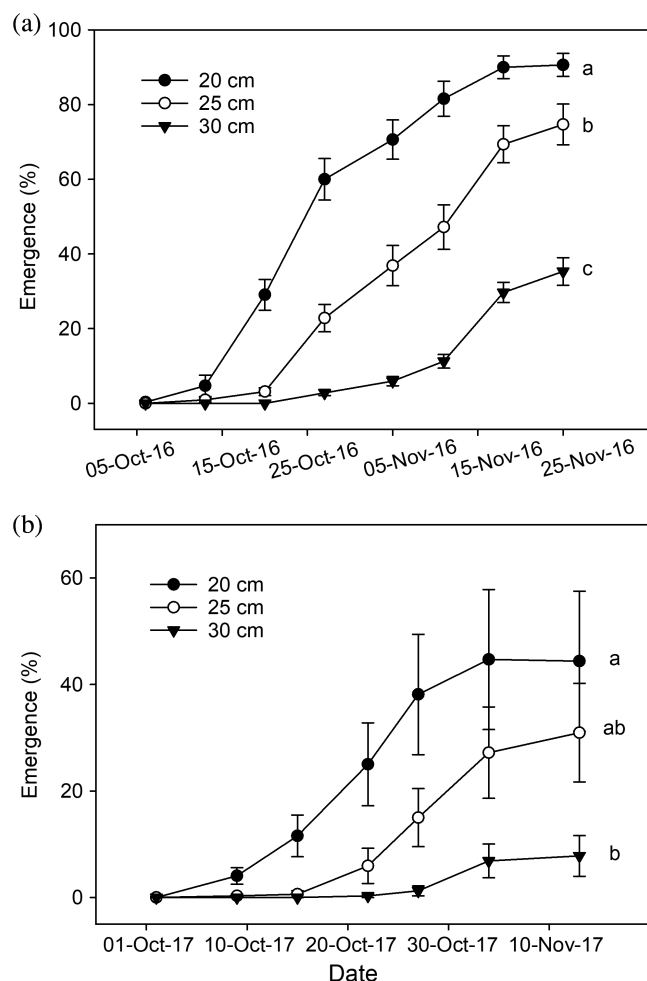
than in 2016 (Fig. 2*b*), yet corms planted closer to the surface emerged earlier than those planted deeper (Table A3). However, at the last recording period, differences were no longer significant (*P* = 0.061; Table 1). During spring 2018, emergence remained very low (mean ± standard error: 24.3% ± 8.0%) and did not differ among planting depth treatments.

Insufficient numbers of flowers were available in the plots in 2016 and 2017 to perform statistical tests (average flowering rates of 2.5% and 0.8%, respectively) and to determine the effect of the treatments on flowering.

We counted the number of shoots per planted corm in spring 2017 as a proxy for the number of corms that had been produced. Corms planted at 20, 25, and 30 cm had produced on average 3.6 ± 0.1 shoots per corm, 3.2 ± 0.2 shoots, and 2.2 ± 0.1 shoots per corm respectively; differences between the 30 cm and the two shallower treatments were significant (Table 1). However, planting depths had no impact on either total corm mass or the

number of corms produced per planted corm, nor on the mass of the largest corm or the number of corms of 8 g or more produced per corm. Across treatment groups, 2.6 corms were produced on average per corm planted. Each planted corm produced on average 13.0 g of replacing corms (fresh mass), and the largest corm produced per planted corm weighed 8.2 g on average. On a planted corm basis, 0.66 corm of 8 g or more was produced in one growing season, i.e., out of six plants harvested per plot, four corms of 8 g or more were recorded. We suspect that subsampling the plots reduced our capacity to perceive differences among treatments, explaining why there were differences in terms of shoots per planted corm but not in terms of corms produced per planted corm.

Senescence was more or less synchronous between planting depth treatments in early summer 2018 (Fig. 3; Table A3; P16 – S17/18), despite differences in the dates of emergence the previous autumn (Fig. 2*b*). Although we did not record leaf senescence on a weekly basis in summer 2017, we did record the date at which all leaves had completely senesced, which was July 25th. June was colder in 2017 than in 2018 (Fig. 1*b*), which likely delayed the entry into senescence of the plants in 2017.

We dug out some corms during the summer 2018 (P16 – S17/18) to find the cause of the low emergence that was observed in spring 2018 (24.3% ± 8.0%). Nematode, fungal, and bacterial isolation tests were performed (Laboratoire d'expertise et de diagnostic en phytoprotection, MAPAQ), which indicated that corm decay was most likely not due to pathogenic fungi, bacteria, or nematodes. We suspect that saffron did not perform well in these plots, due to the nearby forest border, which reduces the duration of daily sun exposure in the autumn, together with possible drainage problems. Therefore, it seems likely that the low emergence observed in autumn 2017 (Fig. 2*b*) reflects not only a delay in emergence for corms that have been deeply planted, but also possible mortality during summer 2017.

Depth of 10, 15, 20, and 25 cm

As observed in the first trial, shoots emerged earlier and attained a higher per cent emergence prior to snowfall for corms that were planted closer to the surface than for those that were planted deeper (Figs. 4*a* and 4*b*; Tables 1 and A2). During autumn 2018 (P18 – S18/19), 90% of the corms planted at 10 cm depth had emerged by early October, whereas about 20% of corms at 20 cm had produced an emerging shoot by the end of October. Despite a high level of leaf emergence for shallow planted corms, none flowered. We suspect that cold temperatures that were recorded during autumn 2018 (Fig. 1*b*) explain the absence of flowers in the plots, despite high leaf emergence. Spring emergence was significantly different among depths the next spring, although most corms had produced at least one shoot, i.e., around 98.3% of corms that were planted at 10 cm

Table 1. Effects of planting depth on saffron phenology, flowering, and corm production for two planting depth trials and for either one or two seasons following planting.

Experiment	Planting depth 20, 25, 30 cm				Planting depth 10 to 25 cm	
Planting year	P16				P18	
Growing season	S16/17		S17/18		S18/19	
Variables	F	P	F	P	F	P
% Emergence in autumn	40.7[a]	**<0.001**	3.27[a]	0.061	72.2[b]	**<0.001**
% Emergence in spring	6.15[a]	**0.009**	0.89[a]	0.430	5.6[b]	**0.009**
Shoots per corm	30.2[a]	**<0.001**	-	-	21.5[c]	**<0.001**
Number of corms	1.4[a]	0.276				
Total corm mass	0.3[a]	0.735	-	-	-	-
Biomass largest corm	0.2[a]	0.807				
Number of corms ≥8 g	0.9[a]	0.424				

Note: F statistics and P values of one-way ANOVAs are presented. Significant results are highlighted in bold. Shoot emergence during autumn was also analysed as repeated measures. See Table A3 for details of these analyses.

[a–c]Degrees of freedom: [a]2, 18; [b]3, 15; [c]3, 18 (only half of the blocks were measured in spring 2019). P, planting year; S, the year data were collected.

Fig. 3. Evolution of leaf senescence in summer 2018 (P16 – S17/18) for corms that were planted at different depths (20, 25, or 30 cm). P, planting year; S, the year data were collected.
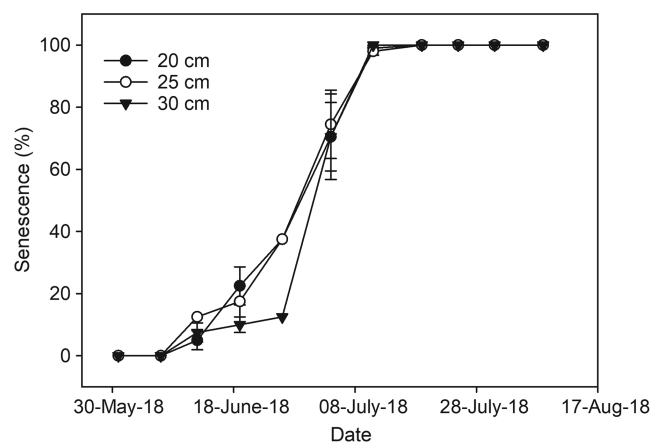
depth and 93.8% of corms that were planted at 25 cm. The effect of planting depth on shoot production was also evident in this experiment, with an increasing number of shoots produced as planting depth decreased, from 1.4 ± 0.05 shoots per corm at 25 cm up to 2.4 ± 0.1 shoots per corm at 10 cm. As corms planted at 10 or 15 cm were exposed for a longer period to 23 °C or more during summer compared with those that were planted deeper (Fig. 1d), this should have improved their flowering rate. Yet, shoot emergence the following autumn (P18 – S19/20) was much lower than in 2018 (Fig. 4) and almost none flowered, despite similar soil temperatures during the autumn of the two years. Differences among depths exhibited very similar patterns in the autumns 2018 and 2019.
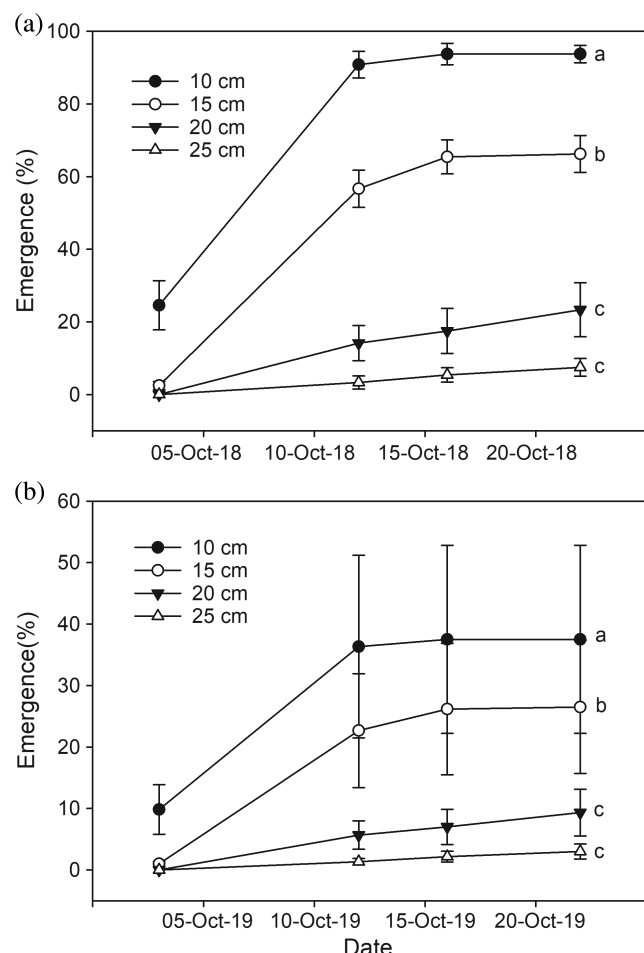
Impact of corm depth on saffron yield and corm growth

Soil temperature decreased with depth during the summer, although to a lower degree than expected. Cavins and Dole (2002) reported much greater differences in temperatures between 15 and 30 cm on raised field beds as soon as the soil warmed in late spring. We suspect that the narrow width (about 1.65 m wide) of the raised beds in the current study strongly increased heat exchange with the air, reducing the difference between depths compared with flat planting conditions. Similarly, winter temperatures only differed by about 0.5 °C, but in this case a heavy snow blanket might explain the homogeneity of temperatures across depths. Nevertheless, planting on raised beds under a humid continental climate is necessary to improve drainage, as saffron is sensitive to wet soil conditions (Kumar and Sharma 2018).

Under warmer summers such as those in Iran, which accounts for 90% of world saffron production (Jahan and Jahani 2007), shoots from deeper corms emerged a few days ahead of those that had been planted closer to the surface (Galavi et al. 2008). The authors attributed this effect to cooler temperature to which the corms are exposed in deeper soil, which can induce faster shoot emergence. Indeed, shoot emergence can take place at a range of temperatures, but cooler temperatures around 17 °C hasten shoot growth compared with either warmer or cooler temperatures (Molina et al. 2010). In the current study, the soil reached near 17 °C at about the same

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Fig. 4. Per cent shoot emergence (mean ± standard error) in autumn 2018 (P18 – S18/19) (*a*) and autumn 2019 (P18 – S19/20); (*b*) for corms planted at 10, 15, 20, and 25 cm depths; N = 6. A posteriori analyses of treatment effects following repeated measure ANOVA are presented as lowercase letters. P, planting year; S, the year data were collected.

the temperature near 0 °C throughout the winter months, even at 10 cm below the soil surface, based on the data that we collected in the 2018–2019 season. Frost risk thus appears to be low even at 10 cm and we could expect that planting saffron 10 to 15 cm deep would not put the plant at great risk of frost damage either during late autumn or early spring in locations where the snow cover establishes early and melts after air temperature has increased above 0 °C. In areas where snow cover is not constant throughout the winter, damage could occur to shallow planted corms, as suggested by Kumar et al. (2009).

Planting depth influences not only shoot emergence, as shown in the current study, but also affects many growth parameters such as leaf width, leaf and flower number, corm diameter, and contractile root production both in saffron (Galavi et al. 2008) and in bulbous species such as *Allium tuncelianum* (Kollmann) Özhatay, B.Mathew & Siraneci (Kizil and Khawar 2015) and *Narcissus* cultivars (Hagiladi et al. 1992). Another response that has been reported repeatedly in saffron is the stimulatory effects that shallow depth has on corm production (Galavi et al. 2008; Kumar et al. 2009). Production of many small corms reduces the size of the main corm (Galavi et al. 2008), thereby negatively affecting the production of saffron spice from the main corms. Small corms (<8 g) may take many years to reach the size where they can successfully flower (Mollafilabi, 2004; Douglas et al. 2014). Yet, they will compete with one another and with the larger corms for nutrients. Therefore, shallow planting might require more frequent excavation and replanting of the corms, but this disadvantage could be more than offset by a higher yearly saffron yield that is associated with earlier emergence under cool climates.

Corm depth did not influence the timing of leaf senescence, which appears to be induced by a period of high temperature in late spring, and early summer. Leaf senescence was greatly delayed in the current study compared with what has been reported in different regions where saffron is grown. In Spain, leaf senescence is completed by early June (Molina et al. 2005), whereas in India and Iran it is completed by early May (Kumar et al. 2009; Fallahi and Mahmoodi 2018). Even in cooler climates such as England, leaf senescence is well advanced by mid-May (Yadollahi et al. 2007). Therefore, it appears that the delay in senescence may be attributed to the long period under snow cover, where carbon fixation cannot take place. Despite warmer temperatures in June, the plant remains active to complete its annual life cycle. As a result, the dormancy period is shortened considerably under humid continental climates.

None of the treatments led to flower and saffron production. In the first trial, the location of the site was apparently not suitable. In the second trial, soil temperatures appeared to be too cold to allow for flower production in the first autumn (P18 – S18/19) and to induce

time at all depths, which occurred between mid-August and early September, depending upon the year (Figs. 1*a*, 1*b*, and 1*e*). The delay in emergence at greater depth is thus most likely due to the greater distance that the shoot must travel before reaching the soil surface, as have been suggested for spring geophytes (Hagiladi et al. 1992; Lubbe and Henry 2019).

In Iran, corms planted at 15 cm produced more flowers than those planted at either 10 or 20 cm (Galavi et al. 2008), while in another Iranian study corms that were planted at 20 cm produced more flowers than either at 10 or 30 cm (Vafabakhsh et al. 2010). In India, it is recommended that growers plant corms 15 cm deep (Alam 2007), whereas in Italy, corms are planted at 10 to 20 cm if they are cultivated as a perennial crop (Gresta et al. 2008*b*). The results from the current study suggest that 10 to 15 cm might also be the optimal planting depth for cultivation of saffron under a colder climate. Snow cover was most likely sufficient on this site to maintain

flower bud formation in the second growing season (P18 – S19/20).

Floral induction

No buds were visible on the corms during the spring and early summer (data not shown). We started noticing the presence of a bud by early August, and the shoot became apparent by mid-September. Flower initiation also occurs in early August in Spain and in Azerbaijan (Azizbekova and Milyaeva 1999); however, in Spain, 2 months elaspe from complete leaf senescence (early June) to floral initiation (early August) during which flower induction takes place (Molina et al. 2004), whereas this period lasted 2 to 3 weeks in the current study. Earlier work has shown that optimal soil temperatures that are required for floral induction vary between 23 and 27 °C, and corms need to remain at these temperatures for 10 to 12 weeks (Molina et al. 2005) to induce maximum flower production. After an exposure to 25 °C for 30 days, plants reached 70% of maximum flowering. We are obviously below these expected conditions, with a mean daily soil temperature of 20 to 22 °C for a duration of 6 weeks from early July to mid-August, depending on the year (Fig. 1). Soil temperature remains too cool in the summer on our site, especially at greater depths, which most likely restricted flower induction. We suspect that autumn temperatures also played a role, as corms conditioned under warmer climates, then planted at the end of the summer, also exhibited very limited flowering before the permanent snow cover was established in this site. Another potential explanation for the low flowering rate recorded the second year could be that the plant had not reached a specific vegetative stage prior to being exposed to the cold weather of the winter. Although we found no specific studies testing the vernalisation requirement in saffron, the plant is grown only under climates with a cool to cold winter.

Planting period

Leaf emergence was affected by corm planting period. In 2017 (P17 – S17/18), shoots of corms that had been planted in early September emerged later compared with those of corms that were planted in late July or August, although at the last recording date (4 November 2017) differences were no longer significant (Fig. 5a; Tables 2 and A3). The planting period also had a significant effect on the number of flowers (Fig. 5b) and the quantity of dry saffron harvested per m^2 (Fig. A1; Table 2). Despite very similar shoot emergence among the three first planting dates, corms planted on late August 2017 produced twice as many flowers and dry saffron compared with those planted at the other three periods. Across all treatments, the average percent flowering for this experiment was 29%.

In 2018 (P18 – S18/19), temperatures during the flowering period were much lower than in the previous year (Fig. 1b). The low percent emergence (Fig. 6) most likely

Fig. 5. (a) Evolution of shoot emergence and (b) cumulative number of flowers harvested in autumn 2017 for corms planted at different dates during the summer (P17 – S17/18). Mean ± standard error; N = 4. P, planting year; S, the year data were collected.
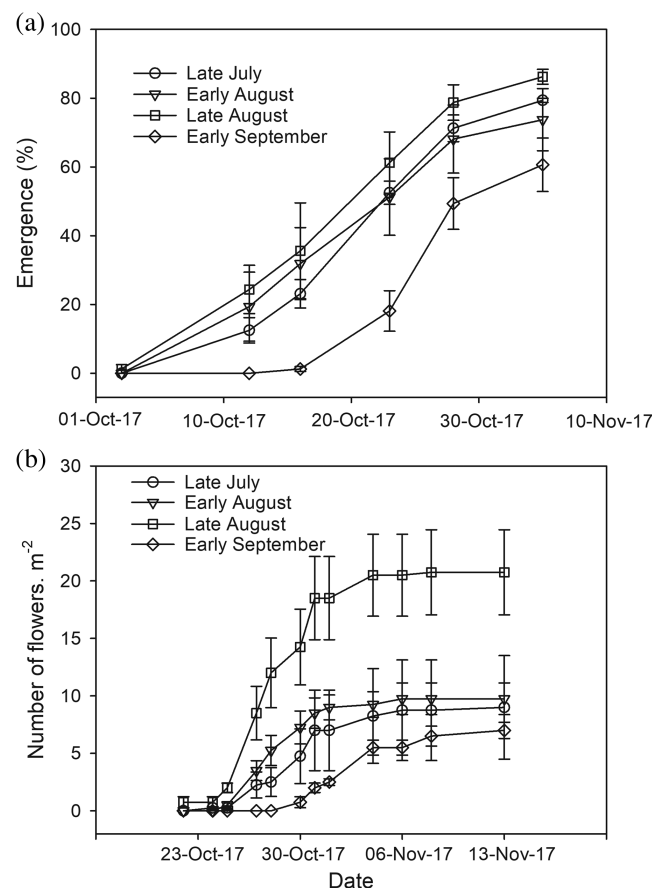
explained the absence of flowers. There were no statistical differences among planting dates on the phenology of leaf emergence in 2018 (Table A3). Yet corms planted in early September exhibited a slight delay in emergence, as was the case for those planted in 2017 (P17 – S17/18), a delay that they recovered towards the end of the autumn similarly to what was observed in 2017.

In both years, shoot emergence continued throughout the winter. By spring 2018 (P17 – S17/18), it had reached around 90% for all planting dates, whereas for corms planted in summer 2018 final emergence averaged 72% the following spring (P18 – S18/19; Fig. 7; Table 2). Shoot emergence from corms that were planted in early September 2018 was lower than for those planted at the end of August 2018, confirming that early September is too late for saffron planting in this location.

As with the planting depth experiment (Fig. 3), leaf senescence in early summer 2018 was relatively synchronous among all planting dates of 2017 (P17 – S17/18; data not shown; Table A3). Weather conditions had a preeminent effect on leaf senescence in early summer, overcoming the effects of variation in date of shoot emergence during the previous autumn. At the end of

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Table 2. Effects of planting date on saffron phenology, flowering, and corm production.

Variables	Planting date[a]		Year of planting		Date × Year	
	F	P	F	P	F	P
% Emergence in autumn[b]	0.18	0.912	3.68	0.067	1.07	0.382
% Emergence in spring[b]	5.69	**0.004**	23.7	**<0.001**	2.79	0.062
Flower number[c]	9.23	**0.002**	-	-	-	-
Dry saffron[c]	5.59	**0.012**	-	-	-	-
Shoots per corm[c]	0.31	0.817	-	-	-	-
Corm number[c]	2.62	0.099	-	-	-	-
Total corm mass[c]	0.20	0.895	-	-	-	-
Mass of the largest corm[c]	0.79	0.522	-	-	-	-
Number of corms ≥8g[c]	1.2	0.386				

Note: *F* statistics and *P* values of two-way ANOVAs are presented for autumn and spring emergence, testing the effect of planting date, year of planting and their interaction. Flower and saffron production, together with corm mass, were monitored only for corms that were planted in 2017 (P17 – S17/18). Significant results are highlighted in bold. Shoot emergence during autumn was also analysed as repeated measures. See Table A3 for details of these analyses. P, planting year; S, the year data were collected.
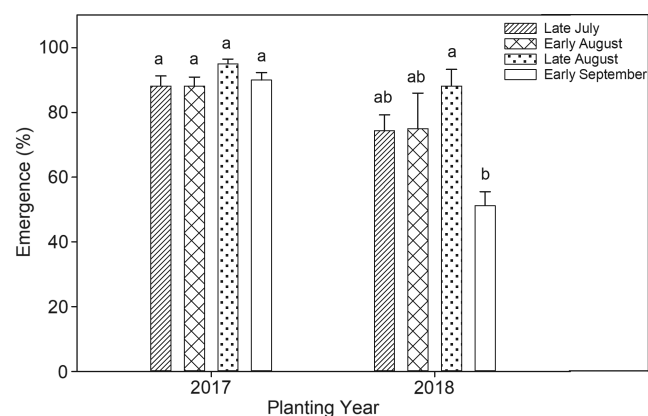
[a]Degrees of freedom for planting date, 3; for year of planting, 1; for the interaction, 3.

[b]Degrees of freedom for the error term, 24. The two years of planting correspond to P17 – S17/18 and P18 – S18/19.

[c]Degrees of freedom for the error term: 12.

Fig. 6. Evolution of shoot emergence in the autumn of 2018 (mean ± standard error) for corms planted at different dates during the summer (P18 – S18/19). N = 4. P, planting year; S, the year data were collected.

Fig. 7. Final shoot emergence (mean ± standard error) the following spring for corms planted in 2017 (P17 – S17/18) and 2018 (P18 – S18/19) according to corm planting dates; N = 4. P, planting year; S, the year data were collected.
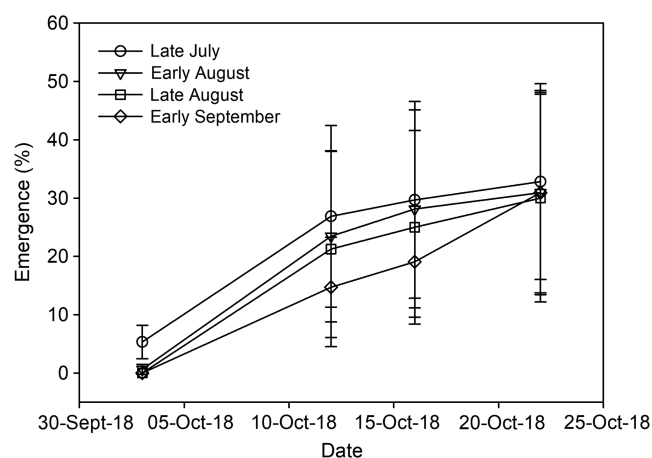
the first growing season (P17 – S17/18), corms had similar masses regardless of planting date (Table 2). On average, 3.3 corms were produced per planted corm, for a total fresh mass of 14.1 g. The largest corm produced per planted corm weighed 8.3 g and 0.57 corm of 8 g or more were produced per planted corm. Therefore, emergence later during the autumn did not influence biomass that the plant had accumulated in the corm by the end of

the season. This is in accordance with what we reported in the planting depth experiment where corms planted deeper emerged later in the autumn but produced similar size corms in the first growing season as those planted at shallower depths (P16 – S16/17).

Some studies have demonstrated that the optimum period for planting saffron corms is between June 5 and July 5, i.e., immediately after corms enter dormancy (Bayat et al. 2016). These periods are suitable for

Fig. 8. Evolution of shoot emergence (mean ± standard error) in autumn 2017 (P17 – S17/18) according to the different treatments of fertilization that were applied. F0, no fertilization; F1, fertilization. N = 4. P, planting year; S, the year data were collected.
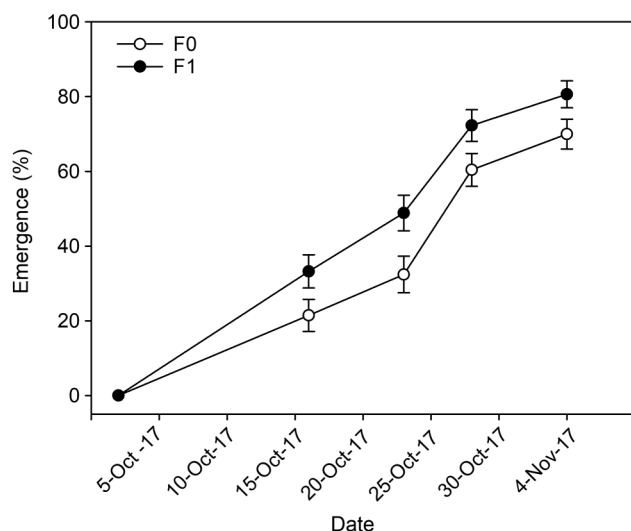
Mediterranean climates where senescence occurs early in the summer, although much later planting dates (mid-September up to October) have been reported in those climates (Gresta et al. 2008b). There are many factors suggesting that early planting is not suitable under colder climates. Temperature of exposure is more easily controlled for corms that are exposed to air than for those exposed to soil temperatures. As stated previously, soil temperature in the present location was cool and the warmest period did not last long enough to optimize floral induction. Keeping the corms at warm temperature indoors and planting them in late summer might be a better strategy to insure sufficient exposure to warm temperatures. In Spain, where corms enter dormancy in early June, excavating corms in mid-July and transferring them to 17 °C — the temperature at which shoot growth is fastest —reduced the number of flowers that were produced to 0.25 flower per corm. However, if these lifted corms are incubated at 25 °C until early September, they can produce 2.25 flowers per corm (Molina et al. 2005). Logistic reasons also favour planting later in the summer under colder climates. As corms enter dormancy in early July in Quebec (G.A. Landry, personal observation, Le ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec), they cannot be harvested, sorted out, and shipped before mid-July at the earliest, which is when we received the corms from a local producer for this experiment; whereas, those from abroad are not available before mid-August. If corms become available earlier in the season, it would nevertheless be preferable to keep them inside under warm temperature and plant them at the end of the season to insure optimal temperature exposure during the summer.
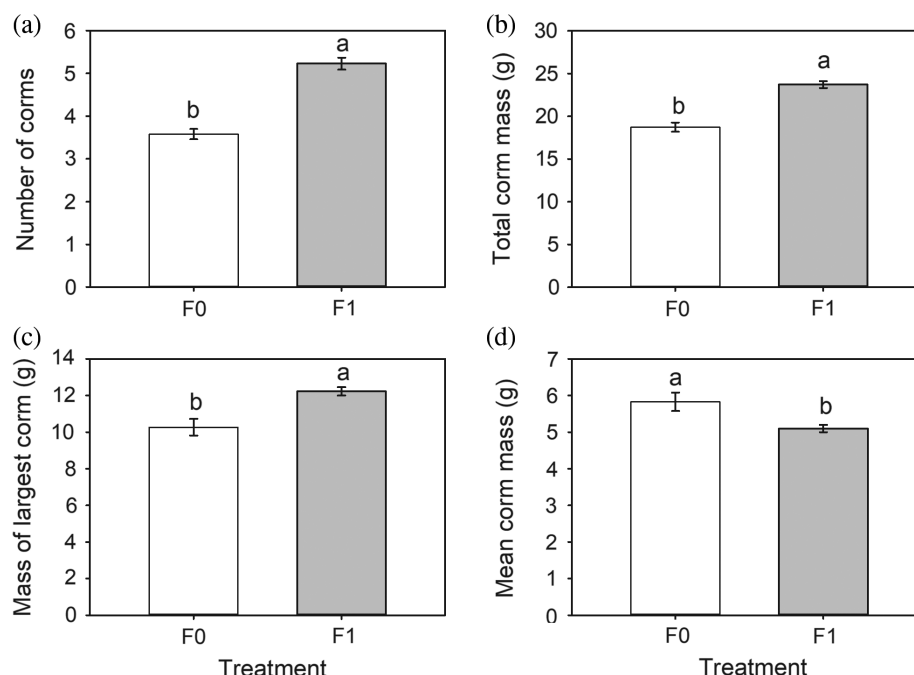
Fertilization

Fertilization stimulated an early shoot emergence during the first autumn (Fig. 8; Table A4). This positive effect of fertilization on emergence was still evident at the last recording date (4 November 2017; Table A5), whereas the addition of mycorrhizal fungi had a marginally positive effect on the emergence at the last recording date in autumn ($P = 0.064$). The average percent flowering in 2017 for this trial was 71%, with an average of 14.1 flowers per m^2. None of the treatments influenced the rate at which flowers were produced (Table A4) or the total number of flowers that were produced (Table A5). The mycorrhiza by biostimulant interaction had a significant effect on dry saffron yield (Fig. A2). Plots where mycorrhizal fungi, but no biostimulants, were added produced more dry saffron (0.113 g m^{-2}) than plots without mycorrhizal fungi and biostimulants (0.086 g m^{-2}) or plots that were treated with Turitek (around 0.076 g m^{-2}; with or without mycorrhizal fungi addition). This effect of mycorrhizal fungi might be due to the slightly higher shoot emergence that was recorded in the plots where their spores had been added. In spring 2018 (P17 – S17/18), regardless of the treatment that was applied, the final emergence was high, exceeding 90% in most plots (data not shown). Differences between fertilized and non-fertilized plots were no longer apparent (Table A5).

Fertilization stimulated the production of corms, both in terms of number and total corm mass (Figs. 9a and 9b; Table A5). Accordingly, fertilized plants produced 3.0 ± 0.1 shoots per planted corm whereas non fertilized plants produced 2.4 ± 0.1 shoots per corm. The largest corm produced per planted corm was also greater in the fertilized than in the non-fertilized plots (Fig. 9c). The only negative effect of the fertilization treatment was a reduction in the mean corm mass (Fig. 9d), most likely due to production of a higher number of very small corms. Indeed, fertilized corms produced on average 5.2 corms at the end of the first growing season which included the replacing corm, whereas non fertilized corms produced 3.6 corms. Knowing that the recommended fresh mass ensuring flower production is 8 g (Mollafilabi 2004; Douglas et al. 2014), the largest corm that is produced is sufficiently large to flower, but many small ones are not. In fertilized plots, 1.1 corms of 8 g or more were produced per planted corm in the first growing season whereas non-fertilized plants produced 0.81 corms of 8 g or more per planted corm. The addition of mycorrhizal fungi or biostimulants did not influence corm production.

As was the case for the other variables, fertilization was the only treatment that did influence nutrient accumulation in corms (Table A5). Under fertilization, absorption of nitrogen and magnesium increased, whereas absorption of phosphorus was reduced (Fig. 10). Potassium and calcium concentrations were not affected by the fertilizer treatment. As has been

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Fig. 9. Effects of fertilization on the number of corms, total corm fresh mass, the fresh mass of the largest corm and the mean corm fresh mass (mean ± standard error) that were harvested at the end of the first growing season (P17 – S17/18). N = 4. F0 = non-fertilized plants; F1 = fertilized plants. P, planting year; S, the year data were collected.

reported for the other experiments (planting depth and planting period), senescence in early summer 2018 was relatively synchronous among treatments (Table A4). By July 25, most plants had reached 100% senescence.

Fertilization also affected root mycorrhizal colonization. In unfertilized plots, the mean mycorrhizal colonization in saffron roots was 20.4% ± 10.2% and about one-fifth of the infected root segments contained arbuscules (20.6% ± 10.6%). In fertilized plots, mean mycorrhizal colonization was much reduced, i.e., at around 5.8% ± 4.9%, and only 6.0% ± 4.3% of the infected segments had arbuscules.

Fertilization hastened shoot emergence the first autumn but had no effect on flower production, contrary to what has been reported by other researchers. Both flower number and saffron yield were improved when complete foliar fertilizer (Hosseini et al. 2004; Khorramdel et al. 2015), manure (Jahan and Jahani 2007; Koocheki and Seyyedi 2015; Ghanbari et al. 2019), a combination of manure and chemical fertilizer (Amiri 2008), or vermicompost was applied (Jami et al. 2020). The effect of vermicompost and mycorrhiza addition on flower production lasted at least 2 years, and leaf area and dry mass were also stimulated. Addition of manure or other sources of organic matter is recommended in saffron production, due to the very low level of organic matter (<0.5%) in most saffron fields (Amiri 2008; Koocheki and Seyyedi 2015; Fallahi and Mahmoodi 2018; Ghanbari et al. 2019). Carbon reached 3.5% in the current study (Table A2). In richer soil containing 1% organic

matter, the addition of nitrogen as urea or NPK induced a higher gain in saffron yield than the addition of manure, whereas in a poorer soil, manure had a greater impact than chemical fertilizer (Behzad et al. 1992). Therefore, application of chemical fertilizers was justified, despite the lack of impact on flowering.

Saffron yield recorded during the first growing season as a function of fertilization or planting period (Figs. A1 and A2) are in the range expected for similar size corm and similar planting density. Our best growing treatment produced 3.5 mg of dry saffron per corm, whereas authors have reported 2 mg per corm (Koocheki et al. 2016), 2.8 mg per corm (Emam et al., 2012), 4.4 mg per corm (Iqbal et al. 2012), and up to 7 mg per planted corm (Caser et al. 2019b) during the first growing season. These results confirm that (*i*) corms were of good quality, (*ii*) that autumn conditions in 2017 allowed the plant to emerge and flower as expected before snow cover and (*iii*) that soil physical and chemical characteristics were most likely adequate as well.

Fertilization improved saffron corm growth and propagation. Corm mass production in fertilized plots was similar to what has been reported in the literature for corms of similar size at planting (i.e., around 15 g) after one growing season. Total corm mass production reported in the literature ranged from 14 g (Khorramdel et al. 2015) to 30 g (Iqbal et al. 2012) while we reported 24 g in fertilized plots (Fig. 9). Only one study reported a much higher corm production, i.e., 53 g (Siracusa et al. 2010). In general, less than 50% of the planted corms

Fig. 10. Effects of fertilization on corm N, K, P, Ca, and Mg concentrations at the end of the first growing season (P17 – S17/18); N = 4. F0 = non-fertilized plants; F1 = fertilized plants. P, planting year; S, the year data were collected; N, nitrogen; K, potassium; P, phosphorus; Ca, calcium; Mg, magnesium.
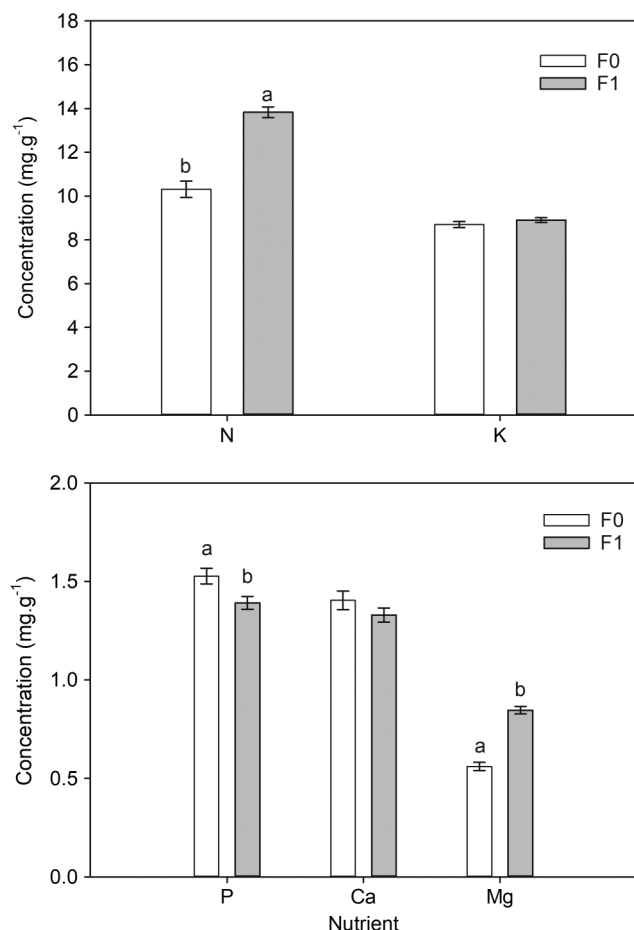
produced corm 8–10 g or more in the first year (0.35 to 0.42 large corms per planted corm; Iqbal et al. 2012; Koocheki and Seyyedi 2015, 2020). We reported 1.1 corms of 8 g or more per planted corm, similarly to Douglas et al (2014), in a study in New Zealand. The number of large corms is expected to increase during the first 4 years before exhibiting a reduction, most likely due to competition, which lead Koocheki and Seyyedi (2020) to recommend harvesting corms at the end of the fourth growing season. We can conclude that the long winter and overall temperature regime colder than the optimal during the summer and autumn does not appear to affect corm production in saffron, at least based on the first growing season.

Fertilization improved nitrogen and magnesium concentrations, indicating that the plants were able to absorb at least part of the nutrients from the fertilizer. Seyyedi et al. (2018) also reported increased nitrogen concentrations in corms that had been fertilized, but the increase was more important in large corms than in smaller daughter corms. We reported nitrogen and

phosphorus concentrations in the same ranges as in other studies (Lone et al. 2016; Seyyedi et al. 2018). The absence of flowers during the second autumn (P17, S18-19) is most likely not due to low nutrient availability, nor to the size of the corms, but rather to low temperatures during either the summer or the autumn as discussed previously.

The addition of mycorrhizal fungi spores at planting did not enhance the colonization, beyond that recorded from natural sources, which is somewhat surprising. These spores contain *R. intraradices* (formerly *Glomus intraradices*), a species that has been reported to infect saffron roots (Caser et al. 2019*a*); however, *R. intraradices* alone did not improve flower and saffron yield or corm size (Caser et al. 2019*b*). Only when *R. intraradices* was combined with *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler (formerly *Glomus mossae*) did flower number and saffron yield increase. In another study, addition of *F. mossae* also improved flower and stigma production in saffron (Ghanbari et al. 2019). The lack of an effect in the current study cannot be related to the intensity of mycorrhizal colonization, as Aimo et al. (2010) reported a mycorrhizal colonization in saffron that varies between 10% and 30%, while ours ranged from 0.07% to 55.2%, with an average of 13%. Further essays could be carried using *F. mossae*, as two studies indicated improved yield when saffron was infected with this mycorrhizal fungus. It would also be worth testing whether local sources of mycorrhizal fungi are sufficient to support a healthy mycorrhizal symbiosis with saffron.

Fertilization depressed the level of arbuscular mycorrhizal colonization in saffron roots, as has been repeatedly demonstrated in the literature (Elbon and Whalen 2015). This reduction in mycorrhizal colonization level in the fertilized plots might explain reductions in phosphorus concentrations, as was observed in fertilized plants. Indeed, positive plant growth responses to arbuscular mycorrhizal colonization are frequently attributed to increased phosphorus uptake through the symbiosis (Smith and Smith 2012). Despite the addition of phosphorus in fertilized plots, the podzolic nature of soils in the area might limit phosphorus availability. Soils in the plots had an acidic pH around 5.3 (Table A2) and are rich in iron and aluminum, which bind phosphorus very strongly and make it unavailable (Penn and Camberato 2019) (Lin, 2020 #7377; Lin, 2020 #7377). Phosphorus added through fertilization might have ended up mostly adsorbed by the soil colloids. We conclude that saffron could benefit from mycorrhizal symbioses, but we still need to determine the best technique, fertilizer composition, and fungal species to ensure arbuscular mycorrhizal colonization of saffron roots at planting.

Biostimulants had very limited impact on saffron. We reported a complex interaction between biostimulants and mycorrhizal fungi addition on saffron yield (Fig. A2), but no other variable was influenced by the presence of either biostimulant. Some studies have

reported positive effects of different biostimulants ([Deh-Arbab et al. 2019](#)), including some that contain *Pseudomonas* ([Aimo et al. 2010](#); [Ambardar and Vakhlu 2013](#)), a bacterium that is present in the EarthAlive biostimulant that we tested. As is the case for fertilizers, mycorrhizal fungi and biostimulant-specific compositions might strongly influence plant response; further tests will be required to identify the best composition that improves plant growth, mineral nutrition and protection against disease.

Conclusion

Shallow planting at 10 to 15 cm depth between late July and the end of August should be favoured to hasten shoot and flower emergence under climatic conditions where snow cover is long lasting. Corm production was in the same range as those reported in the Mediterranean basin after one season, and it can benefit from the application of a fertilizer at planting. Saffron yield per planted corm during the first growing season was also in the same range as reported previously. Yet, the strongly reduced flowering that we observed in all experiments during the second growing season is most likely due to the soil temperature being warm enough for too short a duration to stimulate floral induction in July, or to the fast cooling during autumn. We conclude that this site is beyond the northern limit for saffron flower production beyond the first growing season. Techniques that can raise soil temperature, such as the application of solar mulch during dormancy, as well as plastic tunnels applied during the snow-free period would be worth trying. Other aspects that could influence saffron yield and corm production, such as soil physicochemical properties, would also need to be assessed to obtain a more complete description of the growing conditions that optimize saffron yield and corm production under continental climates.

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Appendices

Table A1. Monthly meteorological conditions in the Baie St-Paul region, averaged over 2016 to 2019 (Historique-Météo.net 2021).

Month	Temp max °C	Temp min °C	Wind max km h^{-1}	Precipitations[a] mm	RH max %	Cloud cover %	Snow[b] mm	Sun hour h
January	−7.8	−14.2	14.6	79.0	90.2	63.9	41.3	5.5
February	−6.4	−13.8	15.2	76.2	90.6	62.5	41.3	6.9
March	−3.0	−9.4	15.3	64.9	94.3	60.3	32.0	8.3
April	3.2	−2.1	15.5	98.2	93.3	62.7	18.5	10.2
May	14.1	7.4	14.9	69.7	81.8	46.4	0.9	12.5
June	18.6	12.5	15.4	78.8	83.1	41.6	0.0	13.1
July	22.3	16.0	14.4	44.7	84.1	35.5	0.0	13.4
August	21.3	15.0	14.2	56.4	86.6	34.4	0.0	13.2
September	17.3	10.5	13.8	68.6	87.5	37.2	0.0	10.0
October	9.9	4.4	15.7	100.0	85.9	48.5	1.3	7.6
November	1.0	−3.7	16.5	91.8	87.6	58.9	22.4	6.1
December	−5.8	−11.6	14.5	59.1	90.7	55.8	24.2	5.8
Annual mean	7.0	0.9	15.0	74.0	88.0	50.6	15.2	9.4

Note: Data represent the monthly mean except for precipitations and snow where the total for the month was used to calculate the mean across the 4 years of the study. RH, relative humidity.

[a]Total annual precipitation averaged 887.4 mm of which 181.9 fell as snow.

[b]The data are reported as their rain equivalent (liquid precipitations). One cm of snow is equivalent to 1 mm of rain (10:1 ratio). This means that the area received an average of 1.82 m of snow annually.

Table A2. Results of soil analysis for the different experiments.

Parameters	Planting depth		Planting period		Fertilization	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
pH water	5.42	0.01	5.45	0.03	5.32	0.02
pH buffer	5.83	0.04	5.83	0.07	6.02	0.05
Organic matter (%)	3.34	0.36	3.68	0.24	3.57	0.21
P (kg/ha)	68.8	15.9	75.2	21.6	24.6	7.7
K (kg/ha)	113.6	7.4	421.8	187.8	402.3	110.1
Ca (kg/ha)	822.9	63.3	1307.2	337.4	753.5	197.2
Mg (kg/ha)	91.4	6.9	166.3	51.2	195.0	58.5
Al (ppm)	2002	24	1951	112	2001	115
CEC	17.4	0.3	19.1	0.5	16.2	0.5
Satur. in P	1.54	0.37	1.79	0.62	0.61	0.17
Cu (ppm)	0.29	0.06	0.66	0.06	0.11	0.01
Fe (ppm)	214.9	5.6	239.4	20.9	226.0	33.2
Mn (ppm)	10.4	0.5	29.9	14.6	38.5	14.3
Zn (ppm)	2.31	0.16	5.11	1.46	2.42	0.66
B (Mehlich ppm)	0.34	0.02	0.56	0.16	0.45	0.16
S (Mehlich ppm)	15.5	0.5	18.6	1.9	47.3	12.1
Na (ppm)	8.3	1.0	21.8	11.3	22.1	4.8
N total (g/kg)	1.49	0.09	1.74	0.12	1.43	0.17
Sand (%)	57.5	3.5	65.0	-	64.5	1.5
Loam (%)	31.0	2.0	26.0	-	21.5	2.5
Clay (%)	11.5	1.5	9.0	-	14.0	1.0

Note: P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; Al, aluminum; CEC, cation exchange capacity; Satur. in P, saturation in phosphorus; Cu, copper; Fe, iron; Mn, manganese; Zn, zinc; B, boron; S, sulfur; Na, sodium; N, nitrogen.

Table A3. Results of repeated measures ANOVA on shoot emergence and flower production during the autumn and for leaf senescence during the following spring/summer in three experiments: (a) planting depth 20 to 30 cm; (b) planting depth 10 to 25 cm; and (c) planting date.

Variable	Treatment		Date of measurement		Treatment × date of measurement	
	F	**P**	**F**	**P**	**F**	**P**
(a) Planting depth 20–25–30 cm						
Emergence in 2016 (P16 – S16/17)	56.9 (2, 21)[a]	**<0.001**	515.4 (7, 147)	**<0.001**	29.8 (14, 147)	**<0.001**
Emergence in 2017 (P16 – S17/18)	5.4 (2, 21)	**0.013**	22.9 (6, 126)	**<0.001**	4.1 (12, 126)	**0.023**
Leaf senescence in 2018 (P16 – S17/18)	0.2 (2, 12)[b]	0.830	240.3 (6, 72)	**<0.001**	0.73 (12, 72)	0.571
(b) Planting depth 10–15–20–25 cm						
Emergence in 2018 (P18 – S18/19)	61.2 (3, 20)	**<0.001**	183.3 (3, 60)	**<0.001**	15.3 (9, 60)	**<0.001**
Emergence in 2019 (P18 – S19/20)	56.3 (3, 20)	**<0.001**	137.0 (3, 60)	**<0.001**	8.5 (9, 60)	**<0.001**
(c) Planting date						
Emergence in 2017 (P17 – S17/18)	4.1 (3, 12)	**0.033**	215.5 (5, 60)	**<0.001**	2.7 (15, 60)	**0.029**
Emergence in 2018 (P18 – S18/19)	0.4 (3, 12)	0.753	49.4 (3, 36)	**<0.001**	0.8 (9, 36)	0.570
Number of flowers in 2017 (P17 – S17/18)	20.4 (3, 12)	**<0.001**	176.5 (11, 132)[c]	**<0.001**	5.6 (33, 132)	**<0.001**
Leaf senescence in 2018 (P17 – S17/18)	0.6 (3, 12)	0.609	252.1 (9, 108)	**<0.001**	0.84 (27, 108)	0.560

Note: The planting year is indicated in parentheses (e.g., P17 means planted in late summer 2017). Given that the assumption of sphericity was not met for any of the variables, we calculated the Geisser-Epsilon F and P values to estimate the effect of date of measurement and of the interaction treatment × date of measurements. Significant results are highlighted in bold.
[a]Degrees of freedom for the treatments and interaction terms followed by the degrees of freedom for the error term are presented in parentheses.
[b]Due to mortality there were a reduced number of plots that were surveyed for leaf senescence than for emergence. Only plots were there were emerged plants were surveyed.
[c]There were more frequent counting of flowers than of leaf emergence in 2017 explaining the higher degrees of freedom for flower.

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Table A4. Results of repeated measures ANOVA on shoot emergence and flower production during the autumn and leaf senescence the following spring/summer in the fertilization experiment.

Effect	Variable					
	Emergence		Number of flowers		Leaf senescence	
	F	P	F	P	F	P
Fertilization (FER)	4.8[a]	**0.034**	0.1[a]	0.788	0.1[a]	0.714
Mycorrhizal fungi (MYC)	2.5[a]	0.127	0.5[a]	0.475	1.1[a]	0.294
Biostimulants (BIO)	0.2[b]	0.847	1.5[b]	0.237	1.1[b]	0.358
FER × MYC	2.2[a]	0.151	0.1[a]	0.953	0.1[a]	0.786
FER × BIO	1.3[b]	0.297	0.9[b]	0.400	0.4[b]	0.693
MYC × BIO	0.2[b]	0.787	1.5[b]	0.244	0.3[b]	0.716
FER × MYC × BIO	3.2[b]	0.052	1.4[b]	0.272	0.9[b]	0.430
Date of measurement (DAY)	190.5[c]	**<0.001**	531.8[e]	**<0.001**	615.4[g]	**<0.001**
FER × DAY	2.0[c]	0.139	0.3[e]	0.731	0.3[g]	0.714
MYC × DAY	1.6[c]	0.201	1.0[e]	0.370	1.7[g]	0.191
BIO × DAY	0.5[d]	0.766	1.0[f]	0.390	0.7[h]	0.599
FER × MYC × DAY	1.7[c]	0.184	0.1[e]	0.929	0.5[g]	0.594
FER × BIO × DAY	0.8[d]	0.514	1.3[f]	0.270	0.8[h]	0.515
MYC × BIO × DAY	0.1[d]	0.985	1.8[f]	0.148	0.4[h]	0.784
FER × MYC × BIO × DAY	1.1[d]	0.390	1.7[f]	0.177	1.0[h]	0.394

Note: Data analyzed are from the 2017/2018 season (P17 – S17/18), as there were a very limited number of shoot and no flower that emerged in the autumn 2018 (P17 – S18/19). Given that the assumption of sphericity was not met for these variables, we calculated the Geisser-Epsilon F and P values to estimate the effect of date of measurement and of all interactions with date of measurements. Significant results are highlighted in bold.

[a-h] Degrees of freedom for the treatments and interaction terms, degrees of freedom for the error term: [a]1, 36; [b]2, 36; [c]4, 144; [d]8, 144; [e] 11, 496; [f] 22, 396; [g] 8, 288; [h] 16, 288.

Table A5. Effects of the application of fertilizers (FER), mycorrhizal fungi (MYC) and biostimulants (BIO) at planting on saffron phenology, flowering, corm production, mycorrhizal colonization, corm biomass and nutrient concentrations.

Variable	Factor[a]													
	FER		**MYC**		**BIO**		**FER × MYC**		**FER × BIO**		**MYC × BIO**		**FER × MYC × BIO**	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Emergence in autumn	6.2	**0.018**	3.67	0.064	0.11	0.897	1.28	0.267	1.21	0.312	0.19	0.828	2.08	0.141
Emergence in spring	2.08	0.159	2.67	0.112	0.21	0.811	0.71	0.404	0.21	0.809	1.32	0.281	4.41	**0.020**
Number of flowers	0.37	0.546	1.13	0.296	2.46	0.101	0.09	0.771	1.75	0.190	2.67	0.084	2.96	0.066
Saffron yield	1.02	0.319	0.43	0.518	4.39	**0.020**	0.14	0.710	2.25	0.121	3.48	**0.042**	2.18	0.129
Number of corms	89.3	**<0.001**	0.14	0.710	0.26	0.769	0.48	0.492	2.86	0.072	0.97	0.390	1.06	0.357
Shoots per corm	9.85	**0.004**	1.88	0.180	0.09	0.910	1.64	0.210	0.89	0.420	0.06	0.946	1.40	0.261
Total corm biomass	27.1	**<0.001**	1.01	0.322	1.21	0.312	0.12	0.730	0.36	0.701	0.28	0.757	0.09	0.918
Biomass largest corm	13.0	**<0.001**	0.07	0.793	1.49	0.240	2.58	0.118	1.00	0.379	0.40	0.674	0.20	0.820
Number of corms ≥ 8g	9.36	**0.004**	0.26	0.613	0.11	0.894	0.04	0.843	0.01	0.989	0.29	0.747	0.14	0.869
Mycorrhizal colonization	36.5	**<0.001**	3.19	0.083	1.72	0.196	0.08	0.780	0.39	0.683	1.78	0.185	3.69	**0.036**
Arbuscular content	33.1	**<0.001**	2.58	0.118	1.77	0.186	0.01	0.941	0.12	0.890	0.37	0.694	2.43	0.103
N	63.3	**<0.001**	0.55	0.465	0.68	0.516	0.04	0.847	1.08	0.351	1.02	0.371	0.11	0.901
P	8.33	**0.007**	0.25	0.618	1.07	0.353	0.14	0.711	0.47	0.632	0.16	0.852	1.30	0.285
K	1.13	0.295	0.47	0.498	0.20	0.818	0.09	0.761	0.79	0.461	0.24	0.787	0.83	0.447
Ca	1.66	0.207	3.65	0.065	0.50	0.611	0.16	0.688	0.13	0.880	1.26	0.297	1.90	0.166
Mg	115.0	**<0.001**	0.13	0.720	1.03	0.367	0.06	0.801	0.55	0.582	0.64	0.533	0.31	0.734

Note: F and P values of three-way ANOVAs are presented. Significant results are highlighted in bold. Planting took place in late summer 2017 and data reported are for the 2017/2018 season (P17 – S17/18). P, planting year; S, the year.

[a]Degrees of freedom for the treatments and interaction terms: FER: 1; MYC: 1; BIO: 2; FER × MYC: 1; FER × BIO: 2; MYC × BIO: 2; FER × MYC × BIO: 2; degrees of freedom for the error term: 33.

Fig. A1. Saffron yield as a function of date of planting in autumn 2017 for corms planted in late summer 2017 (P17 – S17/18). N = 4. P, planting year; S, the year data were collected.
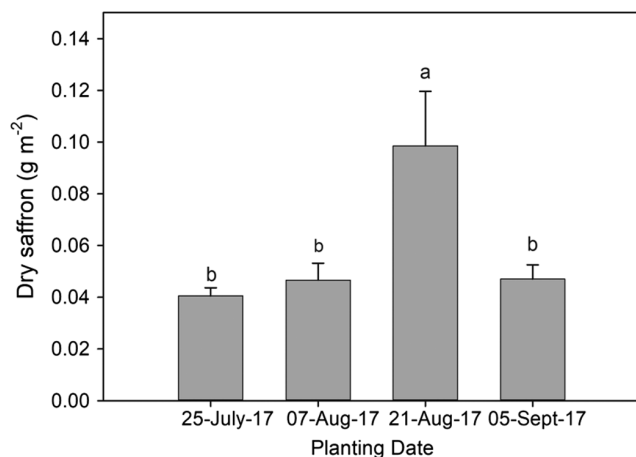
Fig. A2. Effect of the addition of mycorrhizal fungal spores and biostimulants on saffron yield in autumn 2017 for corms planted in late summer 2017 (P17 – S17/18). M0: no mycorrhizal fungal spores added, M1: mycorrhizal fungal spores added; B0: no biostimulants added; B1: Turitek added; B2: EarthAlive soil activator added. N = 4. Different letters indicate significant differences among treatment groups according to Tukey tests. See Table A5 for the ANOVA results. P, planting year; S, the year data were collected.