Boron Applications and Bee Pollinators Increase Strawberry Yields

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ABSTRACT

Several factors influence fruit set and yield in strawberry production. Bees play an important role in pollinating strawberry flowers; however, bee populations are decreasing due to air pollution and high usage of pesticides. Boron (B) can increase fruit set by improving pollen viability, germination, and pollen tube growth. This study aimed to assess how the application of honey bees and B fertilizer affects yields and marketable fruit quality of Fortuna strawberry cultivar. The experiment used two strawberry growing tunnels. One was open, thus allowing bee activity, and the other was closed, which meant that bees could not access the strawberry flowers. Each tunnel had different B applications. The presence of bees increased the total fruit yield per plant by 54% compared to the results of plants that were not pollinated by bees. Plants treated with bees yielded bigger fruit than those without bee treatment, which is likely a result of more achenes having been fertilized. Fruit formation, however, did not depend directly on pollinator activity. Both in the presence and absence of bees, applying B to the soil increased yields and decreased misshapen fruit rates. Applying B to the soil was more effective than B foliar applications. As a result of this study, it is demonstrated that strawberry plants perform at a higher rate with the application of B and the presence of bee activity.

KEYWORDS

Misshapen fruits; pollen; pollination; yield

Introduction

Insects and animals play an important role in the pollination of many major food crops (Klein et al., 2007). The presence of pollinating insects is necessary to ensure successful strawberry production and obtain marketable fruits (Klatt et al., 2014). Bees have been shown to improve strawberry yields, size, and shelf life (Klatt et al., 2014). Pollination and fertilization promote ovules auxin production (Nitsch, 1950), which causes rapid cell division and increases fruit weight (Roussos et al., 2009). For these reasons, strawberries need insect pollination for successful fruit production with minimum misshapen fruit. A lack of pollinators (Klatt et al., 2014) and low temperatures (especially below 7°C) (Ariza et al., 2012) can decrease the number of fertilized ovaries in strawberry fruits. The insufficient fertilization of ovaries results in misshapen strawberry fruit that is small, button-like, and irregular (Carew, Morretini, and Battey, 2003).

Boron (B) is an important nutrient in soil, especially for fruit-growing plants. B is only required in very low levels and there is a narrow range between B deficiency and toxicity. Sometimes, B applications to soil results in toxicity symptoms in plants (Gupta, 1979). B plays a pivotal role in plant development, such as supporting cell wall production, cell division and differentiation, membrane function, root elongation, hormone regulation, and the generative development of plants (Marschner, 1995). B deficiency inhibits pollen germination and pollen tube growth, which results in misshapen fruits (Riggs et al., 1987), low fruit yields, and poorer quality (Guttridge and Turnbull, 1975).

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Typically, the occurrence of misshapen fruit is high early in the season when strawberry prices are two to three times higher than later in the season. For this reason, decreasing the misshapen fruit rate early in the season is essential for gaining a high income from strawberry production. In this study, the application of B and honey bees was evaluated on strawberry yields and their quality. The effects of honey bee activity and B applications on pollen viability and pollen tube growth were also evaluated. To the best of our knowledge, this is the first study to evaluate the combined effects of these two factors on strawberry yields, fruit weight, and the percentage of misshapen fruit.

**Materials and Methods**

**Field and Material Conditions**

This study was performed at Cukurova University between 2017 and 2018, during the growing season in Adana, Turkey. The experiments were carried out with Fortuna strawberry (*Fragaria x ananassa* Duch.) cultivar. Fortuna strawberry cultivar was selected due to its superior fruit quality characteristics. It is a short-day cultivar that tends to yield in winter-spring periods and produces big, attractive, uniform fruit. Additionally, this cultivar has firm flesh and maintains its fruit size and shape during the entire growing period. Plants were planted in late September (Sept 25, 2017) and all mature fruits were harvested until mid-June (June 10, 2018).

The study was conducted in two Spanish type high tunnels that were 6.5 m wide, 2.75 m high, and 40 m long. They were covered with 36-month durable UV, IR, AB, EVA, and LD containing polyethylene. One tunnel was completely covered with a monofilament (UV stabilized, 8–12% shading) white net to prevent pollinators from visiting the flowers. One commercial honey bee hive (*Apis mellifera* L.) was placed near the tunnel that allowed bees to enter for the other tunnel (Figure 1).

Frigo plants were planted on September 25, 2017, in raised beds that were 65–70 cm wide, 35 cm high. Their inter-row spacing was 35–40 cm and the beds were covered with black plastic mulch. Plants were arranged in a triangle formation with the distance between plants at 30 cm. Irrigation was applied through a drip system based on pan evaporation. All necessary cultural practices and plant protection were followed uniformly for all treatments during the season.

Temperature and moisture changes of both tunnels were registered using a digital data logger (HOBO UX120 Plug Logger, Onset, USA) between February 1st, 2018 and June 11th, 2018. Throughout the experiment, minimum and maximum temperatures within the no-bee tunnel were 11.9°C and 31.8°C, while in with bee tunnel the values were 11.3°C and 32.7°C. The average temperatures were 19.8°C and 20.1°C in no-bee and with-bee tunnels, respectively.

**Boron Applications**

B applications were applied five times every three to four weeks, starting from one week before flowering. The first application was applied on January 29th, 2018, and the following applications were performed on February 26th, March 19th, April 9th, and April 30th during the growing season. These applications were based on the plant growth rate that shortened during the growing season due to increasing temperatures that spurred plant growth. In this study, a fertilizer that consisted of 20%
B (Na₂B₄O₇·4H₂O) (EtiDot-67, Etimaden, Turkey) was used as the B source. Applications consisted of (i) Foliar B as 10 g 100 L⁻¹ by foliar sprays, (ii) Soil B as 1 kg ha⁻¹ via the drip irrigation system (iii) Foliar+Soil B as a combination of (i) and (ii) in half doses and (iv) Control plants were sprayed only with water. Foliar sprays were applied with Tween 20.

**Pollen Viability and Germination Rates**

Pollen viability and germination rates were determined 14 days after each application in order to evaluate the effects of B and different growing conditions on pollen quality. To obtain fresh pollen, 30 flowers were collected one day before anthesis from each application. The anthers were removed and left to dehisce at room temperature. Fresh pollen was immediately used for testing pollen viability and germination capacities.

Pollen viability rates were tested with 1% 2,3,5 Triphenyltetrazolium Chloride (Norton, 1966). In the test, dark red colored pollens were recorded as “fully viable”, pink pollen as “semi-viable” and colorless or very light pink as “non-viable”. The total value of fully viable and half the semi-viable pollens were considered to obtain viable rate (Eti, 1991). For each application, viability was recorded in three slide replications by counting at least five fields in each slide until reaching 100 pollen grains for each replication.

Pollen germination rates were tested with the “agar in petri” method with the medium consisting of 1% agar + 10% sucrose at 25°C (Karabıyık et al., 2017). Pollen was recorded as germinated when the length of the pollen tube exceeded the pollen diameter. For each application, germination was recorded in three petri dishes by counting at least five fields in each petri dish until reaching 100 pollen grains for each replication. Germinated pollens were used for calculating the germination percentage.

**Yield, Average Fruit Weight, and Misshapen Fruit Rate**

Fruits were harvested once or twice a week (depending on fruit maturity), counted, and weighed to determine monthly yield, total yield, and individual fruit weights. Misshapen fruits were separated, counted, and weighed at every harvest. Unshaped and button-like fruits were evaluated as misshapen fruits. The rates of monthly and average misshapen fruits were obtained.

**Statistical Analysis**

The data was analyzed by using two-factor randomized complete block design combined over the location (no-bees and bee tunnels) with three replications (Littell et al., 1997). Each replication included 10 plants in two parallels, one for fruit set and other for pollen analysis. Bee activity and B applications on the parameters were also determined separately. JMP 8.0.1 software was used for ANOVA testing and the results were compared with the Least Significant Difference (LSD) by using a ≤ 5% threshold. Arc-sin transformation was used for evaluating percent values before analysis.

**Results**

**Pollen Viability Rates**

Pollen viability rates are presented in Tables 1 and 2. B applications significantly increased pollen viability rates. It was found that neither the absence nor presence of bees made any significant difference in pollen viability. The highest pollen viability occurred on April 23, 2018 (75.9%) and was followed by March 12, 2018 (72.9%).

In the study, growing conditions x application interactions were not important while growing conditions x dates were found to be important (Table 1). The highest pollen viability rate was obtained on the 4th dates in the no-bees tunnel (80.2%). This rate varied between 56.2% and 80.2%. Regarding
Table 1. Effects of B treatments and growing conditions (presence or absences of bee) on the percent pollen viability rates of ‘Fortuna’ strawberry cultivar over the season.

| Treatments          | Sampling Date |          |          |          |          |          |          |
|---------------------|---------------|----------|----------|----------|----------|----------|----------|
|                     | 14 Feb.       | 12 Mar.  | 02 Apr.  | 23 Apr.  | 14 May   | Gr. Cond. x Treat. | Gr. Cond. Av. |
| Growing Conditions  | 1st           | 2nd      | 3rd      | 4th      | 5th      |                      |               |
| Bees                |               |          |          |          |          |                      |               |
| Control             | 59.2 ± q     | 77.7 ± a-f | 56.2 ± pqr | 69.0 ± f-n | 72.1 ± c-i | 66.8               | 68.3          |
| Foliar              | 57.0 ± m-p   | 70.8 ± d-j | 76.8 ± a-g | 83.9 ± a  | 70.3 ± d-l | 71.7               |               |
| Soil                | 65.3 ± l-q   | 70.2 ± d-k | 64.2 ± qr | 54.8 ± qr | 69.7 ± e-m | 64.5               |               |
| Foliar+Soil         | 60.1 ± j-q   | 78.9 ± a-e | 62.5 ± i-q | 78.5 ± a-e | 69.8 ± e-m | 69.9               |               |
| Samp. Date x Gr.    | 60.1 ± cd    | 74.4 ± b  | 64.9 ± c  | 71.5 ± b  | 70.4 ± b  |                      |               |
| Cond. No Bees       |               |          |          |          |          |                      |               |
| Control             | 60.1 ± k-q   | 67.2 ± g-o | 66.8 ± h-o | 75.9 ± a-f | 59.7 ± m-q | 65.9               | 68.6          |
| Foliar              | 55.8 ± pqr   | 72.4 ± c-h | 71.2 ± d-j | 81.3 ± ab | 65.6 ± h-p | 69.2               |               |
| Soil                | 47.8 ± r     | 67.4 ± g-o | 72.6 ± b-i | 80.7 ± abc | 59.9 ± l-q | 65.0               |               |
| Foliar+Soil         | 60.9 ± k-q   | 79.3 ± a-d | 75.6 ± a-f | 83.0 ± a  | 69.6 ± e-n | 73.7               |               |
| Samp. Date x Gr.    | 56.2 ± d     | 71.6 ± b  | 71.5 ± b  | 80.2 ± a  | 63.7 ± c  |                      |               |
| Cond.               |               |          |          |          |          |                      |               |
| Samp. Date Av.      | 58.1 ± c     | 72.9 ± A | 68.2 ± B  | 75.9 ± A  | 67.1 ± B  |                      |               |

LSDgr. cond. = N.S., LSDsamp. date** = 2.22, LSDsamp.date×gr. cond.** = 3.14, LSDgr.cond×treat = N.S., LSDsamp.time×gr. cond.×treat** = 6.28.  
1 Differences between averages showed by different letters are statistically significant by *, P ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. N.S.: Non-Significant. Samp. Date: Sampling date; Gr.Cond.: Growing condition; Av: Average; Treat: Treatment.

Table 2. Effects of B treatments on the percent pollen viability rates of ‘Fortuna’ strawberry cultivar over the season (%).

| Treatment          | Sampling Date |          |          |          |          |          |          |          |
|--------------------|---------------|----------|----------|----------|----------|----------|----------|----------|
|                    | 14 Feb.       | 12 Mar.  | 02 Apr.  | 23 Apr.  | 14 May   | Treat. Av. |          |
|                    | 1st           | 2nd      | 3rd      | 4th      | 5th      |          |          |
| Control            | 59.6 ± h-i    | 72.4 ± bcd | 61.5 ± f-i | 72.5 ± c-d | 65.9 ± d-h | 66.4 B    |          |
| Foliar             | 56.4 ± i     | 71.6 ± cde | 73.9 ± b-c | 82.6 ± a  | 67.9 ± c-q | 70.5 A    |          |
| Soil               | 55.8 ± i     | 68.8 ± c-f | 68.4 ± c-f | 67.8 ± c-f | 64.8 ± e-h | 65.1 B    |          |
| Foliar+Soil        | 60.6 ± ghi   | 79.1 ± ab  | 69.0 ± cde | 80.7 ± a  | 69.7 ± cde | 71.8 A    |          |

LSDtreat*** = 1.98 LSDsamp.date×treat* = 4.44.  
1 Differences between averages showed by different letters are statistically significant by *, P ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. N.S.: Non-Significant. Samp. date: Sampling date; Gr.Cond.: Growing condition; Av: Average; Treat: Treatment.

the triple interaction (growing condition x application x dates), pollen viability levels varied between 47.8% (with-bees x soil B x 1st sample date) and 83.9% (no-bees x foliar x 4th sample date).

The Foliar+Soil B had the highest pollen viability with 71.8% followed by Foliar B (70.5%) (Table 2). The lowest rate was obtained from the Soil B at 65.1%. While sampling date x application, interactions have a significant effect on the average pollen viability rates (Table 2). The highest pollen viability obtained was from Foliar B at 82.6% in the 4th sample date. This value is followed by Foliar+Soil applications from the 4th sample date (80.7%) and the 2nd sample date (79.1%) that placed in the same statistical group. The lowest pollen viability level was found in Soil B application in 1st date at 55.8%.

**Pollen Germination Level**

Tables 3 and 4 show the pollen germination levels of the Fortuna strawberry cultivar. The average values between applications, periods, growing conditions, and all interactions except from growing conditions x application were found to be significantly important (p ≤ 0.05). Within this context, pollen germination levels were higher in bee tunnels (58.5%) compared to the no-bee tunnel (54.1%). The highest level was observed in the second sampling date with 73.5%, whereas the lowest was in the fifth sampling date (48.9%) when the temperatures were high. Like the pollen viability rates, the highest pollen germination level (58.6%) was measured at Foliar+Soil B applications, whereas the
Table 3. Effects of B treatments and growing conditions (presence or absence of bee) on the percent pollen germination rates of ‘Fortuna’ strawberry cultivar over the season (%).

| Growing Conditions | Bees | 14 Feb. (1st) | 12 Mar. (2nd) | 02 Apr. (3rd) | 23 Apr. (4th) | 14 May (5th) | Gr. Cond. x Treat. | Gr. Cond. Av. |
|--------------------|------|---------------|---------------|--------------|--------------|------------|----------------|---------------|
| Control            | 44.2 mn² | 74.1 b | 55.5 f-k | 58.5 d-h | 60.3 d-h | 58.5 AB | 58.5 A |
| Foliar             | 66.1 bcd | 77.5 a | 48.9 j-n | 57.5 e-j | 55.8 f-k | 61.2 A |
| Soil               | 61.9 c-g | 77.6 a | 44.7 lmn | 31.3 p | 49.1 j-n | 52.9 C |
| Foliar+Soil        | 65.4 cde | 73.9 ab | 52.8 h-m | 56.7 f-j | 58.3 d-i | 61.4 A |
| Samp. Date × Gr. Cond. | 59.4 c | 75.8 a | 50.5 e | 50.9 e | 55.9 cd | 52.5 C | 54.1 B |
| No Bees            | 45.5 lmn | 68.9 bc | 49.1 j-n | 56.1 f-j | 42.6 n | 52.5 C | 56.1 BC |
| Foliar             | 53.4 g-f-l | 55.9 f-k | 59.6 d-h | 49.8 i-n | 61.6 c-g | 52.2 C |
| Soil               | 41.9 no | 78.4 a | 62.6 c-f | 47.2 k-n | 30.8 p | 52.2 C |
| Foliar+Soil        | 43.2 n | 81.4 a | 62.7 c-f | 58.4 d-i | 33.4 op | 55.8 BC |
| Samp. Date × Gr. Cond. | 45.9 f | 71.2 b | 58.5 c | 52.8 de | 42.1 f | 52.7 B | 73.5 A | 54.5 B |
| Treatments         | 51.9 B | 51.9 BC | 48.9 C | |

LSD gr.cond.*** = 1.14, LSDsamp. date. *** = 1.81, LSDsamp. date×gr. cond.*** = 2.56, LSDgr.cond.×treat = N.S., LSDsamp. date×gr. cond.×treat*** = 5.12. * Differences between averages shown by different letters are statistically significant by *, P ≤ 0.05; **p ≤ 0.01, ***p ≤ 0.001. N.S.: Non-Significant. Samp. Date: Sampling date; Gr.Cond.: Growing condition; Av: Average; Treat: Treatment.

Table 4. Effects of B treatments on the percent pollen germination rates of ‘Fortuna’ strawberry cultivar over the season (%).

| Treatment         | 14 Feb. (1st) | 12 Mar. (2nd) | 02 Apr. (3rd) | 23 Apr. (4th) | 14 May (5th) | Treat. Av. |
|-------------------|---------------|---------------|--------------|--------------|------------|------------|
| Control           | 44.8 gh       | 71.5 b        | 52.3 de      | 57.3 cde     | 51.4 ef     | 55.5 B     |
| Foliar            | 59.7 c        | 66.8 b        | 54.3 cde     | 53.7 cde     | 58.7 c      | 58.6 A     |
| Soil              | 51.9 def      | 78.0 a        | 53.6 cde     | 39.2 h       | 39.9 gh     | 52.5 C     |
| Foliar+Soil       | 54.3 cde      | 77.7 a        | 57.8 cde     | 57.5 cde     | 45.9 fg      | 58.6 A     |

LSDtreat.*** = 1.62 LSDsamp.date×treat*** = 3.62. * Differences between averages showed by different letters are statistically significant by *, P ≤ 0.05; **p ≤ 0.01, ***p ≤ 0.001. N.S.: Non-Significant. Samp. Date: Sampling date; Gr.Cond.: Growing condition; Av: Average; Treat: Treatment.

lowest was observed from Soil B applications at 52.5%. The triple interaction values ranged from 30.8% (fifth sample date x bees x soil) to 81.4% (second sample date x no bees x Foliar+Soil).

**Fruit Yield and Quality**

**Yield Per Plant**

The monthly and total yield per plant is presented in Tables 5 and 6. With the presence of bees, the total plant yield was 54% higher than when bees are absent. The highest monthly yield was obtained in April 2018. B treatments significantly increased the yield over most harvest dates (Table 6). In terms of the total yield, the application of B to the soil resulted in higher yields, substantiated by a yield 42% larger than the control.

**Average Fruit Weight and Misshapen Fruit Rate**

The average fruit weights are presented in Tables 7 and 8. The differences between fruit weight values in terms of sampling time, growing conditions, applications, and sampling time x growing condition interactions were found to be statistically significant. Like the total fruit yield, fruit weight was also found to be significantly higher in plants grown with B applied to the soil and in tunnels with bees. Fruit weights were as much as 17% greater in tunnels with bees. The highest average fruit weight was obtained in March and decreased as the season progressed.

Misshapen fruit percentages were significantly higher with the presence of bees, as presented in Table
Table 5. Effects of B treatments and growing conditions (presence or absences of bee) on yield over the season (g/plant⁻¹).

| Growing Conditions    | Bees          | Treatments         | March  | April  | May  | June  | Av. | Gr. Cond. x Treat. | Gr. Cond. |
|-----------------------|---------------|---------------------|--------|--------|------|-------|-----|---------------------|------------|
| No Bees               | Control       | 212.9 e-a           | 370.6 b| 162.6 h-l | 179.6 g-k | 925.8 C | 1175.0 A |
|                      | Foliar        | 167.9 g-l           | 412.5 a| 282.2 de  | 243.4 d-g  | 1106.1 B |      |
|                      | Soil          | 372.9 bc            | 637.8 a| 176.6 g-k  | 241.7 d-g  | 1428.9 A |      |
|                      | Foliar+Soil   | 393.2 b             | 410.3 b| 234.3 d-h  | 201.4 f-j  | 1239.2 B |      |
| Samp. Date x Gr. Cond.|               | 286.8 b             | 457.8 a| 213.9 c   | 216.5 c   |        |      |
| Samp. Date Av.       |               | 245.4 B             | 380 A  | 174.2 C   | 169.3 C   |        |      |
| LSDgr.cond.*** = 67.7 | LSDsamp.date*** = 27.8 | LSDsamp.date×gr.cond.* = 39.3 | LSDgr.cond.xtreat* = 135.5 | LSDsamp.date×gr.cond.xtreat* = 78.6. | Differences between averages showed by different letters are statistically significant by *, P ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. N.S.: Non-Significant. Samp. Date: Sampling date; Gr.Cond.: Growing condition; Av: Average; Treat: Treatment.

Table 6. Effects of B treatments on yield over the season (g/plant⁻¹).

| Treatments         | March  | April  | May  | June  | Treatment |
|--------------------|--------|--------|------|-------|-----------|
| Control            | 190.5  | 361.8  | 153.9 d| 143.6 d| 804.9 C   |
| Foliar             | 170.7  | 345.0  | 221.7 c| 175.4 cd| 912.9 B   |
| Soil               | 301.1  | 502.7  | 156.9 d| 194.9 cd| 1156.0 A  |
| Foliar+Soil        | 319.2  | 355.7  | 164.2 d| 163.2 c| 1002.0 B  |

LSDTreat.*** = 95.8, LSDsamp.date×treat.*** = 55.6. Differences between averages showed by different letters are statistically significant by *, P ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. N.S.: Non-Significant. Samp. Date: Sampling date; Gr. Cond.: Growing condition; Av: Average; Treat: Treatment.

Table 7. Effects of B treatments and growing conditions (presence or absences of bee) on average fruit weight over the season (g/fruit).

| Growing Conditions    | Bees          | Treatments         | March  | April  | May  | June  | Av. | Gr. Cond. x Treat. | Gr. Cond. Av. |
|-----------------------|---------------|---------------------|--------|--------|------|-------|-----|---------------------|---------------|
| No Bees               | Control       | 31.5                | 15.4   | 12.7   | 8.7  | 17.1  | 17.6 A |          |
|                      | Foliar        | 31.4                | 16.7   | 14.1   | 9.6  | 17.9  |      |          |
|                      | Soil          | 32.7                | 17.9   | 14.2   | 9.1  | 18.5  |      |          |
|                      | Foliar+Soil   | 30.9                | 14.4   | 13.4   | 8.4  | 16.8  |      |          |
| Samp. Date x Gr. Cond.|               | 31.6 a              | 16.1   | 13.6 d  | 8.9 f| 8.9 f | 15.0 B |      |
| Samp. Date Av.       |               | 27.4 b              | 13.3 d  | 11.5 e  | 8.0 f| 8.0 f | 15.1  |      |
| LSDgr.cond.*** = 67.7 | LSDsamp.date*** = 27.8 | LSDsamp.date×gr.cond.* = 39.3 | LSDgr.cond.xtreat* = 135.5 | LSDsamp.date×gr.cond.xtreat* = 78.6. | Differences between averages showed by different letters are statistically significant by *, P ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. N.S.: Non-Significant. Samp. Date: Sampling date; Gr.Cond.: Growing condition; Av: Average; Treat: Treatment.

9. This result was contrary to our expectations. Harvest dates affected the occurrence of misshapen fruit formations, which was due to the different ecological conditions. The highest percentage of misshapen fruit occurred in June and the lowest was in March when the temperatures were relatively lower than the other sampling times (see Table 9, Figure 2). The results showed that the percentage rate of misshapen fruit decreased the application of B to the soil (see Table 10). There was no significant difference among dates and application interactions that varied between 6.4% and 17.9%.
Table 8. Effects of B treatments on average fruit weight over the season (g/fruit).

| Treatments    | March | April | May  | June | Treat. Av. |
|---------------|-------|-------|------|------|------------|
| Control       | 29.4  | 14.9  | 11.6 | 8.2  | 16.1 B    |
| Foliar        | 28.0  | 14.5  | 12.8 | 8.7  | 15.9 B    |
| Soil          | 30.9  | 15.8  | 13.3 | 8.8  | 17.2 A    |
| Foliar+Soil   | 29.6  | 13.4  | 12.4 | 8.2  | 15.9 B    |

LSDtreat*** = 95.8 LSDsamp.date×treat*** = 55.6. Differences between averages showed by different letters are statistically significant by *, P ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. N.S.: Non-Significant.
Samp. Date: Sampling date; Gr.Cond.: Growing condition; Av: Average; Treat: Treatment.

Table 9. Effects of B treatments and growing conditions (presence or absences of bee) on percent misshapen fruit rates over the season (%).

| Growing Conditions | Bees           | Treatments | March | April | May  | June | Gr. Cond. x Treat. | Gr. Cond. Av. |
|--------------------|----------------|------------|-------|-------|------|------|-------------------|---------------|
| Control            | 14.9           | Control    | 10.5  | 12.8  | 22.4 | 15.2 | 12.4 A            |
| Foliar             | 10.2           | Foliar     | 13.4  | 11.6  | 17.9 | 13.3 |
| Soil               | 8.5            | Soil       | 8.2   | 9.5   | 12.4 | 9.7  |
| Foliar+Soil        | 12.2           | Foliar+Soil| 10.9  | 11.0  | 11.4 | 11.4 |
| Samp. Date x Gr. Cond. | 11.4  | Control    | 10.8  | 11.2  | 16.1 | 7.8  |
| No bees            |                | Foliar     | 5.8   | 6.5   | 15.4 | 8.4  |
|                   |                | Soil       | 4.4   | 6.3   | 9.6  | 6.2  |
|                   |                | Foliar+Soil| 1.6   | 6.5   | 8.5  | 5.8  |
| Samp. Date x Gr. Cond. | 3.9   | Control    | 5.5   | 7.0   | 11.8 |
|                   |                | Foliar     | 4.4   | 6.3   | 9.6  | 6.2  |
|                   |                | Soil       | 1.6   | 6.5   | 8.5  | 5.8  |
|                   |                | Foliar+Soil| 3.9   | 5.5   | 7.0   | 11.8 |
| Samp. Date         | 7.7 B          |            | 8.1 B | 9.1 B | 13.9 A |

LSDgr.cond*** = 1.89, LSDsamp.date*** = 2.67, LSDsamp.date×gr.cond. = N.S. LSDgr.cond×treat = N.S., LSDsamp.date×gr. cond.×treat = N.S. Differences between averages showed by different letters are statistically significant by *, P ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. N.S.: Non-Significant. Samp. Date: Sampling date; Gr.Cond.: Growing condition; Av: Average; Treat: Treatment.

Discussion

Bee activity and the application of B improved strawberry yields and quality in this study. For successful pollination and fertilization, it is important to have high pollen viability and germination rates. This study showed that temperature values affected pollen viability and germination in Fortuna strawberry cultivar. For this cultivar, significantly low pollen viability and germination levels were seen in lower (1.6°C, February 2nd, 2018) and higher temperatures (44.0°C, 4 May) (Figure 2). Similarly, it has been reported that in short-day strawberries, temperatures below 17°C or above 25°C can reduce pollen viability (Braak, 1968; Ledesma and Sugiyama, 2005; Leech et al., 2002; Voyiatzis and Paraskevopoulou-Paroussi, 2002). Ledesma and Sugiyama (2005) also reported a decrease in pollen viability levels in Toyonoka strawberry cultivar under comparatively high temperatures (30/25°C), whereas the control values were 28/13°C. These results are similar to our findings during May.

The optimum temperature and humidity for pollen germination depend on the strawberry cultivar (Koyuncu, 2006; Leech et al., 2002). In one study, the optimum temperature for strawberry pollen germination was reported to be 15–25°C (Hortynski and Zebrowska, 1991). Leech et al. (2002) found that for Florence strawberry cultivar, the optimum temperature for pollen germination was 10°C, while it was 20°C for Pegasus, Tamella, and Marmolada. They also reported that 30°C and 75–85% humidity lowered pollen germination for all cultivars that were evaluated. Karapatzak et al. (2012) found lower germination levels for two strawberry cultivars that were exposed to high temperatures (30/20°C day/night), compared to plants exposed to lower temperatures (22°C/15°C, 26°C/11°C). In another study, the lowest in vitro pollen germination levels were determined at 7°C for Elvira, Selva, and Chandler, and 15°C for Cavendish, Allstar, and Elsanta (Koyuncu, 2006). In the same study, pollen germination levels were reported to be between 27.9% and 64.2%, which is generally in line with our findings. As literature indicates, ecological conditions, such as temperature and humidity impact
strawberry cultivars in different ways. The distinct effects of ecological conditions on pollen viability and germination levels were demonstrated in this study.

Regardless of the growing conditions, Fortuna foliar B applications significantly increased pollen viability and germination rates. Interestingly, although pollen germination was positively influenced by foliar B sprays, germination values were found to be lower when B was applied to the soil. This occurred in the control plants too. Peñaloza and Toloza (2018) reported that high B concentrations affected pollen quality and formation depending on genotypes. In another study, it was found that calcium and B applications decreased pollen viability levels of Sweet Ann strawberry cultivar by about 5% (Karabiýık et al., 2017). These recent studies show that a genotype’s pollen viability responds differently to calcium and B applications (Karabiýık et al., 2017; Peñaloza and Toloza, 2018).

Figure 2. Difference in minimum (Tmin), maximum (Tmax) and average (Tave) temperatures during growing period in covered and uncovered tunnels.
Regarding germination levels, Muengkaew et al. (2017) reported that different doses of B and calcium applications positively affects pollen germination in mango trees. Nyomora et al. (2000) have reported that pollen germination rates and pollen tube growth were significantly increased by applying B to the germination medium. Similarly, Fang et al. (2019) determined a decrease in pollen germination rates and a delay in pollen tube growth of apple trees (Malus domestica) under B-deficient conditions. Another study conducted with beans showed that 2,700 ppm or more of B application caused toxic effect and foliar applications did not influence the total yield (Viçosi et al., 2020). Brown and Hu (1996) reported that B mobility in phloem depends on the sorbitol content of species. They reported that in sorbitol-rich species, B can be transported easily in the phloem. Otherwise, there is no movement inside the phloem. The B doses of this study were designed based on the research of Esringü et al. (2011) and their suggested B treatment strategies for Turkey. Our doses showed no toxicity in our plants and the effects of B was seen clearly.

B applications and bee activity significantly improved fruit yields in most cases. Soil B and the presence of bees, separately and together, increased strawberry yields. Similar results were obtained from Klatt et al. (2014) who found that strawberry fruit quality and marketability could be increased by bee pollination, not only relying on wind or self-pollinating plants. Bartomeus et al. (2014) reported that yield and quality were increased between 18% and 71% with pollinator insect activity, depending on the crop. Moreover, the pollinator insect activity may increase plant yields when compared to wind (11%) and self-pollination (30.3%). Furthermore, Güneş et al. (2016) reported that differently applied Bio-B increased fruit yield, as well as increasing antioxidant enzyme activity and ameliorate cold damage in leaves. Considering the results of this study, it is recommended for strawberry growers to use B via soil application and increase bee activity to gain higher yields.

In the current study, soil B application significantly increased fruit weight, while bees did not affect this trait. The highest average fruit weight was obtained in March, most likely because of decreased fruit-to-fruit competition for resources. The average fruit weight decreased as the season progressed, as the fruit load per plant and temperature increased.

The misshapen fruit rate is one of the most important parameters that affect the commerce of strawberry fruits. It directly correlates with pollination and fertilization properties. The reduction of misshapen fruit with the application of B was an important finding of this study. Additionally, the percentage of misshapen fruit decreased with the application of B to the soil in the presence or absences of bees. This data supports the findings of Riggs et al. (1987), who found positive effects of B on strawberry fruit formation, development, and reduction of misshapen fruit.

Our results showed that bees increased the number of misshapen fruits, which is contrary to previous research (Klatt et al., 2014). However, the percentage of misshapen fruit was very low in this study (less than 13%) and there was only a 5.3% difference in misshapen fruit between the bee and no-bee treatments. Moreover, Fortuna flowers have a narrow receptacle and long filaments, which facilitates pollination. In a recent study, no benefit to bee pollination was found, positing that if there is no insect pollination, the combined effect of gravity and wind leads to high pollination levels, even though the pollination rate of the achenes rarely surpassed 60% (Abrol et al., 2019). However, the high misshapen fruit rate with bees present indicates that there can be a facultative parthenocarpy

| Treatments | Sampling Date | March | April | May | June | Treat. Av. |
|------------|---------------|-------|-------|-----|------|------------|
| Control    |               | 9.5   | 6.8   | 11.7| 17.9 | 11.50 A¹   |
| Foliar     |               | 7.9   | 9.7   | 9.1 | 16.7 | 10.90 AB   |
| Soil       |               | 6.4   | 7.3   | 6.9 | 11.0 | 7.90 B     |
| Foliar+Soil|               | 6.9   | 8.7   | 8.9 | 9.9  | 8.61 B     |

LSD<sup>treat</sup>* = 3.78 LSD<sup>samp.date>treat = N.S. ¹Differences between averages showed by different letters are statistically significant by *, P ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. N.S.: Non-Significant. Samp. Date: Sampling date; Gr.Cond.: Growing condition; Av: Average; Treat: Treatment
caused by cross-pollination by bee activity. Despite low misshapen fruit formations, lower fruit weight occurred under no-bee conditions. This situation also indicates that Fortuna strawberry cultivar may have a parthenocarpic fruit formation ability. This would potentially be due to early ovule degeneration or self-incompatibility properties. However, these results must be further examined in the future with a focus on flower structures, fruit set performance by self and cross-pollination, pollen-pistil interactions, ovule longevity, and parthenocarpic ability of this cultivar.

In various studies, it was reported that ecological conditions (temperature and relative humidity) affect pollen viability, germination, and ovule degeneration, which directly affects fruit development. In this study, ecological conditions affected the occurrence of misshapen fruit formations. The misshapen fruit rate was highest in June, likely due to high temperatures, and lowest in March when temperatures were relatively lower. This result shows that Fortuna is possibly more sensitive to high temperatures than low temperatures. Strawberry cultivar genetics also play a role in the occurrence of misshapen fruit, in addition to environmental factors. Cultivars grown in the same conditions had significantly different misshapen fruit rates (Ariza et al., 2011; Carew et al., 2003). Ariza et al. (2012) reported that misshapen fruit rates were higher in Camarosa (1.156 g/plant−1), having higher yields, than Medina (887 g/plant−1), especially in the early season. They also reported that there was a decrease in misshapen fruit rates by using bee pollination, contrary to what was found in this study. Our findings show a low tendency for Fortuna strawberry cultivar to yield misshapen fruit.

Conclusion

Although pollen viability and germination were negatively influenced by applying B to the soil, the applications increased yields and the average fruit weight as well as decreased the misshapen fruit rate without bee activity. A high yield can even be obtained with the absence of bee activity by only 35% decrease in total yield. Furthermore, in both conditions, the application of B to the soil significantly increased the total yield. At the same time, the occurrence of misshapen fruit formations for this cultivar was found to be higher in high temperatures. Our findings indicate that B fertilization and a bee population are very important for obtaining higher yields in strawberry cultivation.

In this study, it is demonstrated that pollination requirements and the reproductive biology of Fortuna strawberry cultivar can be different from other strawberry cultivars. So, these results should be confirmed by other commercial cultivars.

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