Original Article

RELATIONSHIPS BETWEEN FLOWER OPENING TIME, ENVIRONMENTAL CONDITIONS, COROLLA OPENING SIZE AND NECTAR PRODUCTION IN FIVE WINTER OILSEED RAPE (*BRASSICA NAPUS* L.) CULTIVARS IN CHINA

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Abstract

Oilseed rape is an economically important crop and provides a good nectar resource. In this study, the nectar secretion characteristics of five winter oilseed rape (*Brassica napus* L.) cultivars in China were examined throughout the daytime. The correlations of corolla opening size, air temperature and relative humidity with nectar production were also determined. Different daily patterns of nectar secretion were detected in flowers opening at different times of the day, and similar trends were observed among cultivars. The nectar volume and nectar sugar concentration in flowers that opened before 9:00 peaked on the first day. The nectar volume of flowers that opened at 11:00 peaked on the second day, and the nectar sugar concentration peaked on the first day. The total nectar yield of flowers opening before 9:00 (4.422-5.265 µl) was lower than that of flowers opening at 11:00 (7.982-10.646 µl). The average nectar sugar concentration of flowers opening before 9:00 (18.4-23.3%) was higher than that of flowers opening at 11:00 (15.3-17.5%). The nectar volume was positively correlated with air temperature and relative humidity, while the nectar sugar concentration was positively correlated with air temperature and negatively correlated with relative humidity. The nectar volume and nectar sugar concentration were positively correlated with corolla opening size. The glucose/fructose ratio ranged from 0.89 to 1.44. These results will contribute to a better understanding of the nectar secretion patterns of *B. napus* L. and will support further studies assessing the melliferous potential of *B. napus* L. and the temporal dynamics of plant-pollinator interactions.

Keywords: oilseed rape, nectar volume, sugar concentration, glucose, fructose, temperature

INTRODUCTION

Oilseed rape (*Brassica napus* L.) is the fourth largest oilseed crop in the world in terms of production (Food and Agriculture Organization of the United Nations, 2022), and its sown area and yield in China rank first in the world (She et al., 2015). According to its different sowing dates, oilseed rape can be classified into spring cultivars and winter cultivars (She et al., 2015). In China, approximately 90% of oilseed rape production comes from winter oilseed rape (National Bureau of Statistics of China, 2021), which is normally sown in autumn, blooms in spring, and is mainly grown in the Yangtze River basin (Sichuan, Chongqing, Yunnan, Hunan, Hubei, Jiangxi, Anhui, etc.) (Gan et al., 2022). Oilseed rape, in addition to its role in the production of cooking oil, feed and biofuel, also produces abundant pollen and nectar that are attractive to many insect pollinators that, in turn, help improve the seed quality, grain weight and oil content of oilseed rape (Williams, 1978; Morandin & Winston, 2006; Rosa et al., 2011; Bommarco et al., 2012). Field experiments have indicated that honeybees are the main pollinators of winter oilseed rape (Kołtowski, 2002; Rosa et al., 2011). With approximately 7.0 million ha of cultivated oilseed rape area in China (National Bureau of Statistics of China, 2022), the crop is one of the most important nectar and pollen plant resources for honey, pollen and royal jelly production (Wen et al., 2017).

Researchers have observed and analysed the nectary structure and nectar production in different oilseed rape cultivars (Davis et al., 1996). Nectar is synthesized and secreted by the nectary, a special organ of entomophilous plants. Each flower bears two pairs of nectaries, of which two are inner nectaries and two are outer nectaries. The nectar secretion of oilseed rape flowers changes gradually over the course of the transition from bud dehiscence to gradual opening, blooming and then closing (Chabert et al., 2018). Nectar flow begins at the stage in which the yellow corolla is fully expanded. Initially, only the inner nectaries secrete nectar, and then the outer nectaries begin secretion when the four long stamens curve slightly towards the flower centre (Eisikowitch, 1981). When stigma receptivity decreases and oilseed rape flowers begin to senesce, nectar secretion capacity decreases (Chabert et al., 2018).

Nectar production, synthesis and secretion are linked to genetics and the environment (Liu & Thornburg, 2012; Bodó et al., 2021). Different cultivars of oilseed rape produce different nectar volumes, with various sugar contents and glucose/fructose ratios (G/F ratios) (Picard-Nizou et al., 1995; Pierre et al., 1999; Carruthers et al., 2017). Oilseed rape nectar is very low in sucrose and rich in glucose. Only hexose has been detected in this nectar, and most oilseed rape cultivars produced nectar with G/F ratios exceeding 0.95 (Kevan et al., 1991; Farkas, 2008). The external environment also has a great influence on nectar secretion (Enkegaard et al., 2016). For example, nectar quantity and concentration have been found to be correlated with air temperature and relative humidity (Mohr & Jay, 1990). The amount of nectar sugar per flower depends on the relative rates of secretion and reabsorption, and the sugar concentration of nectar is related to the ambient humidity (Burquez & Corbet, 1991). When the moisture content in the air is too low, the water in the nectar easily evaporates, leading to an increase in nectar sugar concentration (Corbet et al., 1979; Bertsch, 1983; Nicolson, 1998). In recent years, the phenomenon of global warming has gradually intensified, and the impact of rising air temperatures on nectar secretion has received widespread attention (Hassan et al., 2017; Takkis et al., 2018). Moderate increases in air temperature are conducive to the secretion of nectar, but extreme increases in air temperature can have a negative impact on nectar secretion (Petanidou & Smets, 1996; Takkis et al., 2015).

To better understand the nectar secretion patterns and for further assessing of the melliferous potential of oilseed rape cultivars, we carried out the present study under field conditions. For five winter oilseed rape cultivars, first, we assessed nectar secretion at the flower bud stage. Then, we analysed and compared the nectar secretion dynamics in flowers opening at different times throughout the day. The nectar sugar composition was also determined and

compared among cultivars. Finally, the correlations of corolla opening size, air temperature and relative humidity with nectar volume and nectar sugar concentration were determined. The results will provide helpful information for a better understanding of the temporal dynamics of plant-pollinator interactions.

MATERIAL AND METHODS

Study site and plant material

This study was carried out in Luoping County, Yunnan Province (24°53' N; 104°20' E), from February 25th to March 12th, 2021. The local average annual air temperature is 15.1°C, and the precipitation is approximately 1700 mm. The study site was a 0.35-ha field sown with five winter oilseed rape (*B. napus* L.) cultivars ('Aiyouwang', 'Ningde 21', 'Dehuiyou', 'Hengheyou 998' and 'Huayou'), with each cultivar being cultivated in an approximately 0.07-ha field. These cultivars were sown in October 2020. Compound fertilizer (N:P:K=15:15:15) was applied during sowing. Chemical herbicides of glyphosate were used for weed control, imidacloprid insecticide was applied to remove aphids and lambda-cyhalothrin insecticide was applied to remove *Meligethes aeneus* F. in the middle and late flowering periods.

Nectar collection and nectar sugar concentration

During the flowering period, we randomly marked 6-12 flower buds from 3-4 plants of each cultivar every three days, and we repeated this procedure five times. For each cultivar, a total of 42 flower buds were marked and observed. Insect proof meshes were used to bag these buds to prevent visiting insects from collecting nectar and affecting the nectar secretion quantity. The flower buds were bagged at 18:00 in the evening or at 9:00 in the morning. To assess the diurnal nectar secretion dynamics, we collected nectar from individual bagged flowers at 2-h intervals during the daytime (from 9 a.m. to 5 p.m.) and the flowers were re-bagged between nectar extractions. The flower buds bagged at 18:00 were monitored from 9:00 the next day, and the flower buds bagged at 9:00 were monitored from 11:00 on the same day until they began to wilt.

The nectar was collected using a microcapillary tube with a length of 100 mm and an inner diameter of 0.3 mm (Mesquida et al., 1988). Nectar volume, expressed in microliters, was calculated through the multiplication of the basal area and the length of the glass micropipette filled with nectar. The concentration of total sugar in the nectar (percentage of mass sugar/total mass solution) was measured with the use of a hand-held refractometer (0.0-53.0%, wt./total wt.; MASTER-53α, Atago, Japan). In addition, we randomly collected 10-15 flower buds from at least six different plants of each cultivar and assessed the nectar secretion volume andthe nectar sugar concentration.

We determined the nectar volume and nectar sugar concentration per flower at each sampling time point throughout the flower lifetime. The daily nectar volume per flower was the sum of the nectar volume collected on that day, and the daily nectar sugar concentration was the average of several nectar sugar concentrations measured on that day. The total nectar yield per flower was the total quantity of nectar collected throughout the flower lifetime, and the average nectar sugar concentration was the average of the nectar sugar concentrations measured during the flower lifetime.

Corolla opening size and meteorological data

From when flowers opened until they began to wither, we also measured the corolla opening size (the maximum distance between the edges of two opposite petals) (N=42 for each cultivar) at each time point when the nectar was collected. During the experiment, the real-time air temperature and relative humidity from 9:00 to 18:00 were also recorded with the use of a mini data logger (RC-4HC, Elitech, China).

Nectar sugar composition

During the flower blooming period, nectar was collected from 1-50 fully open flowers late in the morning to fill the clean glass

capillary micropipettes to obtain approximately 3.533 µL of nectar per sample. The collected nectar was expelled from the pipettes onto filter paper and allowed to air dry, following the protocol of Kevan et al. (1991) and Davis et al. (1996). Each piece of dried filter paper was placed in a 5 mL centrifuge tube and taken back to the laboratory. Six duplicate biological samples were collected from at least six different plants per cultivar.

The sugar composition of the nectar was studied according to the methods of Sun et al. (2017). The nectar spot was cut from the filter paper and eluted with 0.4 mL distilled water. The sugar content was determined by high-performance liquid chromatography (HPLC) conducted on a Cosmosil Sugar column (Sugar D, 5 µm, 250 mm×4.6 mm i.d.) with a differential refractive index detector (Waters 2695). We injected 15 µL of each sample into the column. The temperature of the column was maintained at 40°C, and the solvent was an acetonitrile:water system (75:25 by volume) at a flow rate of 1 mL/min. Standard solutions of glucose, fructose and sucrose were analysed to identify the specific sugars and calculate their relative amounts in each sample.

Statistical analysis

The Shapiro-Wilk normality test and Levene's test were used to test the normality and homoscedasticity of the data. The differences in nectar volume, nectar sugar concentration and sugar composition were analysed with the use of one-way ANOVA or an independent samples t test when the data met the normality and homoscedasticity requirements. The nonparametric Kruskal-Wallis test or Mann-Whitney U test was used when the data did not meet the parametric assumptions. Generalized linear mixed models were used to analyse the interaction between cultivar and time of day for nectar volume and nectar sugar concentration, the interaction between cultivar and flowering day for daily nectar volume and daily nectar sugar concentration, and the interaction between cultivar and flowering opening time for the total nectar yield and the average nectar sugar concentration. The correlations of corolla opening size with nectar volume and nectar sugar concentration were analysed with the use of the Pearson correlation analysis. Stepwise regression analysis was performed to explore the relationship between nectar secretion and weather condition parameters. The multicollinearity was detected through variance inflation factor (VIF) values. Two-tailed probabilities for Fisher's exact test were used to compare the percentage of flower buds that secreted nectar among the cultivars. All analyses were performed with Statistical Product and Service Solutions (SPSS) v27.0 (Morgan et al., 2004). Data are presented as means±standard errors.

RESULTS

Nectar secretion at the flower bud stage

The analyses showed that 30-75% of the flower buds secreted nectar. No significant differences were detected in the percentage of nectar-secreting flower buds among the five cultivars (Fisher's exact test, p=0.162) (Tab. 1). The nectar volume secreted per bud varied between 0.247±0.020 µL and 0.724±0.355 µL, and there was no significant difference among cultivars (Kruskal-Wallis test: H=4.717, df=4, p=0.318). The nectar sugar concentration of 'Dehuiyou' (7.7±2.3%) was significantly lower than that of 'Hengheyou 998' (15.5±1.5%) (p=0.022). There were no significant differences in the nectar sugar concentration among the other four cultivars (p>0.05) (Tab. 1).

Variation in corolla opening size, nectar volume and nectar sugar concentration during the lifetime of a flower

From flower opening to withering, the corolla opening size of the five cultivars ranged from 0-31 mm (Fig. 1A & 1B). The flower buds that were bagged at 18:00 opened before 9:00 on the next morning and closed at approximately 17:00 on the second day after bagging. On the first flowering day, the corolla opening size gradually increased from 15-21 mm at 9:00 and reached the maximum mean value of 26 mm during the period

Table 1.

Nectar secretion volume (µL) and nectar sugar concentration (%, wt./total wt.) in the five oilseed rape cultivars at the bud stage

Cultivar	Number of flower buds	Percentage of buds secreting nectar (%)	Nectar volume (µL)	Nectar sugar concentration (%)
Aiyouwang	12	33.3[a]	0.724±0.355[a]	11.8±0.4[ab]
Ningde 21	12	75.0[a]	0.330±0.169[a]	10.3±0.9[ab]
Dehuiyou	11	63.6[a]	0.252±0.092[a]	7.7±2.3[b]
Hengheyou 998	10	30.0[a]	0.247±0.020[a]	15.5±1.5[a]
Huayou	15	53.3[a]	0.353±0.115[a]	10.5±0.4[ab]

Different letters in columns indicate significant differences ($p<0.05$).

from 13:00-17:00. By the second day, the corolla opening size had dramatically decreased, and the petals began to close and wither until 17:00 (Fig. 1A). For the flower buds that were bagged at 9:00 and that opened at approximately 11:00, the lifespan of a flower was approximately 50 hours. The corolla opening size showed an increasing trend on the first and second flowering days and reached a maximum mean value ranging from 24-26 mm from 13:00-15:00 on the second day. The petals began to close and wither before 13:00 on the third day (Fig. 1B).

Fluctuations in the nectar volume and the nectar sugar concentration were observed over the course of flowering (Fig. 1C-1F). For the flowers that were bagged at 18:00, there was a significant interaction between cultivar and time of day for nectar volume (Wald χ^2=192.568, df=49, $p<0.001$) and nectar sugar concentration (Wald χ^2=1731.946, df=45, $p<0.001$). The nectar volume showed a decreasing trend (Fig. 1C) and peaked at 9:00 ('Ningde 21': 1.133±0.351 µL; 'Hengheyou 998': 1.062±0.334 µL) or 13:00 ('Aiyouwang': 0.709±0.112 µL) on the first flowering day or at 9:00 ('Dehuiyou': 0.997±0.206 µL; 'Huayou': 0.930±0.272 µL) on the second day. The flowers wilted, and the nectar volume was lowest at 17:00 on the second day (Fig. 1C). The nectar sugar concentration increased between 9:00 and 13:00 on the first day and then decreased to a much lower value on the second day (Fig. 1E). The highest value (49.9%-54.1%) was observed between 13:00 and 15:00 on the first day. The lowest value was detected at 9:00 on the first day for 'Aiyouwang' (4.3±0.9%) and 'Huayou' (5.3±1.5%) and between 9:00 and 15:00 on the second flowering day for the other three cultivars (Fig. 1E).

Also for the flowers that opened at 11:00, significant interactions between cultivar and time of day were found for the nectar secretion volume (Wald χ^2=853.836, df=59, $p<0.001$) and the nectar sugar concentration (Wald χ^2=1229.683, df=59, $p<0.001$). Nectar volume on the second day was highest at 11:00 for 'Hengheyou 998' (1.491±0.161 µL) or at 15:00 for the other four cultivars (1.422±0.179-2.166±0.175 µL) (Fig. 1D). The lowest nectar volume was detected at 11:00 on the first day for 'Dehuiyou' and 'Huayou' (0.120±0.051 µL and 0.127±0.032 µL, respectively). For the other three cultivars, the lowest values were found when the flowers were about to close and wilt during the period from 11:00-13:00 on the third day, range: 0.126±0.051-0.147±0.066 µL (Fig. 1D). The nectar sugar concentration showed a decreasing trend on the first day. Then, on the second day, the nectar sugar concentration first increased at the 9:00 and 15:00 time points and then decreased. On the third day, the nectar sugar concentration slightly increased. The nectar sugar concentration peaked (38.5±5.5%-41.5±5.8%) between 11:00 and 15:00 on the first day and reached the minimum value at 9:00 on the second day for 'Dehuiyou' (4.6±0.3%) and 'Huayou' (3.1±0.2%) or on the third day for the other three cultivars (Fig. 1F).

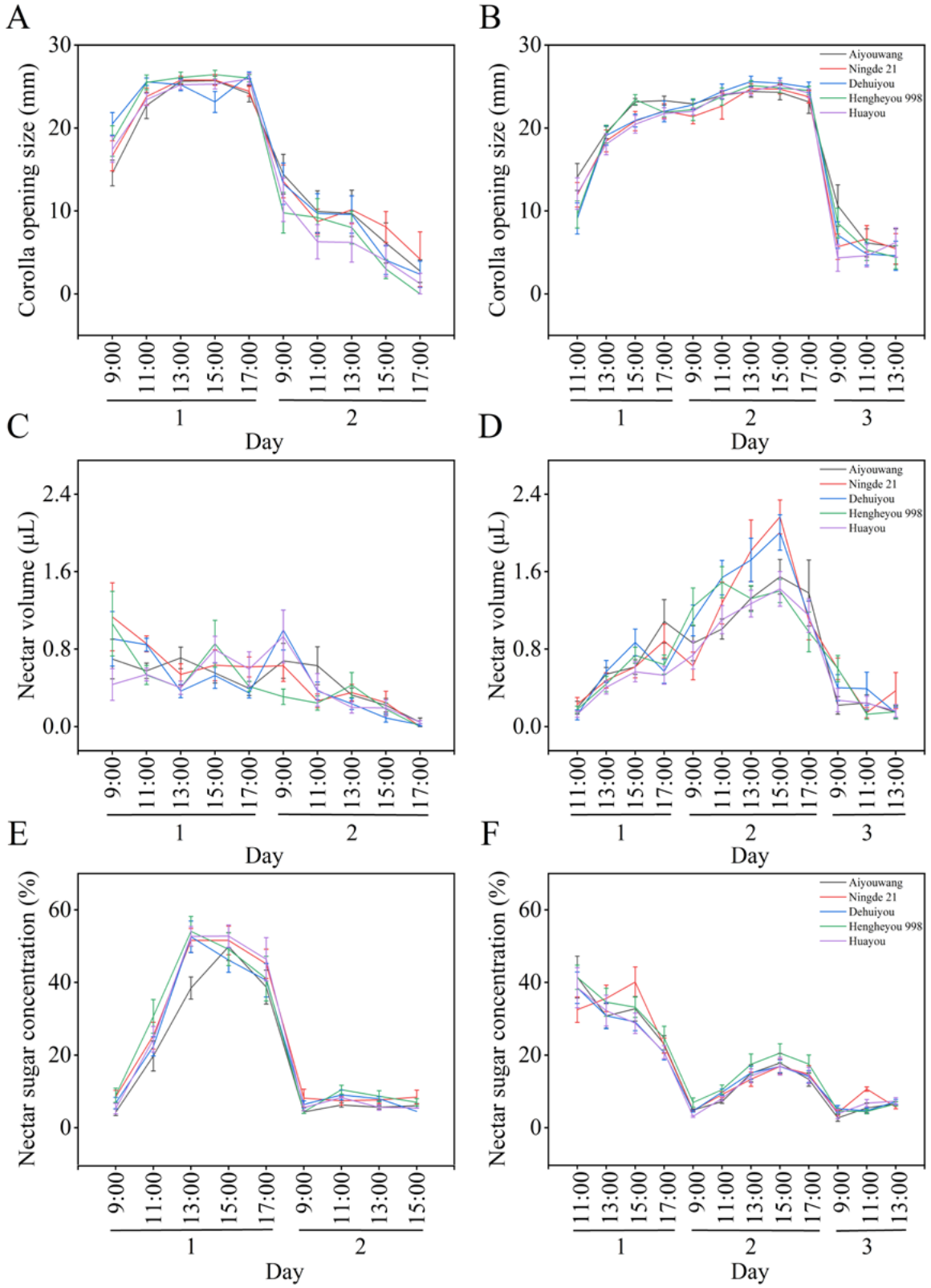


Fig. 1. Corolla opening size, nectar volume and nectar sugar concentration throughout the lifetime of a flower in the five oilseed rape cultivars. A, C & E: flower buds that were bagged at 18:00 and that opened before 9:00 the next morning, B, D & F: flower buds that were bagged at 9:00 and that opened at 11:00.
Data are presented as the means±standard errors.

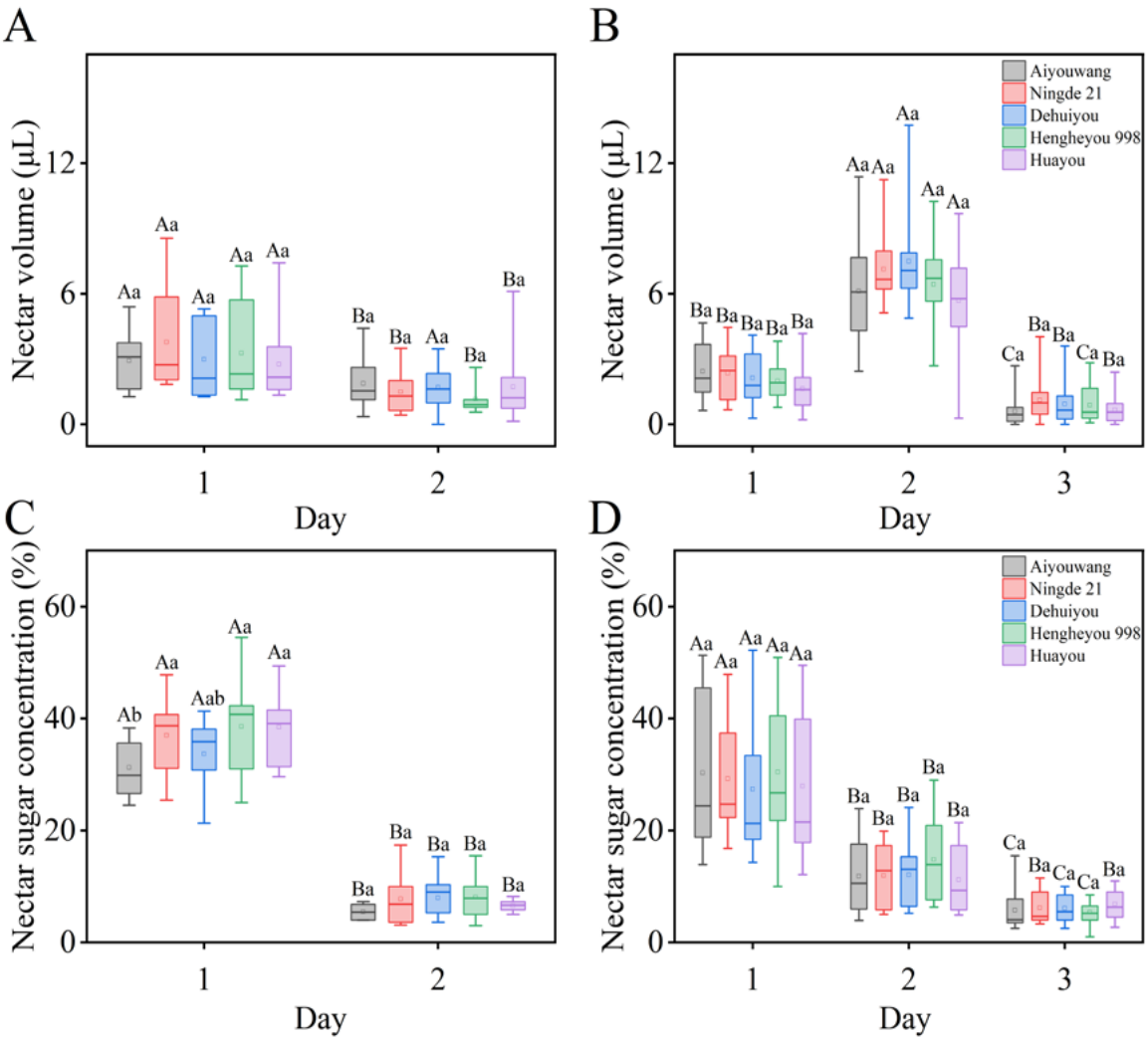


Fig. 2. Daily nectar volume and daily nectar sugar concentration in the five oilseed rape cultivars on different flowering days. A & C: flower buds that were bagged at 18:00 and opened before 9:00 the next morning, B & D: flower buds that were bagged at 9:00 and opened at 11:00. Different lowercase letters indicate significant differences among cultivars on the same flowering day (Kruskal-Wallis test or one-way ANOVA, $p<0.05$). Different capital letters indicate significant differences among different flowering days for the same cultivar (Mann-Whitney U test, independent samples t test or Kruskal-Wallis test; $p<0.05$).

Daily nectar production and the total nectar yield of flowers

The flowers that were bagged at 18:00 secreted nectar for two days (Fig. 1C, Fig. 2A & 2C). There were significant interactions between cultivar and flowering day for daily nectar volume (Wald $\chi^2=44.42$, df=9, $p<0.001$) and daily nectar sugar concentration (Wald $\chi^2=1060.971$, df=9, $p<0.001$). The daily nectar volume and the daily nectar sugar concentration of 'Aiyouwang', 'Ningde 21', 'Hengheyou 998' and 'Huayou' were significantly higher on the first day ($p<0.05$). On both the first and second days, no significant difference was found in the daily nectar volume among cultivars ($p>0.05$) (Fig. 2A). On the first day, the daily nectar sugar concentration of 'Aiyouwang' (31.3±1.3%) was significantly lower than that of 'Ningde 21' (37.0±1.6%), 'Hengheyou 998' (38.6±2.6%) and 'Huayou' (38.4±1.9%) ($p<0.05$) (Fig. 2C).

The flowers that opened at 11:00 secreted nectar for three days (Fig. 1D, Fig. 2B & 2D). Significant interactions between cultivar and flowering day for daily nectar volume (Wald $\chi^2=958.162$, df=14,

p<0.001) and daily nectar sugar concentration (Wald χ^2=411.229, df=14, p<0.001) were detected. The daily nectar secretion volume was significantly higher on the second day for all cultivars (p<0.05) (Fig. 2B). On every flowering day, no significant differences were detected in the daily nectar volume or the daily nectar sugar concentration among cultivars (p>0.05). The daily nectar sugar concentration was significantly higher on the first day for all five cultivars (p<0.05) (Fig. 2D).

There was a significant interaction between cultivar and flower opening time (Wald χ^2=172.346, df=9, p<0.001) for the total nectar yield. For all five cultivars, the total nectar yield of flowers opening before 9:00 (4.422±0.716-5.265±0.804 µL) was significantly lower than that of flowers opening at 11:00 (7.982±0.580-10.646±0.434 µL) (p<0.01) (Fig. 3A). For the flowers opening before 9:00, no significant differences were detected in the total nectar yield among cultivars (Kruskal-Wallis test: H=2.318, df=4, p=0.678). For the flowers opening at 11:00, the total nectar yield of 'Huayou' (7.982±0.580 µL) was significantly lower than that of 'Ningde 21' (10.646±0.434 µL) and 'Dehuiyou' (10.555±0.665 µL) (p<0.01) (Fig. 3A).

For the average nectar sugar concentration, a significant interaction between cultivar and flower opening time (Wald χ^2=91.775, df=9, p<0.001) was detected. Among the cultivars, no significant differences in the average nectar sugar concentration were detected in the flowers that neither opened before 9:00 ($F_{4, 65}$=2.187, p=0.080) nor opened at 11:00 ($F_{4, 90}$=1.447, p=0.225). However, for 'Ningde 21', 'Dehuiyou', 'Hengheyou 998' and 'Huayou', the average nectar sugar concentration of flowers opening before 9:00 was significantly higher than that of flowers opening at 11:00 (p<0.01) (Fig. 3B).

Correlation between corolla opening size and nectar secretion

When the flowers were in bloom, the corolla opening size most frequently ranged between 21 and 27 mm. The nectar volume (r: 0.296-0.436; p<0.001) and the nectar sugar concentration (r: 0.193-0.381; p<0.001) were positively correlated with the corolla opening size in all five cultivars (Fig. 4).

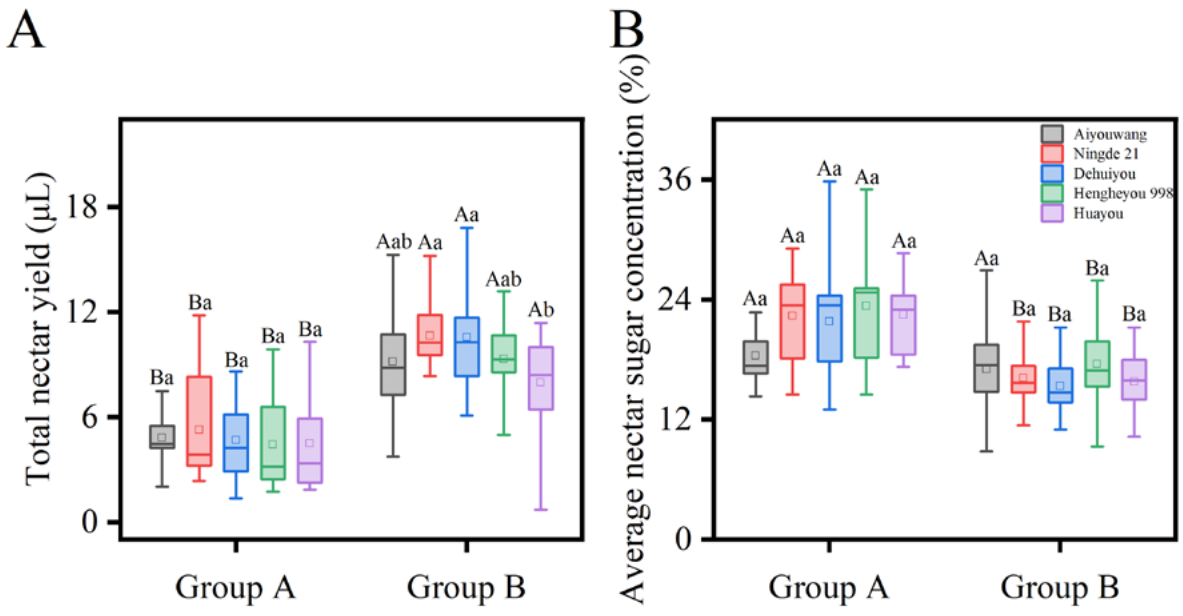


Fig. 3. Total nectar yield and average nectar sugar concentration in the five oilseed rape cultivars. Group A: flower buds that were bagged at 18:00 and opened before 9:00 the next morning, Group B: flower buds that were bagged at 9:00 and opened at 11:00. Different lowercase letters indicate significant differences among cultivars for the flowers opening at the same time (Kruskal-Wallis test or one-way ANOVA, p<0.05). Different capital letters indicate significant differences between flowers of the same cultivar that opened at different times (Mann-Whitney U test or independent samples t test; p<0.05).

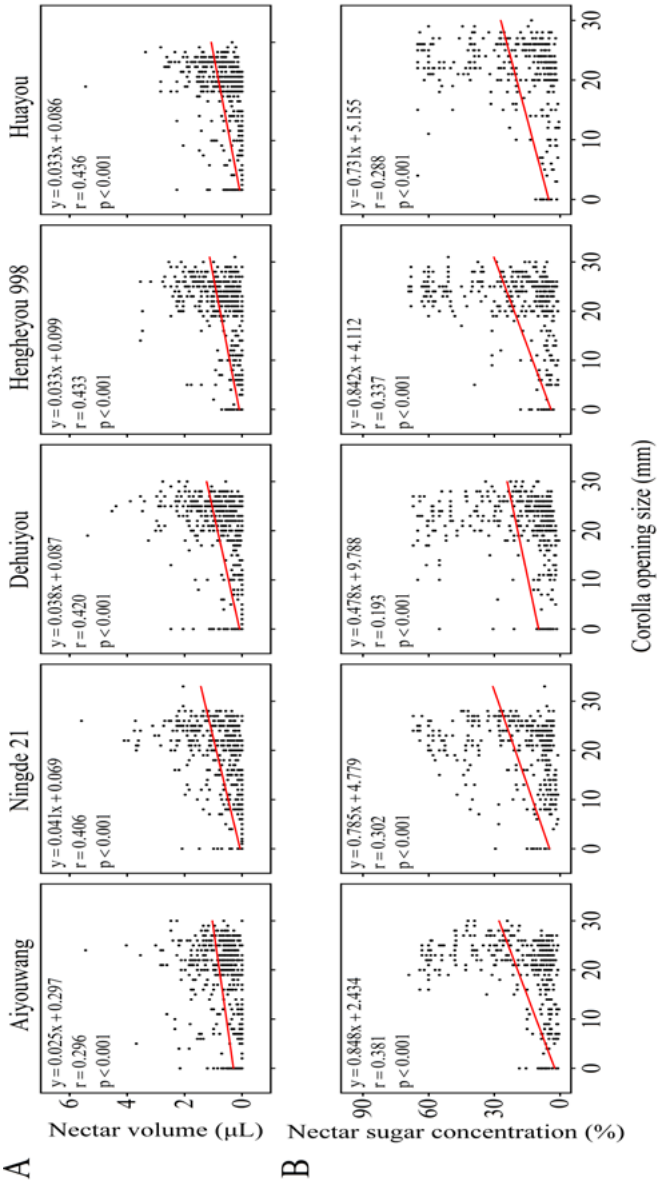


Fig. 4. The relationships of nectar volume (A) and nectar sugar concentration (B) with corolla opening size.

Nectar sugar composition

For all five cultivars, none of the samples contained detectable amounts of sucrose. Nectar carbohydrates consisted almost exclusively of glucose and fructose. There were no significant differences in the contents of fructose (Kruskal-Wallis test: H=2.981, df=4, p=0.561) and glucose ($F_{4, 25}$=1.505, p=0.231) among the five oilseed rape cultivars (Tab. 2). The nectar of 'Dehuiyou' contained less glucose (12.69±2.26 g/100 mL) than the nectar of the other cultivars. The quantities of glucose slightly exceeded those of fructose in 'Ningde 21' and 'Hengheyou 998'. The average G/F ratio ranged from 0.89±0.09 to 1.44±0.38, and no significant differences were detected in the G/F ratios among cultivars ($F_{4, 25}$=0.902, p=0.478) (Tab. 2).

Correlation of air temperature and relative humidity with nectar secretion

Within the air temperature range of 8.6-26.4°C and relative humidity range of 38.3-97.7%, the nectar sugar concentration was significantly positively correlated with air temperature and negatively correlated with relative humidity for all five cultivars (adjusted R^2: 0.433-0.571; p<0.05). For 'Dehuiyou' (adjusted R^2=0.025) and 'Hengheyou 998' (adjusted R^2=0.027), the nectar volume had a significant positive correlation with air temperature and relative humidity (p<0.05). While the nectar volume of 'Ningde 21' had no significant correlation with air temperature and relative humidity (adjusted R^2=0.010), the nectar volume of 'Aiyouwang' (adjusted R^2=0.016) and 'Huayou' (adjusted R^2=0.017) was significantly positively correlated with relative humidity (p<0.01) (Tab. 3).

Table 2.

The nectar sugar composition (g/100 mL) in the five oilseed rape cultivars

Cultivar	Glucose	Fructose	G/F
Aiyouwang	17.22±2.53	20.08±3.03	0.89±0.09
Ningde 21	20.30±2.81	18.40±2.84	1.30±0.29
Dehuiyou	12.69±2.26	15.20±2.81	0.96±0.17
Hengheyou 998	17.56±1.31	16.52±3.69	1.44±0.38
Huayou	14.66±2.66	15.11±2.81	1.04±0.19

Table 3.
Linear regression of environmental parameters correlated with oilseed rape flower nectar production

	Cultivar	Coefficients	Estimate	Std. error	t value	p value	Model fit (adjusted R^2)
Nectar volume	**Aiyouwang**	% Relative humidity	0.007	0.002	2.908	0.004	0.016
	Dehuiyou	% Relative humidity	0.014	0.004	3.753	<0.001	0.025
		Air temperature	0.036	0.013	2.774	0.006	
	Hengheyou 998	% Relative humidity	0.012	0.003	3.762	<0.001	0.027
		Air temperature	0.025	0.011	2.235	0.026	
	Huayou	% Relative humidity	0.007	0.002	2.993	0.003	0.017
Nectar sugar concentration	**Aiyouwang**	% Relative humidity	-0.491	0.069	-7.113	<0.001	0.433
		Air temperature	1.229	0.229	5.380	<0.001	
	Ningde 21	% Relative humidity	-0.517	0.062	-8.364	<0.001	0.571
		Air temperature	1.476	0.216	6.846	<0.001	
	Dehuiyou	% Relative humidity	-0.742	0.067	-11.132	<0.001	0.496
		Air temperature	0.462	0.221	2.084	0.038	
	Hengheyou 998	% Relative humidity	-0.633	0.068	-9.363	<0.001	0.536
		Air temperature	1.226	0.230	5.327	<0.001	
	Huayou	% Relative humidity	-0.670	0.069	-9.739	<0.001	0.524
		Air temperature	0.948	0.232	4.084	<0.001	

Note: In the table above, only the cultivars whose nectar volume and nectar sugar concentration were significantly correlated with air temperature or relative humidity are listed.

DISCUSSION

Nectar secretion patterns are regarded as an adaptive feature of insect-pollinated flowers and are an important aspect of the reproductive biology of plants (Pyke, 2016). Secretion of more nectar helps entomophilous plants to become more attractive to pollinators (Shu et al., 2019). Previous studies have examined the nectary size and the nectar carbohydrate production and composition of *B. napus* (Davis et al., 1994), and have also investigated the quantitative and

qualitative variability of nectar secretion in winter oilseed rape (Pierre et al., 1999), revealing the floral nectar secretion difference between the outer and inner nectary glands or among different genotypes (Eisikowitch, 1981). The variation in nectar secretion and nectar sugar concentration in oilseed rape flowers at different time points and different flowering stages has also been studied (Mohr & Jay, 1990; Farkas, 2008). The number of newly blooming oilseed rape flowers varies at different times of day, with 50-60% flower buds of *B. napus* L. open between 8:00-11:00 in the morning (An et al., 1982). However, whether there is a difference in the nectar secretion of flowers that open at different times of day has not been confirmed experimentally. In this study, we measured the corolla opening size, nectar volume and sugar concentration of the nectar in flowers opening before 9:00 and at 11:00 in the morning. All the measured parameters changed in different patterns over the course of flowering days. However, the investigation was only carried out from 9:00 in the morning and during the daytime; thus, more detailed studies are needed to reveal a more comprehensive comparison of flower opening and the nectar production dynamics of flowers opening at different times of day.

Farkas (2008) studied nectar production and sugar composition in three oilseed rape cultivars in Hungary and reported that in some cases, nectar was found in blossoms between the bud and young flower growth stages. Chabert et al. (2018) also reported that the secretion of nectar in oilseed rape flowers started at the bud stage in a male-fertile hybrid F1 line and in its male-sterile parental line. Although the flower bud samples were limited, it could be confirmed in the present study that nectar secretion in oilseed rape flowers started at the bud stage. With a certain amount of nectar already available to attract pollinators, the onset of nectar secretion at the bud stage should perhaps promote cross-pollination at flower anthesis (Chabert et al., 2018). However, we still do not know precisely the cellular and molecular mechanisms that trigger the beginning and end of nectar secretion. The inconsistency in nectar secretion status may be due to the differences among individual plants or to different positions of the buds as has been shown for the variation in nectar production in *Gymnaconitum gymnandrum* (Lu et al., 2015).

The nectar volume and nectar sugar concentration produced by *Brassica rapa* and *B. napus* have been examined throughout the day, and the flowers were found to produce more nectar, with a lower sugar concentration, in the morning (Mohr & Jay, 1990). Our results for the flowers bagged at 18:00 that opened before 9:00 was consistent with their findings, while different results were found for the flowers opening at 11:00. The nectar sugar concentration peaked between 13:00 and 15:00 whether the flowers opened before 9:00 or at 11:00, which is consistent with the results of previous studies (Mohr & Jay, 1990). This may be due to the high temperature and low humidity of the external environment during this period. In the present study, different tendencies for the nectar amount and nectar sugar concentration were detected during the flowering period between flowers that opened at different times. Therefore, we should consider the nectar secretion volume difference among flowers opening at different times to evaluate their melliferous potential and attractiveness to pollinators.

The investigation was carried out during the daytime, and the potential nectar yield that could be used by pollinators secreted by the flowers opening at different times was compared. For the flowers that were bagged at 18:00 and that opened before 9:00 the next morning, nectar secretion lasted two days, with significantly more nectar secreted on the first day. For the flowers that opened at 11:00, nectar secretion lasted three days, and significantly more nectar was secreted on the second day. This is consistent with the finding that young and pollen-shedding flowers are the best nectar producers (Farkas, 2008). Furthermore, it has been verified that the removal of nectar could promote the secretion of more nectar (Torres & Galetto, 1998), and that when the nectar in the corolla reaches its maximum capacity and is not utilized, it will then be reabsorbed (Burquez & Corbet, 1991; Nepi & Stpiczyńska, 2008). In our study, the total

daytime nectar secretion volume of flowers opening before 9:00 was significantly less ($p<0.01$) than that of flowers opening at 11:00. This might be due to the longer flowering period of flowers opening at 11:00, which allows for more nectar secretion and nectar collection. Our result that the average nectar sugar concentration of flowers opening before 9:00 was higher than that of flowers opening at approximately 11:00 was consistent with the finding of a previous study by Mohr & Jay (1990) that more rounds of extraction increased the amount of nectar but decreased the sugar concentration of nectar.

Chabert et al. (2018) have been reported that nectar secretion is synchronized with stigma receptiveness. In this study, we detected a significant positive correlation between corolla opening size and both nectar volume and the sugar concentration of nectar. However, the relevance of corolla opening size and stigma receptiveness has yet to be established. It is important to note that our study was conducted only during the daytime. The study period did not cover the whole flower lifetime for the flowers opening before 9:00. Further studies are required to determine the period of greatest pistil receptivity and to identify the relationship of the pistil with flower characteristics throughout a flower's lifetime, taking flower longevity variation into account.

Oilseed rape nectar is mainly composed of glucose and fructose (Kevan et al., 1991; Farkas, 2008). In this study, we detected that the nectar carbohydrates of the five cultivars consisted almost exclusively of glucose and fructose. Importantly, honey crystallizes quickly if the G/F ratio is more than 0.90 (Smanalieva & Senge, 2009). Oilseed rape honey tends to granulate readily when the G/F ratio reaches or exceeds 0.90 in most cultivars (Kevan et al., 1991; Farkas, 2008; the present study). With large production and the tendency to granulate quickly, the price of oilseed rape honey is much cheaper than that of the other kinds of honey (Gao & Zhao, 2018). However, not all oilseed rape crops produce honey of equal granulating tendency, as sugar ratios of different varieties vary (Kevan et al., 1991). Information on the sugar composition of different varieties can help breeders to select oilseed rape cultivars that produce honey with a low tendency to granulate.

Microclimatic conditions have been found to influence nectar production (Walker et al., 1974; Pinzauti, 1986; Petanidou & Smets, 1996). When the external temperature has not yet reached the optimal temperature for nectar secretion, a proper increase in temperature can promote secretion (Takkis et al., 2018). Nectar production has been found to be dependent on ambient humidity, with nectar volume increasing with increasing humidity (Bertsch, 1983). In our study, we found that the nectar secretion of oilseed rape flowers is affected by the combination of air temperature and relative humidity. For two of the five cultivars, the nectar secretion volume was positively correlated with air temperature and relative humidity. Slight differential effects of environmental parameters on nectar secretion among cultivars were also detected in this study. Our results indicated that the nectar sugar concentration was positively correlated with air temperature and was negatively correlated with relative humidity. The consistent trend of the correlation between the nectar sugar concentration and the relative humidity has been demonstrated in previous studies on other plant genera (Corbet et al., 1979; Bertsch, 1983).

In the oilseed rape breeding process, higher nectar volumes and nectar sugar concentrations in flowers are more favourable for attracting insects for pollination, which in turn promotes an increase in oilseed rape yield (Bommarco et al., 2012; Shu et al., 2019). Entomophilous plants use nectar to attract pollinating insects, and nectar secretion patterns can be correlated with bee activity to analyse the interactions between pollinators and insect vector plants during pollination. Analysis of nectar yield in flowers can help predict potential honey production and inform strategic allocation of bee colonies.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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