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Thermal Infrared Evaluation of the Influence of Arbuscular Mycorrhizal Fungus and Dark Septate Endophytic Fungus on Maize Growth and Physiology

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Abstract: Thermal infrared imaging technology was used to understand the effects of arbuscular mycorrhizal fungi (AMF) and dark septate endophytic (DSE) fungi, both separately and together, on plant growth and physiological status, and to screen and develop efficient microbial agents in a pot experiment design. Eight treatments comprised the control (CK), AMF inoculation alone, DSE fungal treatments (DSE20%, DSE40% and DSE80%; 2, 4, 8 × 10^5 CFU mL^-1) and combined inoculation treatments (DSE20% + AMF, DSE40% + AMF, and DSE80% + AMF). Canopy temperature (T_{canopy}) and stomatal conductance (gs) were monitored at different growth stages, and plant biomass-related indicators were obtained at harvest. These indicators were used to assess plant growth and the physiological status resulting from the different inoculation treatments. During plant growth, the plant T_{canopy} decreased following inoculation. Differences in T_{canopy} between control and inoculated plants were detected by thermal infrared imaging technology and were −3.8 to + 9.3 °C (control–inoculation treatment). Growth index and T_{canopy} monitoring indicate that the growth-promoting effect of combined inoculation was higher than that of either fungal type alone, with DSE80% + AMF producing the highest growth promotion. During the growth process of inoculated maize, the effect of inoculated AMF on the physiological condition of maize growth can be better monitored by thermal infrared at 10 a.m., 12 p.m., 2 p.m. and 4 p.m. on the 31st–57th days of the growth period. The method and results of this experiment are conducive to the rapid and efficient monitoring of the effects of microorganisms on plant growth and physiological status and can be applied to the screening, application, and promotion of microbial agents.

Keywords: arbuscular mycorrhizal fungi; dark septate endophytic fungi; canopy temperature; thermal infrared; water conditions

1. Introduction

Arbuscular mycorrhizal fungi (AMF) and dark septate endophytic (DSE) fungi are potentially symbiotic microorganisms that occur widely in soils and plant roots in natural habitats [1–3]. They can coexist in the same plant individual but may exert different effects on plants [4].

AMF can form potentially symbiotic relationships with most terrestrial plant species [3]. There is considerable evidence that AMF have the potential to promote plant growth and development [5]. In particular, AMF promote plant nutrient uptake under poor soil...
conditions [3,6], enhance stress tolerance by increasing the net photosynthetic rate and the stomatal conductance (gs) of plant leaves [7,8], and increase yields [9]. The growth-promoting effects of AMF on plants may be due to the ability of AMF to form belowground hyphal networks with host plants and promote the root uptake of water and nutrients, thus encouraging plant growth and development [10–12]. In addition, AMF can also promote vegetation restoration, health, and sustainable development in ecosystems, especially in environmentally damaged areas such as coal-mining areas [13].

DSE fungi are widely distributed endophytic fungi that are conidial or sterile ascomycetes. They usually have brown-to-black hyphae and black septate hyphae [14]. These fungi can intercellularly colonize the root tissues of about 600 plant species belonging to 320 genera and 114 families. DSE fungi can promote plant growth [10,15,16] and are asymptomatic [16,17]. For example, in Gramineae, the DSE fungi isolated from healthy roots of Oryza granulata Nees et Arn. ex Watt. and Oryza glumaepatula Steud. can infect Oryza sativa (L.) and promote its growth without producing any disease symptoms [18,19]. Two DSE fungi isolated from O. glumaepatula and cultured in a previous study [19] increased plant height, nitrogen content, and chlorophyll content compared with uninoculated controls [14].

Both AMF and DSE fungi are prevalent in most terrestrial ecosystems [2,3]. Most previous studies have been concerned with the resource and distribution characteristics of AMF and DSE fungi in different ecosystems [4,20]. AMF and DSE fungi show different responses to the same environmental conditions [4]. For example, AMF are more suited to coastal conditions that have a thin humus layer, but DSE fungi prefer areas with high humus availability [4]. Two dominant plant species, Melandrium apetalum and Poa litwinowiana, have their roots colonized by fungi at an elevation of 5500 m on the glacier front of the Qinghai–Tibetan Plateau. AMF and DSE fungi are dominant in the roots of both species; AMF is dominant in M. apetalum and DSE fungi in P. litwinowiana [20].

Studies indicate that AMF and DSE fungi can be used as effective biological fertilizers or growth-promoting agents [5,14]. It is necessary to screen appropriate fungal agents according to the growth-promoting effect of fungal agents on plants. The purpose is to achieve high growth promotion with the minimum amount of fungal inoculum. Studies on the effects of AMF and DSE fungi, both separately and together in different ratios, on plant growth and physiological status, are, therefore, required to screen and develop new microbial agents. Numerous studies have evaluated the effects of microorganisms on plant growth, photosynthesis, stress tolerance, and gene expression [21,22]. Direct methods are time-consuming, often destructive, and unsuitable for monitoring, and a rapid, indirect monitoring method is therefore needed. Thermal infrared imaging is a rapid, indirect monitoring technology that has often been used to monitor plant physiological status in species such as sesame [23], winter wheat [24], coffee [25], almond trees [26], kiwifruit [27], and grapevine [27,28]. AMF inoculation can increase the stomatal conductance, reduce the leaf temperature, and increase the drought resistance of soybean under drought stress conditions [29].

Here, the effects of an AMF and a DSE fungus and their different ratios in combination on plant growth and physiological status were studied using thermal infrared imaging to address the following issues: (1) whether thermal infrared imaging is suitable for monitoring the physiological status of maize inoculated with AMF and DSE fungi; (2) changes in the physiological status of maize grown after fungal inoculation, with screening for optimum treatment agents or combinations; and (3) appropriate diurnal observation periods and observation times.

2. Materials and Methods

2.1. Pot Experiment

A pot experiment was conducted in the Microbial Reclamation Laboratory of the China University of Mining and Technology (Beijing). The experimental soil was a mixture of clay and sand. The clay was obtained from the Baorixile open-cast mine dump, Inner Mongolia, China, and the sand was river sand. The clay and sand were ground down, screened, then
sterilized at 121 °C for 2 h and dried without supplementary heating. The air-dried clay and sand were thoroughly mixed at a ratio of 1:1 (w/w) to obtain the mixed soil substrate. The plastic plant pots were 23.5 cm in outer diameter, 20.0 cm in inner diameter, 21.5 cm high, and 15.0 cm in bottom diameter. The pots were wiped with 75% (v/v) ethanol in water and rinsed several times with sterile water. Test soil (4.0 kg) was transferred into each pot and watered to 70% of the maximum water-holding capacity. After 48 h, solid NH$_4$NO$_3$, KH$_2$PO$_4$ and KNO$_3$ were added as basal fertilizers and mixed thoroughly to give soil contents of N, P and K of 100, 25 and 150 mg kg$^{-1}$, respectively. Maize (Zhongnuo No. 1) was used as the test plant. Maize seeds were acquired from the Zhongnongzuo Technology Development Co. Ltd., Beijing, China, immersed in 75% (v/v) ethanol solution for 5 min, and washed three times with sterile water. Maize seeds were surface-sterilized in 10% (w/v) sodium hypochlorite solution for 10 min, washed three times with sterile water and germinated in the dark for three days at 25 °C. The sources, propagation, and specifications of AMF and DSE fungi used in the experiment are shown in Table 1. DSE inoculum was prepared by aseptic growth in flasks of modified Melin–Norkrans (MMN) medium [30]. Alternaria sp. culture broth and Diversispora epigaea were sterilized at 121 °C for 30 min to obtain inactivated Alternaria sp. and Diversispora epigaea.

Table 1. Arbuscular mycorrhizal fungus (AMF) and dark septate endophytic (DSE) fungus, as used in the experiment.

| AMF | DSE Fungus |
|-----|------------|
| **Latin name** | Diversispora epigaea (formerly Glomus versiforme) | Alternaria sp. |
| **Source** | Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences | Stipa krylovii Roshev roots isolated from Beidian Shengli mine, China, and deposited in the China General Microbiological Culture Collection Center |
| **Deposit number** | BGC NM04B | CGMCC No. 17463 |
| **Propagation** | Laboratory of Microbial Reclamation, China University of Mining and Technology, Beijing | Laboratory of Microbial Reclamation, China University of Mining and Technology, Beijing |
| **Cultivation specification** | Spore density 26 g$^{-1}$, colonization rate 87%, and hyphal length 3.12 m g$^{-1}$ | 3.11 g dry matter mL$^{-1}$ ($1 \times 10^6$ CFU mL$^{-1}$) |

2.2. Experimental Design

Control (CK), AMF, single-inoculation DSE fungal treatments (DSE20%, DSE40% and DSE80%; 2, 4, 8 $\times$ 10$^5$ CFU mL$^{-1}$) and double-inoculation treatments (DSE20% + AMF, DSE40% + AMF and DSE80% + AMF) were set up (Table 2) with four replicates of each treatment. The additives and the maize seeds for each treatment were added to the corresponding pots with three seeds per pot. After emergence, one seedling was retained in each pot and the other two seedlings were cut into pieces and placed in the pot. The plants were harvested 60 days after emergence. Water was added quantitatively, according to plant growth status.
Table 2. Experimental treatments.

| Treatment         | AMF(g) | DSE (mL) | MMN Medium (mL) |
|-------------------|--------|----------|-----------------|
|                   | Non-   | Inactivated | Non-   | Inactivation | -   |
| Control (CK)      | -                      | - | - | |
| DSE20%            | - 50 | 10        | - 40 |  |
| DSE40%            | - 50 | 20        | - 30 |  |
| DSE80%            | - 50 | 40        | - 10 |  |
| AMF               | 50    | -         | - 50 | - |
| DSE20% + AMF      | 50    | -         | - 40 | - |
| DSE40% + AMF      | 50    | -         | - 30 | - |
| DSE80% + AMF      | 50    | -         | - 10 | - |

2.3. Test Monitoring

The monitoring indexes comprised root, leaf, stem and total fresh biomass, root, leaf, stem and total dry biomass, root, leaf, stem and total moisture content, root/shoot ratio, gs and T\textsubscript{canopy}. The thermal images were collected at 8 a.m., 10 a.m., 12 p.m., 2 p.m., 4 p.m. and 6 p.m. on the 11th, 22nd, 31st, 32nd, 33rd, 35th, 44th and 57th days after emergence, and gs was collected at 12 p.m. on the 44th and 57th days. When the thermal image and gs were collected at the same time, the gs was collected first, followed by the thermal image. The gs of two leaves in the maize canopy was measured. For thermal infrared image shooting, emissivity, air temperature and relative humidity were set in the thermal infrared imager, either to facilitate the correction of thermal picture information or for later image processing. The lens of the thermal infrared imager was perpendicular to the maize canopy and the distance between the lens and the maize canopy was appropriately adjusted; a clear image of the maize canopy was obtained using the manual or automatic focusing function. A FLIR T630sc system (Teledyne FLIR LLC, Wilsonville, OR, USA) was used with the following settings: resolution 640 × 480 pixels, field of view 25° × 19°, focal length 25 mm, temperature reading of −40 °C to +150 °C and accuracy of ±1 °C, thermal sensitivity < 0.03 °C, and spectral range of 7.5–14 µm. Stomatal conductance was measured using a Li-6400XT portable photosynthesis system (Li-Cor Biosciences, Lincoln, NE, USA). The harvest was on the 60th day after seedling emergence and the oven-dried (60 °C, 48 h) separate roots, leaves and stems were weighed and the total dry biomass was calculated. Root, leaf, stem, and total moisture content were calculated as (root, leaf, stem and total fresh biomass—root, leaf, stem and total dry biomass)/root, leaf, stem and total fresh biomass × 100%. The root/shoot ratio was calculated as root dry biomass/(leaf dry biomass + stem dry biomass). The contribution of the fungi to biomass was evaluated in terms of mycorrhizal contribution (MC), calculated as (biomass of inoculated maize—biomass of uninoculated control)/biomass of inoculated maize × 100%.

2.4. Statistical Analysis

Microsoft Excel 2016 was used for data collation and the SPSS 20.0 software package (IBM SPSS Inc., Chicago, IL, USA) was used for the one-way analysis of variance (ANOVA). FLIR ResearchIR Max 4.40.7.26 (Teledyne FLIR LLC, Wilsonville, OR, USA) was used for thermal infrared image processing, and the R (V 3.5.2, https://www.r-project.org/; accessed on 1 April 2022) statistical package was used for interaction analysis. In this experiment, thermal infrared image processing was carried out using FLIR ResearchIR Max to obtain T\textsubscript{canopy}. The target image was imported into FLIR ResearchIR Max to correct the emissivity, air temperature and humidity information, then the region of interest was selected with a rectangular box. Because of maize leaf bending and leaf tip withering, the region of interest selected was the central third of the two leaves at the top of the plant,
and the leaves were not necessarily horizontal in the image. Rotating the rectangular box located the rectangular frame in the middle third of the leaf. The average temperature of each region of interest was calculated by adding an average function, then the average value of the average temperatures of different regions of interest in a plant was taken as the $T_{\text{Canopy}}$ of the plant.

3. Results

3.1. Plant Biomass

Figure 1 shows that not all inoculation treatments promoted plant growth compared with the control. The root, stem, leaf, total fresh biomass, and dry biomass of inoculation DSE20% treatment plants were lower than those in the control; the mycorrhizal contribution rate was negative. The root, stem, leaf and total fresh biomass, root, leaf and total dry biomass of inoculated DSE80%, AMF, DSE20% + AMF, DSE40% + AMF, and DSE80% + AMF treatment plants were significantly higher than those of control plants ($p < 0.05$). The stem biomass of inoculation DSE80% and DSE80% + AMF treatment plants was significantly higher than that of the control plants ($p < 0.05$). However, the root fresh biomass and root dry biomass of inoculation DSE40% treatment plants were significantly higher than those of control plants ($p < 0.05$). The mycorrhizal contribution rates of inoculation DSE20%, DSE40% and DSE80% treatment to root, stem, leaf, total fresh biomass and dry biomass increased with increasing DSE concentration and were significantly lower than the mycorrhizal contribution rate of double-inoculated plants with a corresponding DSE concentration (except stem dry biomass) ($p < 0.05$). The mycorrhizal contribution rate of the inoculated AMF treatment to plant root, stem, leaf and total fresh biomass was significantly higher than that of the inoculated DSE80% treatment ($p < 0.05$). The mycorrhizal contribution rate of double-inoculation treatment to plant root, stem, leaf and total fresh biomass and dry biomass increased with increasing DSE concentration. Compared to the control, inoculation DSE80% + AMF treatment had the greatest promotion effect on root, stem, leaf and total fresh biomass and dry biomass, with significant increases of 533, 368, 279 and 336% (root, stem, leaf and total fresh biomass), and 461, 692, 290 and 399% (root, stem, leaf and total dry biomass) ($p < 0.05$). Moreover, the mycorrhizal contribution rates of inoculation DSE80% + AMF treatment plants to root, stem, leaf and total fresh biomass were 84.3, 78.7, 73.6 and 77.1%, respectively, and to root, stem, leaf and total dry biomass, the rates were 82.2, 87.5, 74.3 and 79.9%, respectively. The root/shoot ratio of inoculation DSE40%, DSE40% + AMF treatment plants were significantly higher than that of the control plants ($p < 0.05$). The variations in root, stem, leaf and total moisture contents of plants in the different treatments were small. The effect of bacterial inoculation on plant moisture content was different from that on fresh and dry biomass. Root moisture contents of inoculation DSE80%, AMF, DSE20% + AMF, DSE40% + AMF, and DSE80% + AMF treatments were not significantly different from the controls.
Figure 1. Plant growth indexes of different inoculation treatments. (a) Root fresh biomass and mycorrhizal contribution (MC); (b) Stem fresh biomass and MC; (c) Leaf fresh biomass and MC; (d) Total
3.2. Plant Canopy Temperature

The values in Figure 2 are the diurnal plant $T_{canopy}$ values at different growth stages. Differences in $T_{canopy}$ between the control and the inoculation treatments were compared by calculation ($T_{canopy}$ of control treatment $-$ $T_{canopy}$ of inoculation treatment) (Figure 3). A value of $>$ 0 indicates that the $T_{canopy}$ of inoculated plants was lower than that of the controls, a value of 0 indicates that the $T_{canopy}$ of inoculated plants was equal to that of the controls, and a value of $<$ 0 indicates that the $T_{canopy}$ of inoculated plants was lower than that of the control plants. Figures 2 and 3 show that differences in $T_{canopy}$ between controls and inoculated plants at 8 a.m., 10 a.m., 12 p.m., 2 p.m., and 6 p.m. were as follows on the 11th day, $-0.5-+0.6^\circ$ C, $-1.4-+0.3^\circ$ C, $-0.7-+1.0^\circ$ C, $-0.8-+0.4^\circ$ C, $-0.3-+0.4^\circ$ C, $-0.7-+0.3^\circ$ C; on the 22nd day, $0-+1.0^\circ$ C, $-1.3-+0.4^\circ$ C, $-1.1-+0.9^\circ$ C, $-0.2-+3.1^\circ$ C, $-0.5-+0.4^\circ$ C, $-0.5-+0.5^\circ$ C; on the 31st day, $-0.5-+0.6^\circ$ C, $+1.5-+7.0^\circ$ C, $-0.6-+1.6^\circ$ C, $-3.8-+2.3^\circ$ C, $-0.3-+1.8^\circ$ C, $-1.7-+0.3^\circ$ C; on the 32nd day, $-0.5-+0.6^\circ$ C, $+0.7-+4.4^\circ$ C, $+1.5-+4.5^\circ$ C, $-0.1-+3.7^\circ$ C, $-0.6-+0.6^\circ$ C, $0.0-+1.6^\circ$ C; on the 33rd day, $-1.7-+0.8^\circ$ C, $+2.5-+8.5^\circ$ C, $+0.3-+5.9^\circ$ C, $+0.2-+9.3^\circ$ C, $+1.0-+2.5^\circ$ C, $+0.3-+1.1^\circ$ C; on the 35th day, $-0.8-+2.1^\circ$ C, $-0.9-+2.4^\circ$ C, $+0.8-+6.1^\circ$ C, $+3.1-+6.1^\circ$ C, $+1.1-+2.2^\circ$ C, $+0.3-+1.7^\circ$ C; on the 44th day, $+0.9-+3.3^\circ$ C, $+0.6-+5.0^\circ$ C, $+0.8-+5.4^\circ$ C, $+0.3-+2.6^\circ$ C, $-0.1-+1.3^\circ$ C, $-0.6-+0.4^\circ$ C; and on the 57th day, $-0.5-+2.4^\circ$ C, $-0.5-+3.2^\circ$ C, $-2.1-+3.5^\circ$ C, $-1.0-+2.4^\circ$ C, $-0.4-+0.4^\circ$ C, $-0.4-+1.1^\circ$ C.

From the 31st day, the $T_{canopy}$ of the controls showed a higher trend than the inoculation treatments (Figure 3). The cooling effect of inoculation on the plant $T_{canopy}$ was monitored from the 31st to the 57th day and helped to indicate the significance of selecting monitoring periods. At 10 a.m. on the 31st day, 12 p.m. and 2 p.m. on the 32nd day, 10 a.m., 12 p.m., 2 p.m. and 4 p.m. on the 33rd day, 12 p.m., 2 p.m. and 4 p.m. on the 35th day, 12 p.m., 2 p.m. and 4 p.m. on the 44th day, and 12 p.m. on the 57th day, significant differences in the $T_{canopy}$ between the control and inoculated plants were detected ($p < 0.05$).

On the 35th, 44th and 57th days, the $T_{canopy}$ of DSE-treated plants decreased with increasing DSE concentration. By comparing the $T_{canopy}$ of plants treated with a single inoculant, it can be observed that the $T_{canopy}$ of plants treated with AMF was significantly lower than that of plants treated with DSE40% at 2 p.m. on the 31st day ($p < 0.05$). At 2 p.m. on the 32nd day, the $T_{canopy}$ of plants treated with AMF was significantly lower than those treated with DSE80% ($p < 0.05$). On the 33rd day, the $T_{canopy}$ of AMF treatment was significantly lower than that of DSE40% or DSE80% treatments at 2 p.m., and significantly lower than that of DSE80% treatment at 4 p.m. ($p < 0.05$). At 12 p.m. and 6 p.m. on the 35th day, the $T_{canopy}$ of the AMF treatment was significantly higher than that of the DSE80% treatment ($p < 0.05$). On the 44th day, the $T_{canopy}$ of the AMF treatment was significantly higher than that of the DSE80% treatment at 2 p.m., those of DSE40% and DSE80% treatments at 4 p.m., and that of the DSE80% treatment at 6 p.m. ($p < 0.05$). On the 57th day, the $T_{canopy}$ of the AMF treatment at 12 p.m. and 6 p.m. was significantly higher than that of DSE80% ($p < 0.05$).
Figure 2. Plant canopy temperature undergoing different treatments on the (a) 11th, (b) 22nd, (c) 31st, (d) 32nd, (e) 33rd, (f) 35th, (g) 44th and (h) 57th days. An analysis of variance (ANOVA) was used to analyze the data at the same time every day. Control (CK), treatments with sterilized DSE and AMF; DSE20%, 2 × 10^5 CFU mL\(^{-1}\) of DSE with sterilized AMF; DSE40%, 4 × 10^5 CFU mL\(^{-1}\) of DSE with sterilized AMF; DSE80%, 8 × 10^5 CFU mL\(^{-1}\) of DSE with sterilized AMF; AMF, sterilized DSE with AMF; DSE20% + AMF → DSE80% + AMF, with different concentrations of DSE and AMF. Different letters above the columns are significantly different at \(p < 0.05\).
Figure 3. Differences in plant canopy temperature between controls and inoculation treatments on the (a) 11th, (b) 22nd, (c) 31st, (d) 32nd, (e) 33rd, (f) 35th, (g) 44th and (h) 57th days. CK—inoculation represents the difference between the mean of CK and inoculation. Control (CK), treatments with sterilized DSE and AMF; DSE20%, $2 \times 10^5$ CFU mL$^{-1}$ of DSE with sterilized AMF; DSE40%, $4 \times 10^5$ CFU mL$^{-1}$ of DSE with sterilized AMF; DSE80%, $8 \times 10^5$ CFU mL$^{-1}$ of DSE with sterilized AMF; AMF, sterilized DSE with AMF; DSE20% + AMF → DSE80% + AMF, different concentration of DSE and AMF.

From the 31st to 57th days, the $T_{\text{canopy}}$ of the double-inoculated plants showed a lower trend than plants treated with the same concentration of DSE. On the 31st day, at 2 p.m., the $T_{\text{canopy}}$ of plants treated with DSE40% + AMF was significantly lower than that of plants treated with DSE40%, and that of plants treated with DSE80% + AMF was significantly lower than that of plants treated with DSE80% ($p < 0.05$). On the 32nd day, the $T_{\text{canopy}}$ of DSE80% + AMF treatment was significantly lower than that of DSE80% treatment at 12 p.m. and 2 p.m. ($p < 0.05$). On the 33rd day, the plant $T_{\text{canopy}}$ at 10 a.m. with DSE20% + AMF treatment was significantly lower than that of DSE20%; at 12 p.m., DSE80% + AMF treatment was significantly lower than that of DSE80%; and at 2 p.m., the DSE40% + AMF treatment was significantly lower than DSE40% ($p < 0.05$).

On the 31st, 32nd, 33rd, 35th, 44th and 57th days, the $T_{\text{canopy}}$ of the double-inoculated plants decreased with the increasing DSE concentration. On the 31st, 35th, 44th and 57th days, the $T_{\text{canopy}}$ of the AMF treatment was higher than that of the double-inoculation treatment (except at 8 a.m. on the 31st and 57th days). At 10 a.m. on the 31st day, 12 p.m.
on the 35th day, 10 a.m., 12 p.m., 2 p.m. and 4 p.m. on the 44th day, and 12 p.m. and 2 p.m. on the 57th day, the T\text{canopy} of DSE80\% + AMF treatment was significantly lower than that of AMF treatment \((p < 0.05)\). During the diurnal cycle, the differences in T\text{canopy} between the control and inoculation treatments were larger at 10 a.m., 12 p.m., 2 p.m. and 4 p.m., at which times the differences between inoculation treatments and controls were easier to detect.

3.3. Plant Stomatal Conductance

Figure 4 shows that on the 44th and 57th days, the gs of plants undergoing different treatments showed different trends. On the 44th day, the gs of plants inoculated with DSE40\% + AMF and DSE80\% + AMF was significantly higher than that of control plants \((p < 0.05)\). On the 57th day, the gs of plants inoculated with AMF was significantly higher than in the other treatments \((p < 0.05)\).

![Figure 4](image)

**Figure 4.** Stomatal conductance \((\text{gs})\) of plants undergoing different treatments on (a) the 44th day and (b) the 57th day. Control (CK), treatments with sterilized DSE and AMF; DSE20\%, 2 \times 10^5 \text{ CFU mL}^{-1} of DSE with sterilized AMF; DSE40\%, 4 \times 10^5 \text{ CFU mL}^{-1} of DSE with sterilized AMF; DSE80\%, 8 \times 10^5 \text{ CFU mL}^{-1} of DSE with sterilized AMF; AMF, sterilized DSE with AMF; DSE20\% + AMF \rightarrow DSE80\% + AMF, different concentration of DSE and AMF. The different letters above the columns are significantly different at \(p < 0.05\).

3.4. Regression Analysis between Canopy Temperature and Stomatal Conductance

According to Figure 5a,b on the 44th day of growth, the correlation between T\text{canopy} and gs of control and inoculated plants was high, and on the 57th day of growth, the correlation between T\text{canopy} and gs of control and inoculated plants was low. However, except for the AMF treatments, the correlation between T\text{canopy} and gs of control and inoculated plants was high on the 44th and 57th days of growth.

3.5. Correlation Analysis between Canopy Temperature, Stomatal Conductance and Biomass

Figure 6a,b shows that there was a significant correlation between \(\text{gs}, \text{T}_{\text{canopy}}\) and the growth indicators \((p < 0.05)\). Considering the gs and T\text{canopy} of inoculation AMF treatment plants, there was no significant correlation between gs and T\text{canopy}. In terms of the gs and T\text{canopy} of inoculated AMF treatment plants, there were significant positive correlations between gs and root, stem, leaf and total fresh biomass, root, leaf and total dry biomass, and leaf and total moisture content \((p < 0.05)\). There were significant negative correlations between T\text{canopy} and stem fresh biomass and dry biomass, as well as a significant positive correlation with stem moisture content \((p < 0.05)\). When the gs and T\text{canopy} data of inoculated AMF treatment plants were removed, there was a significant negative correlation between gs and T\text{canopy} \((p < 0.05)\). In the case of removing the data from the gs and T\text{canopy} of inoculated AMF treatment plants, there were significant positive correlations between
the gs of control and inoculated plants and the root, stem, leaf and total fresh and dry biomass, leaf and total moisture content, and root moisture contents \( (p < 0.05) \). There were significant negative correlations between \( T_{\text{canopy}} \) and root, stem, leaf and total fresh and dry biomass, as well as a significant positive correlation with root moisture content.

**Figure 5.** Regression analysis between canopy temperature and stomatal conductance (gs); (a) control and inoculation treatments on the 44th and (b) 57th days; and control and inoculation treatments without AMF treatment on (c) the 44th day and (d) the 57th day. The points in the dotted box in the Figure are AMF treatment data.

**Figure 6.** (a) Pearson correlation analysis of each factor in the control and inoculation treatments except AMF, (b) Pearson correlation analysis of each factor in control and inoculation treatments. Gs, stomatal conductance; \( T_{\text{canopy}} \), canopy temperature; RFB, root fresh biomass; SFB, stem fresh biomass; LFB, leaf fresh biomass; TFB, total fresh biomass; RDB, root dry biomass; SDB, stem dry biomass; LDB, leaf dry biomass; RSR, root/shoot ratio; TDB, total dry biomass; RMC, root moisture content; SMC, stem moisture content; LMC, leaf moisture content; TMC, total moisture content; ** \( p < 0.01 \); * \( p < 0.05 \).
3.6. Cluster Analysis of the Growth Index, Stomatal Conductance and Canopy Temperature

The cluster analysis results were grouped according to the plant growth index, $g_s$ and $T_{\text{canopy}}$, respectively (Figure 7). According to the growth index, when the distance was 4.26, the treatment was divided into four groups (Figure 7a): group 1 (DSE40%), group 2 (CK, DSE20%), group 3 (DSE80% + AMF), group 4 (DSE80%, AMF, DSE20% + AMF, DSE40% + AMF). According to the clustering results of plant growth status, different treatments were ranked thus: group 3 (DSE80% + AMF) > group 4 (DSE80%, AMF, DSE20% + AMF, DSE40% + AMF) > group 1 (DSE40%) > group 2 (CK, DSE20%). The $g_s$ was a response to the two monitoring results in the late growth stage. According to the $g_s$, when the distance was 1.20, the experimental treatments were divided into five groups (Figure 7b): group 1 (AMF), group 2 (DSE80%, DSE20% + AMF), group 3 (CK, DSE20%), group 4 (DSE40%), group 5 (DSE40% + AMF, DSE80% + AMF). According to the cluster grouping results of the $g_s$ of plant leaves, the different treatments were ranked thus: group 1 (AMF) $\approx$ group 2 (DSE80%, DSE20% + AMF) $\approx$ group 4 (DSE40%) $\approx$ group 5 (DSE40% + AMF, DSE80% + AMF). According to the results of $T_{\text{canopy}}$ clustering, different treatments were ranked thus: group 1 (DSE80% + AMF) > group 2 (DSE80%) > group 4 (DSE20% + AMF, DSE40% + AMF, AMF, DSE20% and DSE40%) > group 3 (CK). The result of the cluster analysis of $g_s$ was different from that of the growth index.
and $T_{\text{canopy}}$, but it showed that the plants treated with DSE80% + AMF grew better, while CK treatment showed a trend of poor plant growth.

![Cluster analysis results](Figure 7). According to the growth index, when the distance was 9.48, the experimental treatments were clustered into four groups (Figure 7a): group 1 (DSE40%), group 2 (CK, DSE20%), group 3 (DSE80% + AMF), and group 4 (DSE40% + AMF). According to the cluster analysis results, different treatments were ranked thus: group 1 (DSE80% + AMF) > group 2 (CK, DSE20%) > group 3 (DSE80% + AMF) > group 4 (DSE40% + AMF).

![Stomatal conductance cluster analysis](Image)

The $T_{\text{canopy}}$ is an efficient and non-contact monitoring method that is widely used in research on plant-water physiological status [26,28]. Stomatal regulation is an important physiological response in plant growth and physiology. When soil moisture is sufficient and the plant-water physiological metabolism is normal, leaf gs is high and transpiration is vigorous; this will maintain the $T_{\text{canopy}}$ as relatively low [31–33]. In contrast, under certain conditions, a lower $T_{\text{canopy}}$ can also reflect a higher gs and vigorous water metabolism by plants. A soil water deficit will lead to the insufficient water supply of plant roots, the low gs of leaves and weak transpiration, making the leaves maintain a relatively high $T_{\text{canopy}}$. However, under certain conditions when the plant $T_{\text{canopy}}$ is high, this may also reflect a low plant gs and the abnormal or slow metabolism of water in the plant. During the period from emergence to the 31st day, the $T_{\text{canopy}}$ monitoring results show little effect from the microorganisms on plant growth. This may be due to the microorganisms colonizing the root, with the microorganisms having no or little plant growth-promoting effect. After the 31st day, the $T_{\text{canopy}}$ of the uninoculated plants was higher than that of inoculated plants and the cooling effect of inoculated plants on the $T_{\text{canopy}}$ was monitored from the 31st to 57th days. This may be due to a stable symbiotic relationship between microorganisms and...
plants. This is consistent with previous studies; Domokos et al. [34] inoculated *Artemisia annua* seedlings at the 5–6-leaf stage (about 35-day-old seedlings) with AMF, and they found that AMF had good colonization in the roots at one week and 8 weeks, while AMF inoculation significantly improved the growth of plant roots, which showed that after *Artemisia annua* seedlings grown for 35 days were inoculated with AMF for one week, AMF could affect the growth of plants. Zhang et al. [35] found that the percentage of fine roots in maize roots inoculated with AMF was significantly higher than that of CK treatment when maize grew for 47 days (one week after simulating root damage); at this time, AMF inoculation significantly increased the contents of indole-3-acetic acid (IAA) and cytokinin (CTK) levels in leaves and root tissues and reduced the content of ABA levels in leaves and root tissues, indicating that the AMF had formed a good symbiotic relationship with maize and that AMF improved plant growth and physiological conditions. Although these potentially symbiotic relationships have high and low differences, even with significant differences between each other, they all show a certain promoting effect. This indicates that thermal infrared testing can be used to monitor the physiological status of maize inoculated with AMF and DSE fungi.

Thapa et al. [24] found that there was a good negative correlation between the decrease in canopy temperature and crop yield in the daytime (10 a.m. to 6 p.m.) of 20 genotypes of winter wheat (except for three genotypes). The regulation of canopy temperature of the three genotypes may have different physiological and/or morphological mechanisms to maintain a cool canopy at the hottest time of the day. We consistently found a high negative correlation between canopy temperature and the stomatal conductance of inoculated and control plants on the 44th day. However, there was a high negative correlation between canopy temperature and stomatal conductance of inoculated (except AMF treatment) and control plants on the 57th day. This may be due to the AMF treatment having different physiological and/or morphological mechanisms for the regulation of plant canopy temperature. Changes in plant leaf temperature are the result of joint regulation by plant physiology and the external environment [36–38]. The AMF inoculation treatment can change the plant canopy structure so that plants can receive more light [39]. While receiving more light, plant leaves will absorb more energy and plant leaf temperature will rise but, at this time, plants inoculated with AMF may increase stomatal conductance and reduce transpiration because of the increase in canopy temperature. This results in higher canopy temperature and stomatal conductance in AMF treatments, which may represent the dynamic regulation of canopy temperature and the stomatal physiological activities of plants inoculated with AMF.

Under certain conditions, higher growth index, stomatal conductance and lower canopy temperature are indicators of healthy plant growth [24]. Biju et al. [40] conducted cluster analysis on plant growth physiological parameters (T_{canopy}, canopy temperature depression (CTD = T_{canopy} – air temperature (T_{air})), crop water stress index (CWSI), root/shoot ratio, relative water content and harvest index), and used these parameters to evaluate the drought resistance of lentil plants, dividing the different genotypes into three groups with different drought tolerance levels. Here, we obtained similar results using cluster analysis of the plant biomass index, T_{canopy} and gs; the different experimental treatments were clustered into different groups representing different physiological conditions of growth. The results of the three cluster analyses had a certain similarity; that is, the results of cluster analysis of the growth index and T_{canopy} showed that DSE80% + AMF treatment had the highest growth status, while the control had the lowest growth status. This also indicates that thermal infrared can be used to monitor the physiological status of maize inoculated with AMF and DSE fungi. The monitoring of plant water status by thermal infrared imaging is different from other technologies, with its dynamic and efficient characteristics. This is because monitoring over a specific period can only reflect the growth and physiological status of plants at that moment in time, and the continuous thermal infrared monitoring of plants for many days can better reflect the physiological status and dynamic change processes of plants [28,41]. This might explain the different
classifications and ranking between the best and worst growth conditions in the cluster analysis results of canopy temperature, stomatal conductance and growth indicators in the current study. This may be because the monitoring frequency of stomatal conductance and growth indicators was less and could not reflect the process of plant growth physiological conditions, whereas the canopy temperature was a comprehensive response to the growth status of different growth periods in the growth cycle. The above discussion suggests that the effect of AMF and DSE fungal inoculation on the growth and physiological status of maize can be assessed by monitoring the plant canopy temperature.

4.2. Changes in the Growth Physiological Status of Maize with Inoculation

Numerous studies have found that AMF can form a potentially symbiotic relationship with plant roots, forming a large hyphal network, promoting plant nutrition and water uptake, increasing plant growth, and improving physiological conditions [8,42–44]. DSE fungi also show similar growth-promoting effects to AMF [14,18,19]. Xie et al. [30] found that simultaneous inoculation with AMF and a high-concentration DSE bacterial solution can change the level of plant hormones, increase the IAA, CTK and gibberellin accumulation (GA) in the aboveground parts of plants, and promote plant growth. Inoculating the same plant species with different microorganisms can give different plant growth-promoting effects; this may be related to microbial species, microbial dosage and environmental factors [4,20,45]. This was verified here, with plants inoculated with different doses and with different types of microorganisms having different growth conditions. The DSE80% + AMF inoculation treatment had the greatest promotional effect on plant growth and the most vigorous plant growth.

Some studies have found no relationship between the growth status of plants and the amount of AMF added. That is to say, under certain circumstances, the effects of higher and lower doses of AMF inoculation on plant growth are similar and there may be a threshold above which the growth promotion effect will not continue to increase [45]. This situation may be related to soil and environmental factors. Here, the growth promotion effect on T_{canopy} and growth indicators did not change in proportion to an increasing concentration of a single inoculation with DSE fungi, but in the case of a double inoculation with AMF and DSE fungi, as the proportion of DSE concentration increased, the effect on T_{canopy} and growth indicators did not change proportionally. There may, therefore, be a threshold above which the growth promotion effect does not increase proportionally, and this may have important implications for the screening of bacterial agents.

It was found that a single inoculation of AMF or DSE fungi or a double inoculation of AMF and DSE fungi could promote plant material accumulation, but it was also found that the inoculation of low-concentration DSE fungi bacterial solution did not promote plant material accumulation and nutrient absorption, but could increase IAA, CTK and reduce abscisic acid (ABA) [30]. Similar results were obtained in this study; on the whole, the monitoring results of single-inoculation microorganisms showed that the inoculation of DSE20%, DSE40%, DSE80% and AMF decreased the plant canopy temperature. Except for DSE20%, other single-inoculation treatments promoted the accumulation of plant biomass; the growth-promoting effect of inoculation with DSE20% + AMF, DSE40% + AMF and DSE80% + AMF was higher than that of a single inoculation.

Although the method proposed in this paper was fast, efficient and non-contact, there was a key problem. When working only from an analysis of the monitoring results, it was impossible to determine what kind of treatment was best; however, by analyzing the results of experimental treatment and monitoring results, the best combination of fungi agents can be selected for production practice, and this method had strong practicability. The results showed that the DSE80% + AMF treatment had the best growth-promoting effect.

4.3. Optimum Observation Period and Time in Diurnal Cycle during the Growth Period

Predecessors have explored the T_{canopy} monitoring of plants during different growth periods. García-Tejero et al. [26] evaluated the water status of almond trees by monitoring
the T\textsubscript{canopy} of plants under different irrigation strategies at different periods and found that the longer the water control time of regulated-deficit-irrigation plants, the higher the T\textsubscript{canopy} compared to with the normal water supply to the plants. Santesteban et al. [28] considered that the acquisition of thermal infrared images on several consecutive days in summer is conducive to monitoring the dynamic changes in plant water levels. Hou et al. [46] found that monitoring the T\textsubscript{canopy} and canopy cooling at the flowering and pod-setting stages is an effective growth period for predicting soybean yield. The plant T\textsubscript{canopy} more closely reflects the difference in physiological status between inoculated and uninoculated plants from the 31st to the 57th day after emergence. This may be related to the establishment of a symbiotic relationship between the inoculated microorganisms and host plants and the function of the symbiotic system.

The appropriate monitoring time in the daily cycle is conducive to the accurate judgment of plant water status and the management of plant water-related decisions through monitoring plant water status. Previous researchers studied the physiological status of plant water by monitoring plant thermal indicators with thermal infrared imaging technology and reached a consistent conclusion that the thermal indicators obtained at noon or at the time of higher temperatures in the day can better reflect the physiological status of plant water [26,46]. García-Tejero et al. [26] found that there was a significant correlation between the T\textsubscript{canopy} and gs of almond tree leaves, measured at 11.30 a.m., 2.30 p.m. and 5.30 p.m. in the daily cycle. The T\textsubscript{canopy} measured at these times can be used to evaluate the water status of almond trees. Hou et al. [46] found that the maximum plant T\textsubscript{canopy} and CTD generally occur at noon. Thapa et al. [47] used thermal infrared imaging technology to select the hottest time of the day to monitor the maize T\textsubscript{canopy} under different geometric planting models (clump, cluster, skip-row) under drought or limited irrigation conditions for water management and with a geometric planting model evaluation. Costa et al. [48] found that the plant T\textsubscript{canopy} was higher than the optimum temperature for leaf photosynthesis between 11 a.m. and 5 p.m., while the highest T\textsubscript{canopy} was measured at 5 p.m., and the maximum plant T\textsubscript{canopy} was delayed relative to the maximum T\textsubscript{air}. Here, the T\textsubscript{canopy} difference between the uninoculated control and the inoculated treatments was larger at 10 a.m., 12 p.m., 2 p.m. and 4 p.m. during the daily cycle, and it was easier to monitor the difference between the inoculated treatments and the uninoculated control, which finding is similar to the results of previous studies.

5. Conclusions

Here, we have explored the feasibility of inoculating plants with AMF and DSE fungi and using thermal infrared imaging technology to monitor the growth and physiological status of maize. Thermal infrared imaging technology can be used to monitor the physiological status of maize inoculated with AMF and different concentrations of DSE fungi. Cluster analysis gave a growth indices trend: DSE80% + AMF > DSE80%, AMF, DSE20% + AMF, DSE40% + AMF > DSE40% > control, DSE20%. The T\textsubscript{canopy} cluster analysis results showed the trend: DSE80% + AMF > DSE80% > DSE20% + AMF, DSE40% + AMF, AMF, DSE20%, DSE40% > control. The order of gs cluster analysis was: AMF ≈ DSE80%, DSE20% + AMF ≈ DSE40% ≈ DSE40% + AMF, DSE80% + AMF > control, DSE20%. During the growth period, the best observation period for monitoring the impact of inoculation on plant growth physiological conditions through T\textsubscript{canopy} was the 31st–57th days, while the best observation times of the daily cycle were 10 a.m., 12 p.m., 2 p.m. and 4 p.m. Here, we used only maize as the host plant to monitor the growth physiological status of AMF and DSE fungi and screened the fungal agents. The effect of AMF and DSE fungi on the growth and physiological status of other plant species inoculated with AMF and DSE fungi remains unknown, and this requires further study.

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