Effects of downy mildew fungi spore infection on cucumber plants under nitrogen stress

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Abstract: Investigating the interplay between biotic stress from downy mildew and abiotic stress from nitrogen deficiency is crucial for improving crop management measures and enhancing cucumber production. A greenhouse experiment was conducted using two pathogen treatments—non-infected (B1) and infected with Pseudoperonospora cubensis (B0)—across three nitrogen levels: deficiency (N1: 50%), optimal (N2: 100%), and excess (N3: 150%). The resulting six treatment combinations (B1N1, B1N2/Control, B1N3, B0N1, B0N2, and B0N3) revealed that downy mildew infection and nitrogen application rates significantly influenced key physiological and biochemical parameters (p<0.05). These included sucrose, soluble sugar, hydrogen peroxide (H_2O_2), catalase (CAT), superoxide dismutase (SOD), polyphenol oxidase (PPO), and malondialdehyde (MDA), as well as the fresh and dry weights of the leaves, stems, and roots. Among all groups, the combination of infection and nitrogen deficiency (B0N1) had the most significant impact on biomass accumulation and hormone metabolism. Compared to the B1N2 control, B0N1 led to substantial reductions in sucrose (52.83%), soluble sugar (68.67%), leaf fresh weight (56.67%), leaf dry weight (55.51%), stem fresh weight (52.82%), stem dry weight (57.28%), root fresh weight (32.46%), and root dry weight (54.07%). This study clarifies the interactive physiological responses of cucumbers to combined biotic and abiotic stress. It is of great significance for facilitating the control of downy mildew and the improvement of cucumber yield in sustainable agriculture.

Keywords: *Cucumis sativus* L., biotic stress, abiotic stress, physiological metabolism, substance accumulation
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1 Introduction

Cucumber (*Cucumis sativus* L.) is a globally important vegetable crop that occupies a prominent position in protected agriculture[1]. Valued for its vitamins, minerals, and diverse biologically active substances, the cucumber holds high economic significance[2]. However, with the continuous expansion of protected cultivation areas, cucumber production faces various stresses[3]. Among them, downy mildew and nitrogen imbalance are critical factors limiting yield and quality[4]. Investigating the effects of downy mildew and nitrogen stress on the physiological metabolism and biomass accumulation of cucumbers is essential for revealing stress-response mechanisms and optimizing cultivation strategies[5].

Studies demonstrate that downy mildew significantly reduces photosynthetic efficiency, disrupts chloroplast ultrastructure, and compromises synthesis and stability of photosynthetic pigments. Furthermore, infection disrupts carbon metabolism, leading to an imbalance in the synthesis, transport, and distribution of carbohydrates, thereby stunting fruit development and yield[6]. Under downy mildew stress, the antioxidant system of cucumber plants undergoes dramatic alterations, and oxidative damage, driven by the imbalance of reactive oxygen species (ROS) metabolism, further exacerbates plant degradation[7]. Nitrogen, a vital macronutrient for plant growth and development, plays a key role in the physiological metabolism and material accumulation of cucumbers[8]. As an essential element, it is the main component of proteins, nucleic acids, chlorophyll, and various secondary metabolites. Nitrogen is also directly involved in essential physiological processes such as photosynthesis, respiration, and nitrogen metabolism[9]. An adequate nitrogen supply can stimulate the vegetative growth of cucumber plants, increase leaf area, and enhance photosynthetic capacity, thereby providing the necessary substances and energy for fruit development[10]. However, in current agronomic practice, excessive nitrogen application is prevalent due to imprecise fertilization strategies and suboptimal management[11]. This not only leads to low nitrogen utilization rate, causing resource waste and environmental pollution, but also triggers problems such as excessive growth of cucumber plants and decreased disease resistance[12]. On the contrary, nitrogen deficiency will cause slow growth of plants, yellowing of leaves, decreased synthesis of photosynthetic products, hindered fruit development, and significantly decreased yield and quality. Research indicates that nitrogen stress (including nitrogen deficiency and excess nitrogen) can affect the activities of nitrogen metabolism-related enzymes in

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cucumbers, such as nitrate reductase (NR) and glutamine synthase (GS), thus changing the synthesis and degradation rates of amino acids and proteins[13]. At the same time, nitrogen nutrition status is also closely related to the balance of carbon and nitrogen metabolism. Nitrogen stress can disrupt the coordination between these metabolic pathways, affecting the accumulation and distribution of carbohydrates[14,15].

While the individual effects of downy mildew and nitrogen stress on cucumber growth have been extensively studied globally, crops frequently encounter multiple concurrent stresses under field conditions. However, the combined impact of downy mildew and nitrogen stress on physiological metabolism and biomass accumulation of cucumbers remains insufficiently explored. Therefore, this study investigates the interactive effects of these two factors on cucumber physiology and material accumulation, providing a critical reference for developing integrated pest control and fertilization management strategies.

2 Materials and methods

2.1 Study site and experiment design

The experiment was conducted in a Venlo-type greenhouse at Jiangsu University. The facility is equipped with a thermal insulation shield, pipeline heating, and a cooling system comprising shading nets and evaporative wet curtain fans. Environmental parameters were regulated by a computerized automatic control system. Cucumber cultivar 'Jinyou No.1' provided by Tianjin Academy of Agricultural Sciences was used. A two-factor factorial design was adopted: two pathogen treatments (inoculated B0, non-inoculated B1) and three nitrogen levels, forming six treatments: B0N1, B0N2, B0N3, B1N1, B1N2 (control), B1N3. Each treatment contained 6 plants, totaling 36 plants. To prevent cross infection, non-inoculated groups (B1N1, B1N2, B1N3) were placed in three cultivation troughs on the west side of the greenhouse, while inoculated groups (B0N1, B0N2, B0N3) were arranged on the east side. Plants were cultivated in double rows per trough, with trough spacing 60 cm, in-row spacing 50 cm, and inter-row spacing 32 cm. The experimental design is shown in Figure 1.

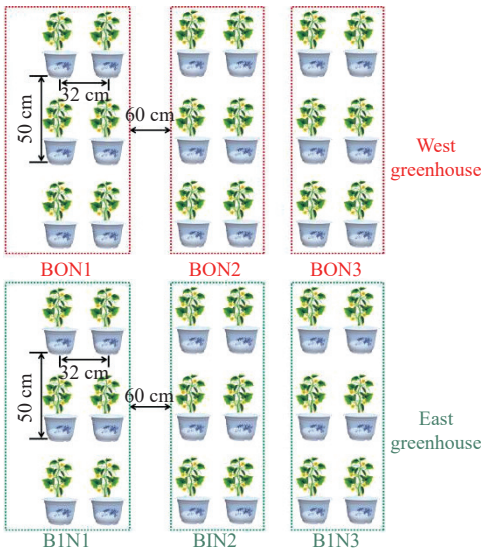


Figure 1 Schematic diagram design of the experimental cucumber planting

The experiment lasted from July 25 to August 18, 2024, with average temperature 25.80°C (16.83°C-36.47°C) and average relative humidity 85.6% (23.7%-95.6%). Perlite was used as the cultivation substrate to reduce matrix interference. Perlite was rinsed and placed into 10 L pots on July 29, 2024. Uniform cucumber seedlings at two-true-leaf stage were transplanted on July 30. Three nitrogen levels (50%, 100%, 150%) were set with reference to previous literature (Table 1)[6]. Nutrient solutions were diluted 100 times and evenly applied to ensure nutrient consistency. Each plant was supplied with 400 mL of nutrient solution daily between 8:00 and 9:00 am.

Table 1 Components of nutrient solution

Nutrient solution group	Chemical reagent	N-50%	N-100%	N-150%
A	$Ca(NO_3)_2·4H_2O$	413 g	826 g	826 g
	KNO_3	303 g	606 g	606 g
B	$NH_4H_2PO_4$	57 g	114 g	144 g
	$MgSO_4·7H_2O$	492 g	492 g	492 g
	$NaNO_3$	0	0	552 g
	KCl	223 g	0	0
	$CaCl_2$	194 g	0	0
C	NaFe-EDTA	7.00 g	7.00 g	7.00 g
	$MnSO_4$	1.70 g	1.70 g	1.70 g
	$ZnSO_4$	1.45 g	1.45 g	1.45 g
	$CuSO_4$	0.19 g	0.19 g	0.19 g
	Na_2MoO_4	0.12 g	0.12 g	0.12 g
	$Na_2B_4O_7$	2.45 g	2.45 g	2.45 g

Fresh infected cucumber leaves were collected, and typical downy mildew lesions were cut and soaked in sterile water to prepare spore suspension. On August 6 afternoon, the spore suspension ($1×10^6$ spores/mL) was sprayed onto the abaxial leaf surface of inoculated plants, while control plants were sprayed with sterile water. Disease severity was graded according to GB/T 17980.26-2000 (Table 2)[16].

Table 2 Grading of cucumber downy mildew severity

Disease level	Symptoms described
Level 0	Asymptomatic
Level 1	Diseased spot area occupies less than 5% of the leaf area
Level 3	Diseased area accounts for 6% to 10% of the leaf area
Level 5	Diseased area accounts for 11% to 25% of the leaf area
Level 7	Diseased area accounts for 26% to 50% of the leaf area
Level 9	Diseased spot area accounts for more than 50% of the leaf area

2.2 Experimental data acquisition

On the 15th day after transplantation (Level 3), leaf physiological indices were determined. MDA content was measured by thiobarbituric acid (TBA) method; SOD activity was determined using cytochrome C reduction method; H_2O_2 content and CAT activity were detected by ammonium molybdate colorimetry; PPO activity was assayed by spectrophotometry at 420 nm using catechol as substrate; soluble sugar content was measured by anthrone colorimetry; sucrose content was determined at 480 nm by resorcinol color reaction. Determination kits for malondialdehyde (MDA), superoxide dismutase (SOD), hydrogen peroxide (H_2O_2), catalase (CAT), polyphenol oxidase (PPO), and soluble sugar in cucumber leaves were purchased from Chengjian Institute of Bioengineering. The kit for determining sucrose content in cucumber leaves was purchased from Beijing Suoleibao Technology Co., Ltd., and the detection equipment was 721 visible spectrophotometers (Shanghai Youke Instrument Co., Ltd.).

On the 20th day after transplantation (Level 7), leaves, stems, and roots were separately sampled. Fresh weights of leaves and stems were weighed with 0.01 g precision electronic balance; root

fresh weight was measured after absorbing surface water with 0.001 g precision balance. Samples were heated at 105°C for 15 min to inactivate enzymes, then dried to constant weight at 80°C, and dry weights were recorded with 0.001 g precision balance.

2.3 Statistical analyses

Experimental data were statistically analyzed using SPSS 29.0.2.0. One-way ANOVA and LSD test were performed to evaluate significant differences (p<0.05). Figures were drawn using Origin 2018. Error bars represent standard deviations, and different lowercase letters indicate significant differences at p<0.05.

3 Results and discussion

3.1 Sucrose and soluble sugar

Downy mildew and nitrogen stress significantly altered the concentrations of sucrose and soluble sugar in cucumber leaves (p<0.05). Furthermore, a significant interaction was observed between downy mildew infection and nitrogen levels regarding their impact on carbohydrate content.

As illustrated in Figure 2a, sucrose levels in cucumber leaves were significantly lower across all treatments compared to the B1N2 control. Specifically, sucrose content decreased by 52.8%, 31.5%, 39.9%, 32.4%, and 14.9% in the B0N1, B0N2, B0N3, B1N1, and B1N3 treatments, respectively. Similarly, soluble sugar content followed a comparable trend (Figure 2b), with reductions of 68.7%, 37.8%, 60.6%, 34.6%, and 17.1% observed in the B0N1, B0N2, B0N3, B1N1, and B1N3 groups, respectively, relative to the B1N2 control.

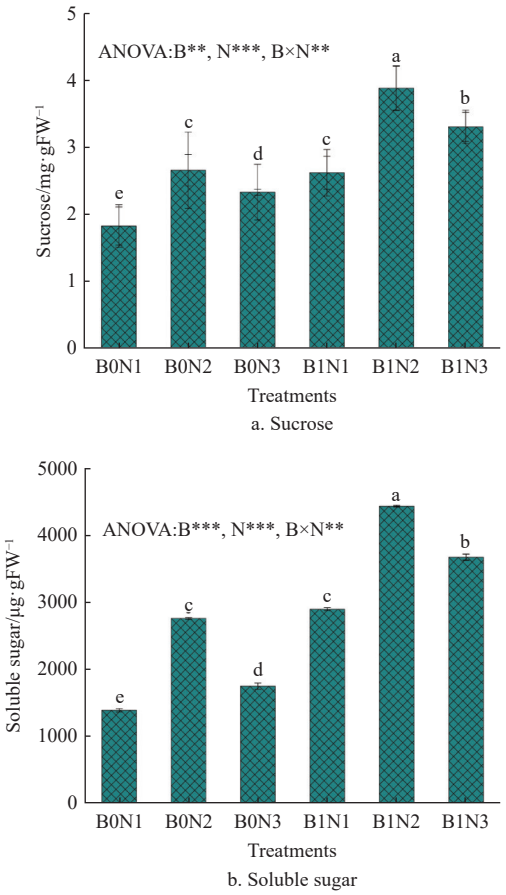


Figure 2 Effects of downy mildew infection and nitrogen stress on sucrose and soluble sugar for cucumber plants

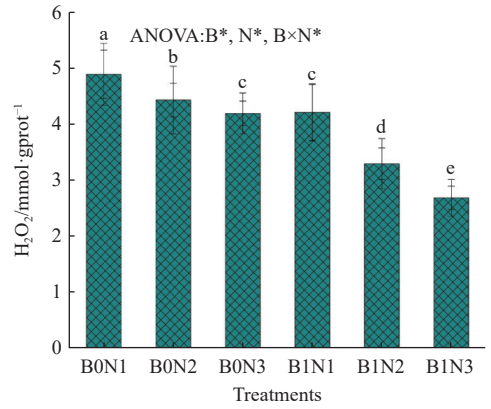
Upon infection with downy mildew, in order to defend against the invasion of spores, cucumbers will activate a series of defense responses to thwart pathogen proliferation, consuming a large amount of energy and substances[17]. Consequently, photosynthetic products originally destined for sucrose and soluble sugar synthesis are prioritized for defense-related pathways, leading to a concomitant decrease in primary carbohydrate content[18]. Under nitrogen deficiency, vegetative growth slows, protein synthesis is impaired, and both cell division and elongation are hindered. This leads to an inadequate accumulation of photosynthetic products and a subsequent decrease in the raw materials needed for the synthesis of soluble sugars and sucrose, ultimately reducing their content. In contrast, when nitrogen is excessive, cucumbers prioritize the utilization of photosynthetic products for the synthesis of nitrogen-containing compounds, such as proteins and amino acids. This shift in metabolic partitioning diminishes the conversion and distribution of photosynthetic products into sucrose and soluble sugar is diminished[19]. This also leads to a decrease in the content of sucrose and soluble sugar. When downy mildew and nitrogen stress coexist, the two will produce a synergistic effect, and the impact on sucrose and soluble sugar in cucumbers is more complex and severe. Meanwhile, under the combined effect of the two stresses, the plants' stress resistance significantly decreases, causing their physiological regulatory mechanisms to prioritize maintaining basic life functions and defense responses. This shift further inhibits the synthesis and accumulation of sucrose and soluble sugars.

3.2 Hydrogen peroxide (H_2O_2) and catalase (CAT)

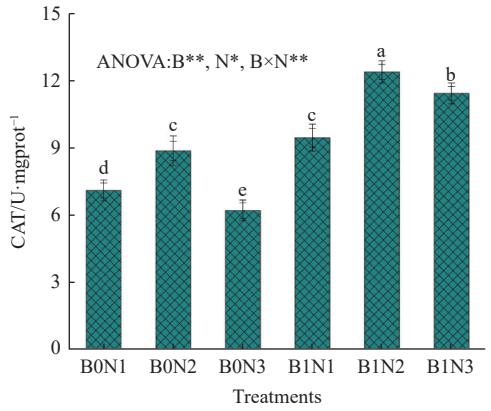
The impacts of downy mildew and nitrogen stress on the concentrations of hydrogen peroxide (H_2O_2) and catalase (CAT) in cucumber leaves are presented in Figure 3. Both stressors significantly affected these parameters (p<0.05), and a significant interaction was observed between downy mildew infection and nitrogen application levels regarding accumulation and CAT enzymatic activity.

As shown in Figure 3a, levels in the B0N1, B0N2, B0N3, and B1N1 treatments increased significantly by 48.4%, 34.4%, 27.2%, and 27.8%, respectively, relative to the B1N2 control. In contrast, the content in the B1N3 group decreased by 18.4%. Correspondingly, CAT enzymatic activities in the B0N1, B0N2, B0N3, B1N1, and B1N3 treatments exhibited reductions of 42.7%, 28.4%, 49.9%, 23.7%, and 7.7%, respectively, compared to the B1N2 control.

Once cucumbers are infected with downy mildew, a range of physiological changes occurs within the host plant. The invasion of pathogenic spores can trigger the defense response of cucumbers, which includes the production of reactive oxygen species[18]. At the early stage of the disease, cucumber plants will actively accumulate H_2O_2, which serves as a signaling molecule to activate the plant's defense genes and initiate protective mechanisms to resist the pathogenic invasion. However, as the disease progresses, the accumulation of H_2O_2 may surpass the plant's scavenging capacity, resulting in excessive buildup within the cells. This surplus of H_2O_2 causes oxidative damage, including the disruption of cell membrane integrity and harm to cellular organelles. To cope with this oxidative stress, the activity of antioxidant enzymes in cucumbers undergoes significant shifts. In the early phase of the disease, the activity of these enzymes increases rapidly to neutralize excess H_2O_2 and maintain cellular redox balance. Conversely, as the condition worsens, enzymatic activity may be inhibited[20]. This is attributed to the massive reproduction of pathogenic spores and the secretion of toxins, which disrupt normal cellular metabolism and impair the synthesis and function of enzymes. This subsequently leads to a decrease in antioxidant efficiency, compromising H_2O_2 clearance and further exacerbating oxidative cellular damage[21].



a. Hydrogen peroxide (H_2O_2)



b. Catalase (CAT)

Figure 3 Effects of downy mildew infection and nitrogen stress on hydrogen peroxide (H_2O_2) and catalase (CAT) for cucumber plants

When downy mildew and nitrogen stress occur simultaneously, cucumber plants undergo multifaceted physiological strain, leading to complex and intensified fluctuations in H_2O_2 levels and antioxidant enzyme activities. The pathogen invasion and subsequent defense response triggered by downy mildew induce a large-scale surge in H_2O_2 production[22]. The synergistic effect of these dual stressors leads to a sharp increase in intracellular H_2O_2 concentrations. Regarding the H_2O_2 enzyme, although the enzyme activity attempts to increase in the early stage of stress to clear H_2O_2, due to the severe damage caused by downy mildew and nitrogen stress to the growth, metabolism, and cell structure of plants, the synthesis of enzymes, the maintenance of active centers, and the stability of enzyme proteins are all greatly affected, resulting in a rapid decline in the activity of H_2O_2 enzyme during the later stages of stress. This leads to the ineffective removal of excessive H_2O_2. The resultant accumulation, coupled with the diminished antioxidant capacity, causes severe oxidative damage to cucumber plants. This further inhibits growth and development, potentially leading to systemic plant mortality[23].

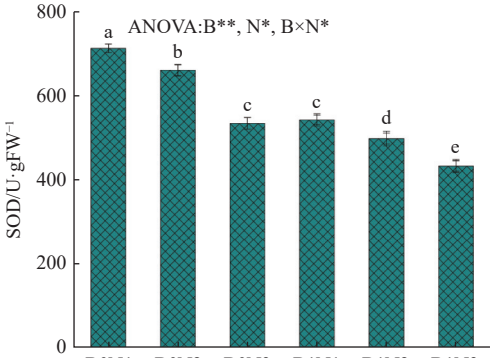
3.3 Superoxide dismutase (SOD), polyphenol oxidase (PPO), malondialdehyde (MDA)

The activities of superoxide dismutase (SOD) and polyphenol oxidase (PPO), alongside malondialdehyde (MDA) levels in cucumber leaves, are presented in Figure 4. Downy mildew and nitrogen stress significantly influenced SOD, PPO, and MDA concentrations (p<0.05). The effects of downy mildew and nitrogen fertilizer stress on the enzymatic activities of SOD, PPO, and MDA in cucumber plants have a significant interactive effect. The SOD activities in cucumber leaves of treatments B0N1, B0N2, B0N3, and B1N1 increased by 42.93%, 32.51%, 7.21%, and 8.90%, respectively, compared to B1N2, as shown in Figure 4a. In contrast, the SOD activities in treatment B1N3 decreased by 13.05%. The PPO activities in cucumber leaves increased by 31.84%, 20.81%, and 8.31% for B0N1, B0N2, and B1N1, respectively, compared with B1N2, as shown in Figure 4b. In contrast, the PPO activities of B0N3 and B1N3 decreased by 3.74% and 12.19%. The MDA activities of treatments B0N1, B0N2, B0N3, B1N1, and B1N3 increased by 47.54%, 9.95%, 34.5%, 30.39%, and 21.92%, respectively, compared with B1N2, as shown in Figure 4c. Furthermore, Figure 4 demonstrates that the trends of SOD and PPO enzymatic responses under downy mildew and nitrogen stress are consistent. However, under downy mildew infection (B0N2 treatment), the synergy between the pathogen and nitrogen stress had the most pronounced impact on MDA levels. Notably, the MDA content in this treatment group was significantly lower than that observed in the B0N1 and B0N3 experimental groups.

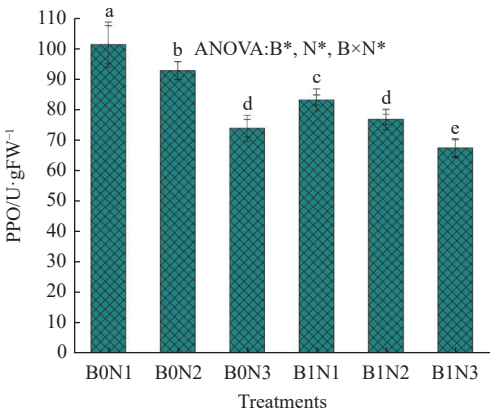
Following the invasion of cucumber plants by the downy mildew pathogen, PPO activity increases significantly during the initial stages of infection, maintaining a relatively high level for a sustained duration[21]. However, if the infection becomes severe and cellular structure is extensively compromised, PPO activity gradually declines due to the degradation of the enzyme's protein structure. Nitrogen stress—encompassing both deficiency and excess—disrupts the physiological metabolism of cucumbers, thereby modulating PPO activity. Under conditions of nitrogen excess, plant growth is stunted, protein synthesis is inhibited, and the overall metabolic rate diminishes. This leads to a compromised defense capacity and an inadequate response to external stressors, ultimately resulting in decreased PPO activity. Conversely, during nitrogen deficiency, although vegetative growth may appear robust, the imbalance between carbon and nitrogen metabolism interferes with the synthesis and allosteric regulation of PPO[17]. Under the concomitant pressure of downy mildew and nitrogen stress, the synergy between pathogenic colonization and nitrogen-induced metabolic disorders initially triggers the plant's defense mechanisms[23]. However, as the stress duration extends and intensity escalates, physiological functions are severely impaired. The confluence of insufficient nutrient supply, cellular disintegration, and disrupted enzymatic homeostasis causes PPO activity to plummet rapidly, thereby drastically weakening the plant's immune resilience[19].

As a pivotal antioxidant enzyme for scavenging superoxide anion radicals in cucumbers, SOD plays a critical regulatory role under various biotic and abiotic stresses. At the early stage of disease, the plants induce a rapid increase in SOD activity, which disproportionates harmful substances into hydrogen peroxide and oxygen to alleviate damage. However, with the continuous infection of pathogenic spores, the cell structure and metabolic function are damaged, affecting the synthesis of SOD and the stability of active centers, suppressing its activity and intensifying the accumulation of reactive oxygen species[6,17]. Initially, the plant upregulates SOD activity to eliminate free radicals. However, if nutrient deficiency is prolonged or severe, the scarcity of precursors for enzyme protein synthesis leads to a decline in activity. Conversely, nitrogen excess can trigger metabolic disorders and an overproduction of ROS, initially stimulating SOD activity. In the later stages, due to aggravated physiological damage—such as ion imbalance—this activity is inhibited[20]. When downy mildew occurs simultaneously with nitrogen stress, the production of reactive oxygen species far exceeds that under single stress. The activity of SOD initially

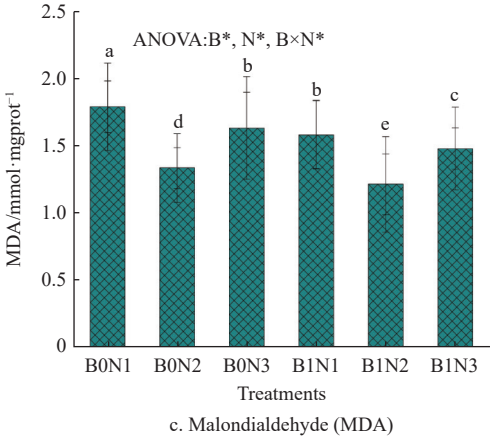
increases significantly to cope, but due to the severe impact of the double stress on its synthesis, active centers, and enzyme protein structure, it briefly increases and then rapidly decreases, resulting in a sharp decline in the plant's ability to scavenge free radicals and a large accumulation of reactive oxygen species, triggering a severe oxidative stress response and causing irreversible cell damage[21].



a. Superoxide dismutase (SOD)



b. Polyphenol oxidase (PPO)



c. Malondialdehyde (MDA)

Figure 4 Effects of downy mildew infection and nitrogen stress on superoxide dismutase (SOD), polyphenol oxidase (PPO), and malondialdehyde (MDA) for cucumber plants

Malondialdehyde (MDA) is the product of lipid peroxidation in cell membranes, and its content level can reflect the degree of cell membrane damage. As the disease progresses, the infection of pathogenic spores and the oxidation of reactive oxygen species will severely damage the structure and function of the plasma membrane, leading to a continuous rise in MDA content[23]. This indicates that the degree of cell membrane damage is constantly intensifying, and the normal physiological functions of cells are seriously affected, which in turn affects the growth and development as well as the disease resistance of cucumber plants. Nitrogen stress can destroy the stability of the cell membrane of cucumber plants, resulting in changes in the content of MDA. The synergistic effect of downy mildew and nitrogen stress will intensify the lipid peroxidation degree of the cell membrane of cucumber plants, and the MDA content will show an explosive increase. The infection of pathogenic spores destroys the structure of cell membranes[19]. Nitrogen stress affects the composition and function of cell membranes. Meanwhile, the massive accumulation of reactive oxygen species accelerates lipid peroxidation reactions. The significant surge in MDA content serves as a hallmark of severe membrane damage, signaling that intracellular physiological and biochemical processes have been disrupted. Consequently, plant development is severely inhibited, immune and stress tolerance capacities decline sharply, and the accumulation of such damage may ultimately lead to systemic senescence or plant mortality[20].

3.4 Substance accumulation

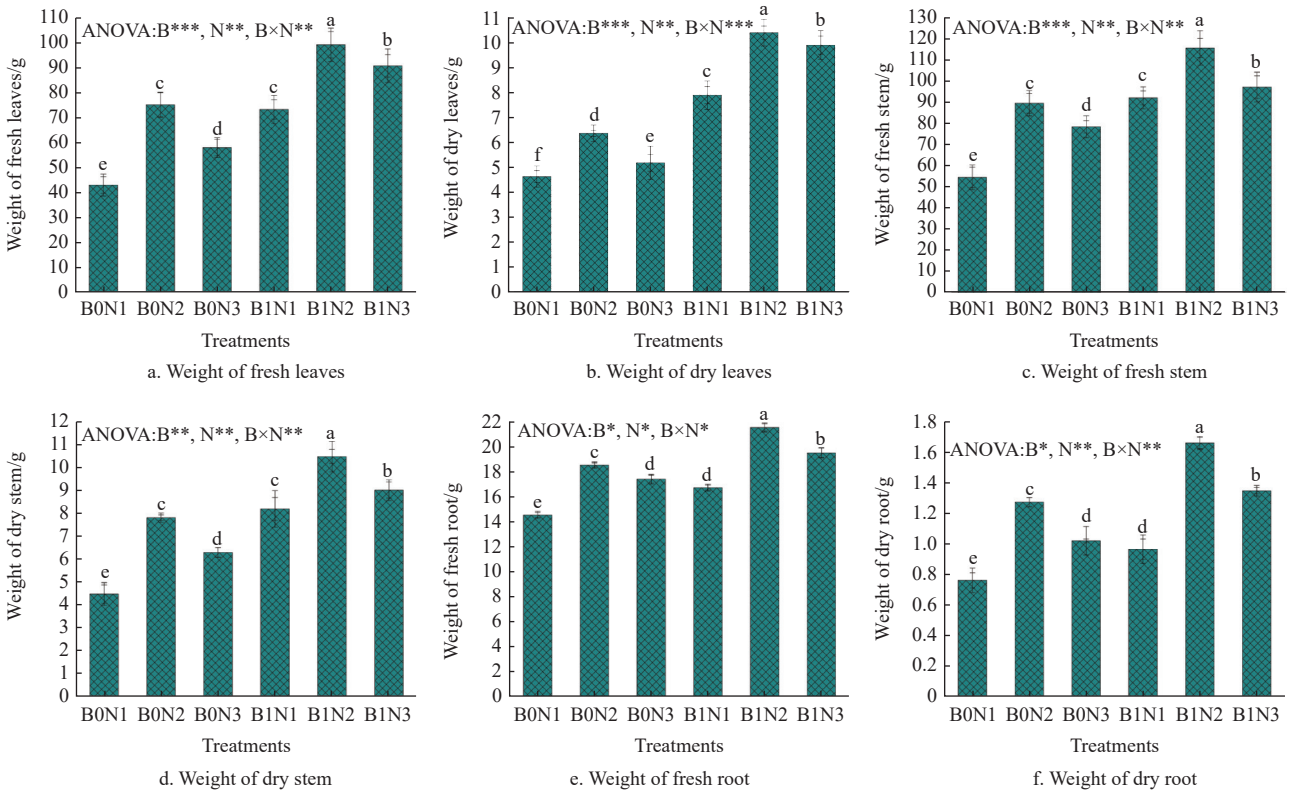
The dry and fresh biomass of cucumber leaves, stems, and roots are presented in Figure 5. Both nitrogen stress and downy mildew infection exerted significant effects on the biomass accumulation of all plant organs (p<0.05). Furthermore, a significant interaction effect was observed between downy mildew infection and nitrogen fertilization levels regarding leaf, stem, and root biomass. As shown in Figure 5a, compared to the B1N2 control treatment, the fresh leaf weight in the B0N1, B0N2, B0N3, B1N1, and B1N3 groups decreased by 56.67%, 24.25%, 41.55%, 26.14%, and 8.49%, respectively. Correspondingly, the dry leaf weight (Figure 5b) decreased by 55.51%, 38.76%, 50.13%, 24.08%, and 4.81% across the same treatments relative to B1N2. Regarding the aerial support structure, the fresh stem weight in B0N1, B0N2, B0N3, B1N1, and B1N3 decreased by 52.82%, 22.55%, 32.15%, 20.34%, and 15.93%, respectively, compared to B1N2 (Figure 5c). Similarly, the dry stem weight in these treatments exhibited reductions of 57.28%, 25.43%, 39.92%, 21.81%, and 13.98%, respectively (Figure 5d). The below-ground biomass followed a similar trend; fresh root weight decreased by 32.46%, 13.94%, 19.16%, 22.28%, and 9.38% under B0N1, B0N2, B0N3, B1N1, and B1N3 treatments, respectively, in comparison with B1N2 (Figure 5e). Finally, as illustrated in Figure 5f, the dry root weight for these treatments showed substantial declines of 54.07%, 23.28%, 38.5%, 41.8%, and 18.8%, respectively, relative to the control.

Upon infection with downy mildew, the leaves represent the primary site of physiological impairment. Due to compromised photosynthetic efficiency, the translocation of photoassimilates to the stems is significantly reduced. Consequently, stem growth is inhibited, manifesting as stunted elongation and reduced girth. Furthermore, the accumulation of both fresh and dry biomass in the stems is markedly lower than that of healthy control plants[19,24]. Similarly, the diminished supply of carbohydrates transported to the below-ground organs deprives the root system of essential nutrients, thereby suppressing growth and decreasing both fresh and dry root biomass[6]. Concurrently, the retardation of the above-ground organs exerts a negative feedback effect on the roots, impairing their capacity for water and nutrient uptake and leading to a decline in root vitality. This impediment to root development often results in morphological alterations, such as reduced lateral root density or restricted primary root elongation.

Under conditions of excess nitrogen, leaves frequently exhibit excessive elongation and reduced thickness, accompanied by increased succulence. Although fresh biomass may increase, the

accumulation of dry matter is relatively deficient, leading to a decreased dry-to-fresh weight ratio[25]. Nitrogen deficiency results in stunted stem growth, characterized by attenuated and weakened stems with shortened internodes, where the increase in biomass is negligible. This is attributed to insufficient nitrogen levels impairing the synthesis of proteins and other biomacromolecules, thereby inhibiting cellular division and elongation within the stem. In contrast, excessive nitrogen may induce vigorous vegetative growth[9].

Regarding root architecture, nitrogen deficiency prompts the root system to proliferate deeper into the soil matrix to forage for nutrients. This results in increased primary root length but a concomitant reduction in root diameter and lateral root density, ultimately limiting total root biomass. Conversely, excessive nitrogen inhibits root morphogenesis, leading to poorly developed architecture, altered structural integrity, and diminished root vitality. This weakens the uptake capacity for water and nutrients, further constraining fresh and dry root biomass[26].



a. Weight of fresh leaves b. Weight of dry leaves c. Weight of fresh stem

d. Weight of dry stem e. Weight of fresh root f. Weight of dry root

Figure 5 Effects of downy mildew infection and nitrogen stress on substance accumulation for cucumber plants

The concomitant effects of downy mildew and nitrogen stress exacerbate damage to the foliage. While downy mildew stunts above-ground growth and curtails the translocation of photoassimilates to the roots, nitrogen stress simultaneously impairs nutrient acquisition. This dual inhibition severely arrests root system development[14]. Consequently, the root system becomes increasingly attenuated, with a significant reduction in both density and longitudinal growth, alongside a notable decline in biomass. The resulting loss of root vitality and diminished absorptive capacity lead to systemic growth retardation and compromised stress resilience, rendering the plant highly susceptible to secondary pests and diseases[6,26].

4 Conclusions

To investigate the physiological responses of cucumber (*Cucumis sativus* L.) to biotic and abiotic stressors, two levels of downy mildew infection (infected, B0; non-infected, B1) and three nitrogen application rates—N1 (50% N, representing deficiency), N2 (100% N, representing optimal supply), and N3 (150% N, representing excess)—were evaluated under greenhouse conditions. The combination of these factors resulted in six experimental treatments: B0N1, B0N2, B0N3, B1N1, B1N2 (Control, CK), and B1N3. The findings demonstrate that downy mildew infection and divergent nitrogen fertilizer regimes exert significant effects (p<0.05) on key physiological and biochemical parameters in cucumber leaves.

While this study elucidated the effects of nitrogen stress and downy mildew infection on intracellular physiological metabolism and biomass accumulation, the scope was limited to the vegetative growth phase and did not account for impacts on reproductive development or marketable yield. Future research should extend these investigations to evaluate how synergistic stressors—including diverse pathogens and soil salinity—influence photosynthetic efficiency, overall plant architecture, and fruit quality. Such studies will provide a more robust theoretical framework for optimizing cucumber cultivation strategies and enhancing crop resilience.

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