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Effects of Nutrient Solution Irrigation Quantity and Downy Mildew Infection on Growth and Physiological Traits of Greenhouse Cucumber

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Abstract: Abiotic and biotic stresses both decrease the quality and quantity of cultivated plants. In this study, in order to see the responses of cucumber plants to drought stress and cucumber downy mildew infection, downy mildew infestation at different two levels, B1 (disease infestation) and B2 (no disease infestation), along with three fertigation requirement levels, full fertigation T1, moderate nutrient solution deficit T2 and severe nutrient solution deficit T3, were applied in a greenhouse. Thus, six treatments, i.e., B1T1, B1T2, B1T3, B2T1, B2T2 and B2T3, were set. The leaf gas-exchange parameters were significantly increased under CK (control experiment, B2T1: no disease infestation and full irrigation) treatment, and leaf photosynthesis rate, transpiration rate and stomatal conductance were significantly decreased under the B1T1 treatment. Leaf intercellular CO_2 concentration was significantly increased under B1T1 treatment. Leaf photosynthesis rate, transpiration rate, intercellular CO_2 concentration and stomatal conductance were significantly decreased under B1T2, B1T3, B2T2 and B2T3 treatments. Compared with treatment CK (B2T1), the plant height of cucumber under B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 11.41%, 19.05%, 27.48%, 7.55% and 10.62%, respectively; the stem diameter of cucumber under B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 5.70%, 13.45%, 23.03%, 9.46% and 15.74%, respectively; and leaf area of cucumber under B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 22.79%, 38.68%, 58.28%, 13.76% and 29.96%, respectively. The root–shoot ratio of cucumber under B1T1, B1T2, B1T3, B2T1, B2T2 and B2T3 treatments was 3.16%, 2.99%, 4.11%, 3.92%, 3.13% and 3.63%, respectively. The root–shoot ratio of cucumber was the highest under the B1T3 treatment.

Keywords: greenhouse; cucumber; drought stress; downy mildew; growth; photosynthesis

1. Introduction

With the continuously increasing global population, the demand for food production has increased [1–3]. Greenhouses are conducive to the growth of vegetables and are hardly affected by the external environment, which plays an important role in improving vegetable yield [4,5]. At present, China's greenhouse cultivation area measures more than 4 million hectares [6,7]. Cucumber, as an important economic crop, is one of the main vegetables widely planted in the world. It has a status second only to tomato in fruit vegetables [8,9]. Because of its rich taste and nutritional value, cucumber is deeply loved by consumers and plays an increasingly important role in agricultural

structure adjustment and farmers' income increase. China is the largest cucumber-producing country in the world in terms of scale and yield. In 2018, the cucumber cultivation area in China was 1.05 million hm^2, accounting for half of the world's cucumber cultivation area, and the annual yield was about 56.29 million t [10–12].

Cucumbers, as they grow, are unavoidably exposed to a combination of abiotic stresses (including salt, drought, heat, chilling and UV-B) and biotic stresses (including bacteria and fungi) [13,14]. These environmental stressors have caused extensive worldwide agricultural losses and posed a major challenge in the face of an ever-increasing world population. These environmental stressors can result in the loss of cucumber yield [15,16]. Therefore, understanding stress responses and adaptation mechanisms against the combination of different stresses is rather vital in terms of improving production efficiency by changing plant management conditions [17].

Effects of deficit irrigation on many vegetables and field crops growth and productivity have been reported by several researchers [7,18,19]. Different drought treatments affect cucumbers' photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration and then affect cucumber plant height, stem thickness and leaf area [20]. Cucumber yield is significantly affected by irrigation water amount at all growth stages [21]. However, unlike drought stress, the oxidative metabolism of different pathogens during infection is carried out by a limited pathological system [7,22,23]. Downy mildew, caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov., is a wide-spread disease of greenhouse and field-grown cucumber plants and it can lead to a significant yield loss [24]. The severity and progress of the disease depend on favorable conditions, such as high humidity, temperature, light intensity and source of inoculum. The preliminary symptoms appear on the upper surface of mature leaves as yellow angular spots and chlorotic lesions on the opposite side of the spot. Severely infected plants produce retarded/deformed fruits, which leads to a considerable loss of production. As the disease progress, the yellow spots became brown and then necrotic, which leads to leaf fall and death [8,25]. Infection by downy mildew pathogens results in many changes in the plant's metabolic processes, including changes in the physiological structure of the leaves, which may affect the stomata that regulate transpiration and water loss from plants [26].

The effect of a single stress on plants has been studied. However, analyzing the impact of a single stress on plants may be very different from a situation where plants encounter multiple different stresses at the same time in the field. Considering this fact, in this study, in order to observe the plant's response to multiple stresses, we applied drought stress and cucumber downy mildew infection at the same time, which represents the situation encountered by cucumber plants under field conditions.

2. Materials and Methods

2.1. Study Site and Treatment Details

The experiment was performed in a Venlo-type greenhouse at the Key Laboratory of Modern Agricultural Equipment and Technology, Ministry of Education, Jiangsu University, Zhenjiang, China (119°45′ E, 32°20′ N). The average greenhouse air temperature was 24.5 °C (the range was from 14.57 to 38.69 °C). The relative humidity of the greenhouse was 82.6%. The experiment was conducted from 24 August 2020 to 27 September 2020.

The cucumber seeds, "Jinyou 1" (provided by the cucumber research institute in Tianjin Academy of Agricultural Sciences, Tianjin, China), were sowed in a plug tray on 24 August 2020. Plastic pots (having dimensions of 20.9 cm in height and 32 cm in diameter) were filled with perlite substrate (the perlite substrate in the plastic pots was washed with running water), used as a growing medium for the plants, on 30 August 2020. Cucumber seedlings were transplanted into plastic pots on 31 August 2020. Seedlings planting density was 5.54 plants/m^2. Full fertigation was applied in order to ensure growth of cucumber seedlings. Cucumber seedlings were roped by a nylon cord vertically. Pruning was done to maintain the cucumber plants' growth by following agronomic

requirements. The cucumbers were picked at the flowering stage (70% of the cucumbers were in bloom) on 27 September 2020.

2.2. Treatments and Experiment Design

To investigate the effect of downy mildew infestation and nutrient solution irrigation (Kawasaki nutrient solution; the composition of the nutrient solution is shown in Supplementary Table S1) on crop growth, leaf gas-exchange, material accumulation (including leaf fresh weight, leaf dry weight, stem fresh weight, stem dry weight, root fresh weight and root dry weight of cucumber plants) and parameters of root systems of cucumber plants, as shown in Figure 1, the experimental design consisted of 6 treatments, each of which was repeated 6 times. Downy mildew infestation at different two levels, B1 (disease infestation) and B2 (no disease infestation), along with three fertigation requirement levels, full fertigation T1, moderate nutrient solution deficit T2 and severe nutrient solution deficit T3, were applied in the greenhouse. Thus, six treatments, i.e., B1T1 (disease infestation and full fertigation), B1T2 (disease infestation and moderate nutrient solution deficit), B1T3 (disease infestation and severe nutrient solution deficit), B2T1 (no disease infestation and full fertigation), B2T2 (no disease infestation and moderate nutrient solution deficit) and B2T3 (no disease infestation and severe nutrient solution deficit), were set. T1, T2 and T3 were 600 (field water capacity), 400 and 200 mL during the whole experiment period, respectively. The nutrient solution was irrigated at 8:00–9:00 in the morning. The cucumber was treated with different nutrient solution irrigation treatments and downy mildew infection on the 9th day after transplanting.

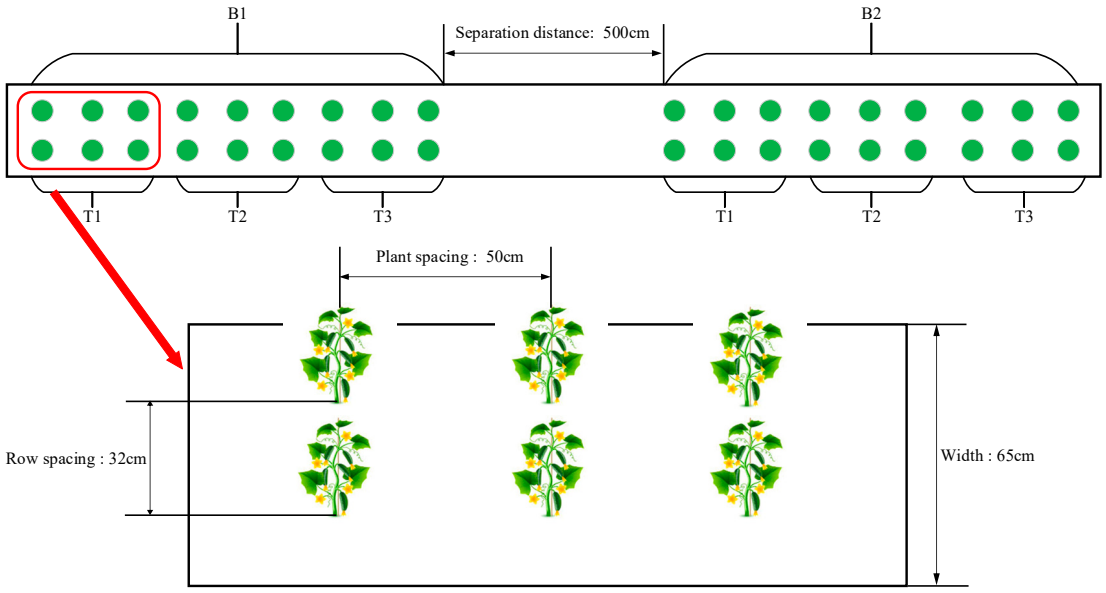

Figure 1. Diagram of cucumber planting.

2.3. Extraction and Inoculation of Pathogenic Fungi

In May 2020, cucumber downy mildew sporangiospores were collected in the Venlo-type greenhouse of the Key Laboratory of Modern Agricultural Equipment and Technology of the Ministry of Education of Jiangsu University, China. First, we rinsed the aged spores and bacteria on the plants' leaves, which were cleaned with sterile water. The leaves were placed on a potato dextrose agar (PDA; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) medium. When the PDA medium was covered with spore colonies, we used sterile inoculation needles to select different colonies and inoculated them on a PDA medium containing cucumber leaves until only one colony was growing on the PDA medium. Then, the colonies on the PDA medium were rinsed with sterile water to prepare a sporangia or conidia suspension. Finally, the morphology of spores was observed under an ultra-deep

three-dimensional microscope (VHX-900F, made by KEYENCE Co., Osaka, Japan) with a cell-counting plate, and pathogens were screened out according to relevant data. Then, the extracted cucumber downy mold spore was subcultured on cucumber leaves (as shown in Supplementary Figure S1) [27]. When the fifth leaf of cucumber was grown (8 September 2020), cucumber downy mold spores were prepared into a spore suspension of 1×10^6 spores/mL with sterile water. Cucumber leaves were inoculated in the evening with a disposable sterile syringe with a capacity of 2 mL (each leaf was inoculated with 2 mL spore suspension), and sterile water was set as the control [28].

2.4. Leaf Gas-Exchange Parameters

Leaf gas-exchange parameters consisting of leaf photosynthesis rate, transpiration rate, intercellular CO_2 concentration and stomatal conductance were measured using a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA). The disease index of cucumber downy mildew was graded according to GB/T17980.26—2000. The severity of cucumber downy mildew grading criteria is shown in Supplementary Table S2.

The measurements were taken on the 9th (asymptomatic), 14th (diseased area accounts for 6% to 10% of the leaf area) and 19th (diseased area accounts for 26% to 50% of the leaf area) day after the seedlings were transplanted into plastic pots. Each selected date for measurement of leaf gas-exchange was parallel to the leaf disease degree. The measurements were taken on sunny days at 9:00–11:00 local time. The position of the selected leaf was kept similar in each plant.

2.5. Crop Growth Parameters and Material Accumulation

Plant height (cm, from perlite substrate surface to cucumber plant top) and stem diameter (mm, at marked point of 10 cm height from perlite substrate surface) of the cucumber plants were measured using a metric ruler and vernier caliper, respectively. The leaf area of each plant was measured after the experiment. In this study, we used a protective cleaning method to wash and tidy the root system; then, chose the Perfection V700 photo scanner (made by EPSON Co., Nagano, Japan) and the WinRHIZO root analysis software (professional version) to measure the root system parameters, including total length, average diameter, surface area, total volume and total tip number. The fresh weights of the leaves, stems and roots of each plant were measured at the end of the experiment. Then, they were put in an oven which was set at 105 °C for 15 min, after which the temperature would be reset to 80 °C, followed by keeping them drying until the weight was constant; a precision electronic scale (0.0001 g) was used to measure the dry weight of leaves, stems and roots [1]. Finally, the root–shoot ratio for the crops was calculated according to the following formula (1):

$$Root - shoot\ ratio = \frac{DW(root)}{DW(leaves) + DW(stem)} \quad (1)$$

where DW (*leaves*) represents the dry weight of leaves, DW (*stem*) represents the dry weight of stems, and DW (*root*) represents the dry weight of roots.

2.6. Statistical Analyses

Data were analyzed using SPSS 16 (SPSS Inc., Chicago, IL, USA) analysis of variance (ANOVA) to study the combined effects of downy mildew infestation and nutrient solution irrigation. The least significant difference (LSD) test was used to determine significance at a significance level of $p < 0.05$.

3. Results

3.1. Leaf Gas-Exchange Parameters

The effects of different nutrient solution irrigation treatments and different stages after downy mildew infection on (9, 14 and 19 days after transplanting) gas-exchange parameters of cucumber leaves

are shown in Figure 2. As we can see from Figure 2, the leaf gas-exchange parameters were significantly increased under CK (B2T1) treatment, and leaf photosynthesis rate, transpiration rate and stomatal conductance were significantly decreased under the B1T1 treatment. However, leaf intercellular CO_2 concentration was significantly increased under the B1T1 treatment during the whole experiment period. Leaf photosynthesis rate, transpiration rate, intercellular CO_2 concentration and stomatal conductance were significantly decreased under the B1T2, B1T3, B2T2 and B2T3 treatments during the whole experiment period. There was no significant difference found in the gas-exchange parameters of cucumber leaves when measured on the 9th day (that is, before different nutrient solution irrigation treatments and downy mildew infection) after transplanting.

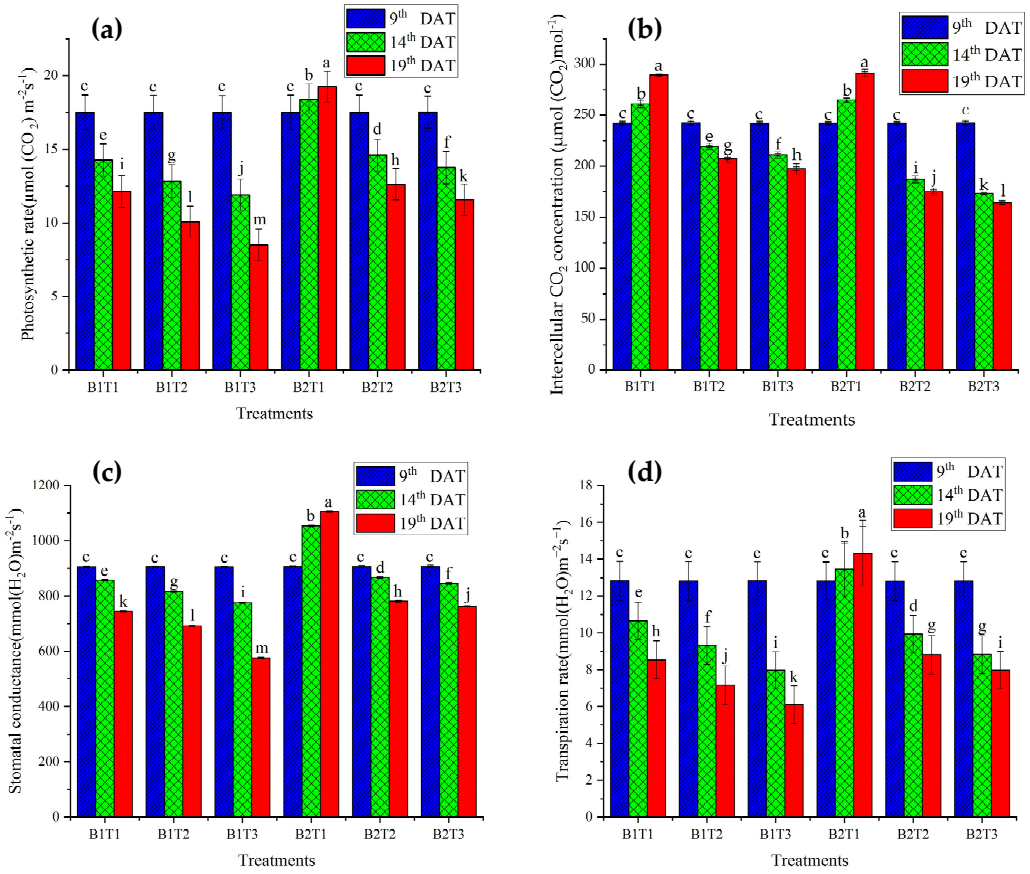

Figure 2. The effects of different water treatments and downy mildew infection on leaf photosynthetic parameters of cucumber plants. Note: Error bars indicate standard deviations, with different lowercase letters between treatments indicating significant differences at ($p < 0.05$). (**a**) Photosynthetic rate; (**b**) Intercellular CO_2 concentration; (**c**) Stomatal conductance; (**d**) Transpiration rate.

From Figure 2a, it can be seen that at the 14th day after transplanting, compared with treatment B2T1, the photosynthetic rate of leaves under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 22.46%, 30.17%, 35.35%, 20.55% and 27.31%, respectively. Furthermore, at the 19th day after transplanting, compared with treatment B2T1, the photosynthetic rate of leaves under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 36.94%, 47.58%, 55.84%, 35.67% and 39.89%, respectively. From Figure 2c, it can be seen that at the 14th day after transplanting, compared with treatment B2T1, the stomatal conductance of leaves under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 18.63%, 22.61%, 26.36%, 17.66% and 19.81%, respectively. Furthermore, at the 19th day after transplanting, compared with treatment B2T1, the stomatal conductance of leaves under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 32.64%, 37.38%, 47.9%, 29.27% and 31.07%, respectively. After downy mildew infects cucumber plants, the hyphae enter the tissue through

the stomata. In this process, the stomata shrink due to damage, and the stomata conductance decreases. Outside CO_2 cannot enter through these deformed stomata, which limits the transmission of CO_2 to chloroplasts, resulting in the decrease in its photosynthetic rate and the increase in its intercellular CO_2 concentration [27]. With the aggravation of drought degree, the photosynthetic rate, stomatal conductance and intercellular CO_2 concentration of cucumber leaves decreased gradually. It shows that drought stress is not conducive to the synthesis of organic matter in cucumber plants, and at the same time, it hinders the transportation of water and organic matter. Compared with water stress treatment alone, downy mildew infection and water stress treatment had greater effects on the photosynthetic rate, stomatal conductance and intercellular CO_2 concentration of cucumber leaves. The results of this experiment are consistent with previous studies [23,29].

From Figure 2b, it can be seen that at 14 days after transplanting, compared with treatment B2T1, the intercellular CO_2 concentration of leaves under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 1.21%, 17.19%, 20.31%, 29.41% and 34.61%, respectively. Additionally, at 19 days after transplanting, compared with treatment B2T1, the intercellular CO_2 concentration of leaves under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 0.63%, 28.75%, 32.22%, 39.94% and 43.67%, respectively. After downy mildew infected the cucumber plants, the intercellular CO_2 concentration of cucumber leaves increased gradually [29]. Therefore, there was no significant difference found in intercellular CO_2 concentration parameters of cucumber leaves under B1T1 and B2T1 treatments when measured 14 and 19 days after transplanting, respectively. Different from downy mold infection, with the aggravation of drought degree, the intercellular CO_2 concentration of cucumber leaves decreased gradually [30]. However, when downy mildew infection and drought stress occurred simultaneously, it can be seen from Figure 2b that drought stress had a greater impact on the intercellular CO_2 concentration of cucumber leaves.

From Figure 2d, it can be seen that at 14 days after transplanting, compared with treatment B2T1, the transpiration rate of leaves under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 20.81%, 30.63%, 40.74%, 26.17% and 34.42%, respectively, and at 19 days after transplanting, compared with treatment B2T1, the transpiration rate of leaves under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 40.47%, 50.03%, 57.36%, 38.52% and 44.38%, respectively. Cucumber leaves infected with downy mildew will increase the water loss of the leaves by increasing the transpiration rate. There are some differences between the results of this study and those of a related study [23]. The inconsistency between the results of this study and their results may be due to the short research period of this experiment. At the same time, these are also affected by many environmental factors and agricultural practices, such as atmospheric CO_2 concentration, light incidence, nutrient solution irrigation and agricultural tillage [31–33].

3.2. Cucumber Plants Growth Parameters

The influence of different nutrient solution irrigation treatments and downy mildew infection on the plant height, stem diameter and leaf area of cucumber is shown in Table 1. The plant height, stem diameter and leaf area showed different responses under different nutrient solution irrigation treatments and downy mildew infection. Compared with treatment CK (B2T1), the plant height of cucumber under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 11.41%, 19.05%, 27.48%, 7.55% and 10.62%, respectively; the stem diameter of cucumber under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 5.70%, 13.45%, 23.03%, 9.46% and 15.74%, respectively; the leaf area of cucumber under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 22.79%, 38.68%, 58.28%, 13.76% and 29.96%, respectively. However, there was no significant difference ($p > 0.05$) in the plant height and stem diameter of cucumber under the B2T2 and B2T3 treatments, and there was no significant difference ($p > 0.05$) in the plant height and leaf area of cucumber under the B1T1 and B2T3 treatments. With the increase in drought stress, cucumber plant height, stem diameter and leaf area showed a downward trend. However, compared with drought stress treatment, the plant height, stem diameter and leaf area of cucumber decreased more obviously when downy mildew infection and

drought stress occurred simultaneously. From the above analysis, we can know that when drought stress occurred or downy mildew infection and drought stress occurred at the same time, the effect on cucumber leaf area was the greatest, while the effect on the cucumber stem diameter was the weakest. The results of plant height in this experiment are consistent with previous studies. The results of stem diameter and leaf area, however, are slightly different [21,34]. The reason may be that previous studies mainly focused on abiotic stress or a single biological stress. The experimental conditions were different [28,35,36]. In addition, it may also be related to the different cucumber varieties studied. Previous studies mainly focused on the effect of downy mildew infection on the disease-resistant and non-disease-resistant varieties of cucumber [37].

Table 1. The influence of different nutrient solution irrigation treatments and downy mildew infection on growth parameters of cucumber plants.

Sample	Plant Height (cm)	Stem Diameter (mm)	Leaf Area (cm^2)
B1T1	100.9 ± 1.35 c	8.27 ± 0.36 b	3036.99 ± 172.06 bc
B1T2	92.2 ± 4.95 d	7.59 ± 0.38 c	2412.13 ± 183.69 c
B1T3	82.6 ± 1.75 e	6.75 ± 0.12 d	1641.23 ± 233.46 d
B2T1	113.9 ± 6.55 a	8.77 ± 0.39 a	3933.67 ± 198.06 a
B2T2	105.3 ± 1.93 b	7.94 ± 0.10 bc	3392.46 ± 253.85 b
B2T3	101.8 ± 1.43 bc	7.39 ± 0.52 c	2755.29 ± 272.14 c

Note: Values within the same columns followed with different lowercase letters are significantly different at ($p < 0.05$).

3.3. Parameters of Root Systems

The influence of different nutrient solution irrigation treatments and downy mildew infection on the root system parameters of cucumber are shown in Table 2. Under different treatment conditions, there were significant differences ($p < 0.05$) in the total length of root, the surface area of root and the total tips of root. Compared with treatment CK (B2T1), the total length of root under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 32.98%, 42.96%, 48.62%, 9.62% and 21.54%, respectively; the surface area of root under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 49.97%, 52.07%, 52.75%, 9.04% and 26.39%, respectively; the average diameter of root under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 46.51%, 56.17%, 62.38%, 19.07% and 28.28%, respectively; the total volume of root under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 56.36%, 59.63%, 64.03%, 11.69% and 31.21%, respectively; the total tips of root under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 48.72%, 53.92%, 59.87%, 16.55% and 28.10%, respectively. From the above analysis, we can know that the total length, surface area, average diameter, total volume and total tips of the root showed different responses under different nutrient solution irrigation treatments and downy mildew infection, and unlike drought stress, there is no significant difference ($p > 0.05$) in surface area, average diameter and total volume of root when downy mildew infection and drought stress occur simultaneously. The results are different from total length and total tips of root in this experiment. Although we used a protective cleaning method to wash and tidy the root system, experimental errors may also occur when root washing is performed [1,38].

Table 2. The influence of different nutrient solution irrigation treatments and downy mildew infection on root systems parameters of cucumber plants.

Samples	Total Length (cm)	Surface Area (cm^2)	Average Diameter (mm)	Total Volume (cm^3)	Total Tips
B1T1	1567.826 ± 51.426 d	314.157 ± 23.175 d	1.672 ± 0.095 c	6.148 ± 0.864 c	5147 ± 194 d
B1T2	1334.482 ± 34.571 e	300.972 ± 30.509 d	1.370 ± 0.072 c	5.688 ± 1.424 c	4626 ± 53 de
B1T3	1202.089 ± 4.481 f	296.701 ± 12.071 d	1.176 ± 0.012 c	5.067 ± 0.708 c	4208 ± 318 e
B2T1	2339.397 ± 41.638 a	627.987 ± 24.295 a	3.126 ± 0.521 a	14.088 ± 1.721 a	10,038 ± 931 a
B2T2	2114.360 ± 78.358 b	571.236 ± 16.316 b	2.530 ± 0.035 b	12.441 ± 0.631 a	8377 ± 328 b
B2T3	1835.504 ± 53.806 c	462.242 ± 7.401 c	2.242 ± 0.190 b	9.691 ± 0.598 b	7217 ± 490 c

Note: Values within the same columns followed by different lowercase letters are significantly different at ($p < 0.05$).

3.4. Cucumber Plant Parameters

The effects of nutrient solution irrigation treatments and downy mildew infection on the biomass of cucumber plants are shown in Figure 3. As we can see from Figure 3, the biomass of cucumber plants showed different responses under different nutrient solution irrigation treatments and downy mildew infection. Compared with treatment CK (B2T1), the fresh weight of cucumber leaves under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 17.1%, 27.75%, 46.28%, 13.74% and 30.97%, respectively; the dry weight of cucumber leaves under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 18.52%, 32.80%, 55.03%, 11.77% and 25.77%, respectively; the fresh weight of cucumber stem under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 23.22%, 24.46%, 49.65%, 3.83% and 27.10%, respectively; the dry weight of cucumber stem under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 27.72%, 37.82%, 53.37%, 8.03% and 34.20%, respectively; the fresh weight of cucumber root under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 48.27%, 57.02%, 71.07%, 13.87% and 40.34%, respectively; the dry weight of cucumber root under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 36.94%, 50.17%, 52.34%, 28.63% and 34.04%, respectively. It can be seen from Figure 3 that there was no significant difference ($p > 0.05$) in the fresh weight and dry weight of cucumber leaves or in the fresh weight and dry weight of cucumber stems under the B1T1, B1T2 and B2T3 treatments, and there was no significant difference ($p > 0.05$) in the fresh weight and dry weight of cucumber roots under the B1T1 and B1T2 treatments.

The photosynthetic rate and formation rate and the amount of assimilate decreased when the plants were infected by pathogens and under water-deficit stress. In order to adapt to the new living environment, on the one hand, plants use limited assimilates to synthesize some substances to maintain certain permeability of cells and tissues to adapt to pathogen infection and drought environment, on the other hand, they continue to provide other cells and tissues to maintain a certain growth rate. Pathogen infection and drought inhibited the outward transportation of sucrose from the mesophyll cells but promoted the loading of the phloem, while the long-distance transportation of sucrose was not affected. Pathogen infestation and drought lead to a decrease in the rate of assimilation formation and the preferential distribution of assimilation to synthetic osmotic adjustment substances will inevitably affect the root–shoot ratio of plants. The influence of different nutrient solution irrigation treatments and downy mildew infection on the root–shoot ratio of cucumber is shown in Figure 4.

As we can see from Figure 4, the root–shoot ratio of cucumber showed different responses under different nutrient solution irrigation treatments and downy mildew infection. The root–shoot ratio of cucumber under the B1T1, B1T2, B1T3, B2T1, B2T2 and B2T3 treatments was 3.16%, 2.99%, 4.11%, 3.92%, 3.13% and 3.63%, respectively. The root–shoot ratio of cucumber was the highest under the B1T3 treatments. When plants are infected by pathogenic and water-deficit stress, the degree of inhibition of root growth is much lower than that of aboveground growth. Therefore, limited assimilation is preferentially distributed to the root system, resulting in an increase in root–shoot ratio [21,39]. Leaf water status is severely affected by pathogenic and water-deficit stress at the cellular levels and encompasses disruptions in plant metabolism. On the other hand, due to the destruction of the leaf cuticle and increase in cell membrane permeability, water loss from the infected leaf areas was increased in plants which were infected by pathogens [40]. Therefore, growth is inhibited under the stress of water and pathogenic bacteria, which may be due to the increase in respiration rate and the decrease in photosynthetic activity, resulting in a decrease in total biomass.

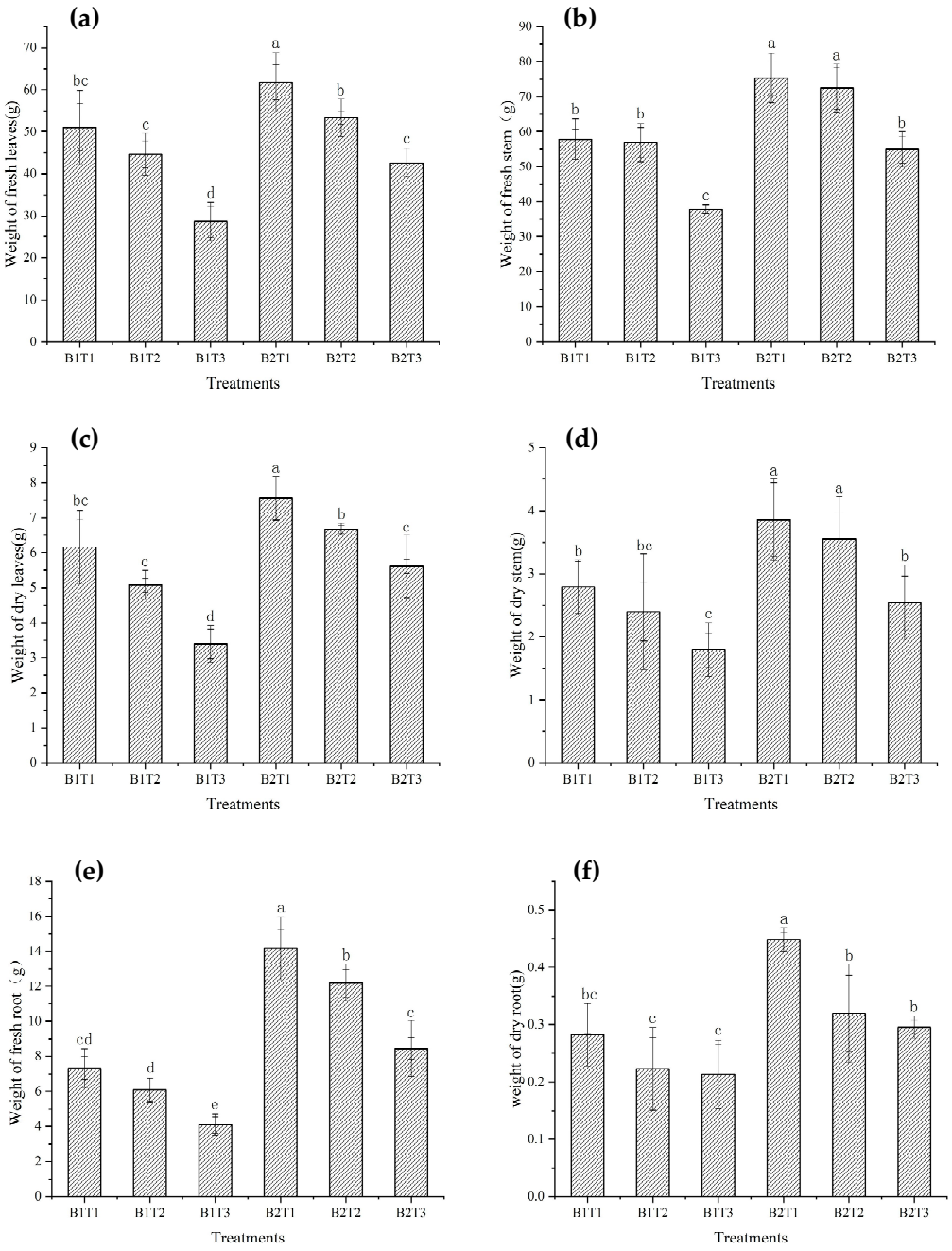

Figure 3. The influence of different nutrient solution irrigation treatments and downy mildew infection on biomass of cucumber plants. Note: Error bars indicate standard deviations, with different lowercase letters between treatments indicating significant differences at ($p < 0.05$). (**a**) Weight of fresh leaves; (**b**) Weight of fresh stem; (**c**) Weight of dry leaves; (**d**) Weight of dry stem; (**e**) Weight of fresh root; (**f**) Weight of dry root.

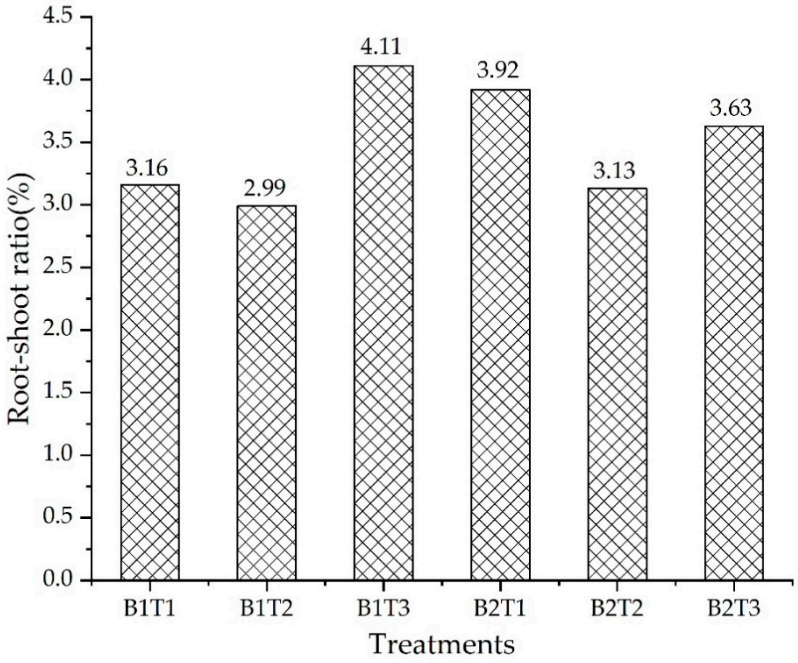

Figure 4. The influence of different nutrient solution irrigation treatments and downy mildew infection on the root–shoot ratio of cucumber.

4. Conclusions

Six treatments, i.e., B1T1, B1T2, B1T3, B2T1, B2T2 and B2T3, with downy mildew infestation at two levels, B1 (disease infestation) and B2 (no disease infestation), along with three fertigation requirement levels, full fertigation T1, moderate nutrient solution deficit T2 and severe nutrient solution deficit T3, influenced crop growth, leaf gas-exchange, material accumulation and parameters of root systems of cucumber grown in a greenhouse. The leaf gas-exchange parameters were significantly increased under the CK (B2T1) treatment, and leaf photosynthesis rate, transpiration rate and stomatal conductance were significantly decreased under the B1T1 treatment. Leaf intercellular CO_2 concentration was significantly increased under the B1T1 treatment during the whole experiment period. Leaf photosynthesis rate, transpiration rate, intercellular CO_2 concentration and stomatal conductance were significantly decreased under the B1T2, B1T3, B2T2 and B2T3 treatments during the whole experiment period. Compared with treatment CK (B2T1), the plant height of cucumbers under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 11.41%, 19.05%, 27.48%, 7.55% and 10.62%, respectively; the stem diameter of cucumber plants under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 5.70%, 13.45%, 23.03%, 9.46% and 15.74%, respectively; and the leaf area of cucumber plants under the B1T1, B1T2, B1T3, B2T2 and B2T3 treatments decreased by 22.79%, 38.68%, 58.28%, 13.76% and 29.96%, respectively. The root–shoot ratio of cucumber showed different responses under different nutrient solution irrigation treatments and downy mildew infection. The root–shoot ratio of cucumber under the B1T1, B1T2, B1T3, B2T1, B2T2 and B2T3 treatments was 3.16%, 2.99%, 4.11%, 3.92%, 3.13% and 3.63%, respectively. The root–shoot ratio of cucumber was the highest under disease infestation and the severe nutrient solution deficit treatment.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/12/1921/s1, Table S1: Component of standard nutrient solution; Table S2: Grading of cucumber downy mildew severity; Figure S1: Extraction of cucumber downy mildew spores.

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