Effect of Shungite Application on the Temperature Sensitivity of Allium cepa Respiration under Two Soil Water Regimes †

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Abstract: This study aimed to evaluate whether shungite application to Umbric Podzols may affect leaf and root mitochondrial respiratory pathways, and the leaf response to a temperature change. A pot culture experiment was conducted with Allium cepa L. seedlings, using soil shungite concentrations of 0, 5, 10, and 20 g kg⁻¹ and two soil water regimes: well-watered (WW) and drying–wetting (DW) cycles. The soil water deficit increased the total respiration (Vₜ) of onion leaves, but not roots, under low (13°C) and high (33°C) measurement temperatures. Shungite application affected leaf Vₜ only at 13°C: it increased the Vₜ rate under WW and decreased it under DW. An increase in the measurement temperature to 33°C enhanced the sensitivity of leaf respiration to the inhibitor of the alternative respiratory pathway (salicylhydroxamic acid, SHAM). Shungite application increased the contribution of the SHAM-sensitive pathway to the leaf Vₜ rate under WW, but not under the DW regime, regardless of the leaf temperature. In contrast to the SHAM-resistant pathway, the temperature sensitivity of the SHAM-sensitive rate decreased following the decrease in soil water availability. Shungite application increased the temperature sensitivity of both SHAM-sensitive and SHAM-resistant leaf respiration under DW, and significantly decreased these parameters under WW. In summary, the decrease of temperature sensitivity of the alternative SHAM-sensitive respiratory pathway with a decrease of soil water availability or the shungite-related decrease of both SHAM-sensitive and SHAM-resistant leaf respiration may play an important role in enhancing the resistance of plant respiration to stress temperature.

Keywords: onion; rock powder; respiratory pathways; soil water deficit

1. Introduction

Rocks containing a multitude of nutrients have been proposed as a slow-release fertilizer that allows nutrients to remain in the top soil for a long time [1]. Therefore, the possibility of using rock powders as an alternative source of nutrients for agriculture practice has been widely discussed [2]. When initial nutrient levels in agricultural soils are low, the application of rock powders may improve not only the ion and cation exchange capacity [3] but also the physical properties of soils, as was shown for inorganic carbon [4].

Shungite rocks formed mainly on a silicate basis are carbon-bearing sedimentary-volcanic rocks widely distributed in the Lake Onega area. The carbonaceous matter characterized by a globular fullerene-like molecular structure is one of the main components of the shungite [5]. Along with inorganic carbon, some macro- and micro-nutrients, such as Si, K, Ca, Mg, Na, Cu, and others, were found in the shungite rocks [5]. Since most of the nutrient elements are prevalent soil elements that beneficially affect the physiological state
of plants, maintaining an adequate plant nutritional status may improve the physiological resistance of plants under stress situations, including stress temperatures [6] and soil water deficit [7].

Soils developed in the areas of shungite rocks have long attracted the attention of farmers and researchers [8]. Centuries-old human activity has revealed that the content of shungite in the soil can enhance the yield of agricultural crops, which could be associated with an improvement of the thermal regime or fertility of shungite soils [9]. Experimental evidence shows that shungite application can promote the plant growth rate, yield, and pathogen resistance for some species [10], but no information is available concerning the effects of shungite rocks on plant respiration, respiration pathways, and their temperature response.

It is well-documented that in agricultural practice, not only the low natural fertility of soils but also climatic factors such as stress temperature or an inadequate soil water regime have long been recognized as the main determining stress factors challenging current agricultural productivity. Along with photosynthesis, respiration is one of the main physiological processes responsible for plant growth and development. While the CO$_2$ assimilation rate has been shown to be strongly suppressed by drought [11], the impact of the soil water deficit on plant respiration may be multidirectional: it decreases in the initial phase of water stress, and increases, as an acclimation mechanism, under lower soil water availability [12].

Plant respiratory metabolism is altered such that under stress conditions, other pathways, besides the cytochrome c oxidase (Cyt) pathway, are induced to provide alternative respiratory substrates to the respiratory processes [13,14]. Compelling evidence has recently demonstrated that alternative pathway respiration (Alt) associated with alternative oxidase activity provides flexibility in cellular energy and carbon metabolism, thus contributing to increased resistance of plants to stress conditions, including soil water deficit and low or high temperatures [13]. The drought-related increase of plant respiration rate described in [12] can be connected with the enhanced capacity of Alt, as was shown by Feng et al. [15]. However, the adjustment of respiratory metabolism, as a plant response to changes in growth conditions, can be connected with increased electron partitioning to the Alt and decreased to the Cyt pathway, with the total leaf respiration not affected [16]. A recent study of the effect of both soil water regime and shungite application on physiological traits of onion seedlings showed that the first one has a much stronger effect on leaf respiration than the second [17]. Although the leaf respiration rate was not significantly affected by shungite application to the soil neither under sufficient nor low soil water availability, shungite improved the nutrient status of onion leaves and plant resistance to a water deficit [17]. This may be due to an impact of shungite on the activity of respiratory pathways, and/or change the partitioning between them, however, there has been no published evidence supporting this state. In this study, we hypothesized that the earlier reported positive effects of shungite rock on plant resistance to the soil water deficit could be connected with its impact on respiratory pathways.

Plant respiration is a temperature-sensitive process, with the temperature sensitivity being referred to as the temperature coefficient (Q$_{10}$), defined as a proportional change in respiration rate per 10 °C change in temperature. The Alt and Cyt pathways have been shown to differ in their sensitivities to short-term changes in temperature [18]. It was proposed that the Alt pathway may maintain mitochondrial electron transport and protect against harmful reactive O$_2$ generation in the cold due to this pathway being less temperature-sensitive (lower Q$_{10}$) than the Cyt pathway [19]. However, some studies found little difference in the Q$_{10}$ values between the Alt and Cyt pathways [20], or a more sensitive Alt than Cyt pathway [18,21]. The shift of the temperature sensitivity of these respiratory pathways under changed conditions, for example, soil water or nutrient availability, can alter the partitioning between the pathways, and consequently, plant resistance to temperature stress.
To estimate the pathways' activity at different temperatures, the respiratory inhibitors are widely used in studies investigating the temperature sensitivity of the Alt and Cyt pathways [20,21]. Specifically, the Alt pathway is sensitive to salicylhydroxamic acid (SHAM), commonly used as an alternative oxide inhibitor. Although SHAM not only affects alternative oxide activity and can slightly modulate the Cyt pathway, it is conventionally accepted that SHAM-sensitive respiration is a measure for the contribution of the Alt pathway to total respiration. The SHAM-resistant respiration corresponds to the sum of cytochrome-related electron transport and the residual non-mitochondrial respiration, which constitutes not more than 10% of the total respiration [22].

Our study investigates the effect of short-term changes in temperature on respiration in intact tissues of onion leaves. We examine whether the $Q_{10}$ values of SHAM-resistant and SHAM-sensitive respiration differ and how shungite application to soil affects the temperature sensitivity of both SHAM-resistant and SHAM-sensitive respiratory pathways. Moreover, we establish the extent to which the shungite dependence on SHAM-resistant and SHAM-sensitive pathways is affected by a change in the soil water availability.

2. Materials and Methods

2.1. Soil Substrate Preparation

The soil used in this study was collected from the 0–30 cm topsoil layer of Umbric Podzols from the Korza valley, the northwest of Russia. Umbric Podzols, as stated earlier [23], are characterized by low natural fertility, thin layers (10–20 cm), and low content (0.5–2.5%) of humus, as well as low pH (4–5). The soil was collected randomly, air-dried, and sieved with a 2 mm sieve. Shungite rock was taken from the Zazhogino deposit (Karelia, Russia) and crushed to a size of 0.5 mm. The shungite rock used in this study contained, in the dry solid: 48.9% of SiO$_2$, 36.9% of C, 5.6% of Al$_2$O$_3$, 2.4% of F$_2$O$_3$, 1.8% of K$_2$O, 0.95% of MgO, 0.84% of S, and 0.14% of CaO. Water extraction from shungite had 0.40 ppm of SO$_4^{2-}$, 0.06 ppm of Ca$^+$, 0.03 ppm of Mg$^{2+}$, 0.014 ppm of K$^+$, 0.003 ppm of NH$_4^+$, 0.0072 ppm of F$^-$, and pH was 4.3. The entire volume of the dry soil was divided into four parts and mixed with shungite powder. Four concentrations of shungite powder were used in this experiment: 0, 5, 10, and 20 g of shungite per 1 kg of dry soil, designated as 0S, 5S, 10S, and 20S, respectively. Before seed sowing, all soils were incubated under 21–23 °C and 70–80% of the maximum soil water-holding capacity for 90 days.

2.2. Plant Growth Conditions

The soil substrates were placed into plastic pots (12 cm wide, 16 cm height). Before sowing, uniform seeds of onion (Allium cepa L., var. Sturon) were imbibed in water for 3 h and sown with 6 seeds per pot. All pots were subjected to a controlled climate chamber (VötschBioLine, Balingen, Germany) with conditions of 23/20 °C day/night temperature, 70% relative air humidity, a 16 h photoperiod, and 300 µmol m$^{-2}$ s$^{-1}$ of photosynthetic photon flux density. All pots were maintained well-watered for one week until seedlings were thinned to three seedlings per pot.

One week after sowing, the pots of the 0S, 5S, and 10S treatments were randomly divided into two blocks and two watering treatments were applied: well-watered (WW) and drying–wetting cycles (DW). The WW seedlings were watered daily to maintain the soil moisture content at the level of about 80% of the water-holding capacity. The DW seedlings were watered once every five days, so from the beginning of the watering treatment before measurements, the onion plants were WW two times, on the 12th and 17th days after sowing. In this way, the completely randomized experimental design included four levels of shungite content in the soil (0S, 5S, 10S, and 20S) and two water regimes among the 0S, 5S, and 10S treatments. For the 20S treatment, only the WW regime was applied. Each treatment included eight pots.
2.3. Total and SHAM-Resistant Respiration Measurement

In addition to the cytochrome (Cyt) chain, plant mitochondria respiration is characterized by the presence of an alternative (Alt) pathway associated with the activity of alternative oxidase. Both pathways use electrons from the ubiquinone pool and support the cell energy balance, but unlike the Cyt pathway, the alternative pathway does not couple electron transport to the generation of a proton motive force for the synthesis of ATP. SHAM is used in studies of plant respiratory metabolism as a specific inhibitor of alternative oxidase. The sensitivity of respiration in intact plant tissues to SHAM provides indirect evidence for electron transport through the alternative oxidase-mediated pathway in the respiratory electron transport chain. Total respiration of leaves and roots and SHAM-respiration, which is resistant to salicylhydroxamic acid, were measured using a Clark-type oxygen electrode (Oxygraph Plus, Hansatech, Norfolk, UK) at 23 °C. Before the onset of respiration measurements, the plants were kept in the dark for 15 min. A leaf sample (about 0.01 g of DW) was harvested with a razor blade, cut into small pieces, and suspended in 2 mL of air-saturated 100 mM HEPES buffer (pH 7.5) in the reaction vessel of the electrode unit. The O₂ uptake rate was measured in the presence of SHAM, an agent commonly used as an inhibitor of alternative pathway respiration (Alt), or in the absence of SHAM. The roots were carefully washed to remove soil and each whole root system was divided into two halves, for the buffer with or without SHAM. The plant samples were kept in a buffer solution in darkness for approximately 15 min until the process rate was stabilized, and then the O₂ uptake rate was measured for 5 min. The rate of oxygen uptake by plant samples in the SHAM-free buffer solution was defined as total respiration (Vₜ), and included both SHAM-resistant and SHAM-sensitive respiratory pathways. The O₂ uptake rate in the SHAM-containing buffer was defined as SHAM-resistant respiration (V_{SHAM-res}), and reflected mainly the Cyt pathway, and the difference between Vₜ and V_{SHAM-res} was defined as SHAM-sensitive respiration (V_{SHAM-sens}), and reflected the Alt pathway associated with alternative oxidase activity. By neglecting the influence of SHAM on Cyt pathway activity, the contribution of V_{SHAM-sens} to the Vₜ rate (%) was calculated as V_{SHAM-sens}/Vₜ ratio. While using this index, we realized possible limitations of the adopted approach. We assumed that SHAM-sensitive O₂ uptake systems capable of mimicking the SHAM-inhibited respiration have a minor partitioning in the total oxygen uptake and are temperature-independent.

2.4. Temperature Response of O₂ Uptake Rates

To determine the temperature response of Vₜ, V_{SHAM-res}, and V_{SHAM-sens} respiratory pathways of onion leaves, O₂ uptake rates were measured at a buffer solution temperature of 13, 23, and 33 °C. The required temperature was attained by connecting the reaction vessel with a water-bath thermostat (VEB MLW Prufgerate-Werk, GDR). The Clark-type oxygen electrode calibration was carried out at each measurement temperature.

The temperature sensitivity of O₂ uptake rates was evaluated using the temperature coefficient (Q₁₀), which shows the proportional change in the respiration rate with a 10 °C increase in temperature. The Q₁₀ values were determined by approximating the plots of respiration rates at different temperatures with a power function.

2.5. Statistical Analysis

For each treatment, the means ± SE were determined with at least six replicates. To assess the significant difference between the treatments, the least significant difference (LSD) of the ANOVA was used at the p < 0.05 level. To ensure the normality and homogeneity of variances, the data were log-transformed if necessary. The effects of shungite concentration, water regime, and their interaction were analyzed using a two-way ANOVA, separately for each measuring temperature. All statistical tests were carried out with Statistica software (v. 8.0.550.0, StatSoft, Inc., Tulsa, OK, USA). When the differences between O₂ uptake rates in the absence and presence of SHAM were statistically insignificant, the V_{SHAM-sens} value was assumed to be zero.
3. Results
3.1. Total and SHAM-Resistant Respiration

For the 0S seedlings, a significant impact of the soil water deficit on total \( V_t \), but not SHAM-resistant \( V_{\text{SHAM-res}} \), respiration was found under low \( (13 ^\circ \text{C}) \) and high \( (33 ^\circ \text{C}) \) measurement temperatures (Figure 1a,c). The leaf \( V_t \) rate was higher in 0S seedlings grown under the DW than the WW condition. On the contrary, at 23 \(^\circ\text{C}\), no significant differences in the \( V_t \) rates of leaves and roots were found between 0S seedlings grown under the DW and WW regimes, but \( V_{\text{SHAM-res}} \) was higher in DW than WW leaves (Figure 1b). According to the two-way ANOVA, the \( V_{\text{SHAM-res}} \) rate of both leaves and roots was significantly affected by soil water availability, but the effect of shungite application was not significant for both \( V_t \) and \( V_{\text{SHAM-res}} \) rates at all measurement temperatures (Table 1). However, for certain conditions of temperature and soil water availability, this effect was significant. Therefore, shungite application decreased leaf \( V_t \) and \( V_{\text{SHAM-res}} \) under DW and increased \( V_t \) under the WW regime at 13 \(^\circ\text{C}\) (Figure 1a). Moreover, under the WW regime and at the temperatures of 23 and 33 \(^\circ\text{C}\), seedlings grown in the soil containing shungite had lower \( V_{\text{SHAM-res}} \) values than 0S seedlings (Figure 1b,c). For the roots, both \( V_t \) and \( V_{\text{SHAM-res}} \) rates of 5S and 10S seedlings were lower under DW and higher under WW than those of 0S seedlings, but these differences were not large enough to be statistically significant (Figure 1d).

![Graphs showing O2 uptake rates](image)

**Figure 1.** Total respiration (white bars) and SHAM-resistant respiration (black bars) for onion leaves (a–c) and roots (d) grown on the Umbric Podzols with shungite concentrations of 0 (0S), 5 (5S), 10 (10S), and 20 (20S) g kg\(^{-1}\) under drying–wetting (DW) or well-watered (WW) regimes. During the measurements, the temperature was kept at 13 (a), 23 (b), or 33 (c) \(^\circ\text{C}\). Different letters indicate significant differences: lower case is used for the white bars, capitals are used for the black bars. * Indicates significant differences between \( V_t \) and \( V_{\text{SHAM-res}} \) at \( p < 0.05 \).
Table 1. Statistical results (p-value) of two-way ANOVA for the parameters shown in Figures 1 and 2.

| Variables          | Treatment Factor, Interaction |
|--------------------|-------------------------------|
|                    | Shungite | Water Regime | Shungite + Water Regime |
| Leaves             |           |              |                          |
| 13 °C              |           |              |                          |
| $V_t$              | 0.233 ns  | 0.194 ns     | 0.052 ns                 |
| $V_{SHAM-res}$     | 0.310 ns  | 0.192 ns     | 0.118 ns                 |
| $V_{SHAM-sen}/V_t$ | <0.001 ***| 0.702 ns     | 0.052 ns                 |
| 23 °C              |           |              |                          |
| $V_t$              | 0.606 ns  | 0.093 ns     | 0.420 ns                 |
| $V_{SHAM-res}$     | 0.231 ns  | <0.001 ***   | 0.582 ns                 |
| $V_{SHAM-sen}/V_t$ | 0.049 *   | 0.039 *      | 0.030 *                  |
| 33 °C              |           |              |                          |
| $V_t$              | 0.551 ns  | 0.093 ns     | 0.394 ns                 |
| $V_{SHAM-res}$     | 0.231 ns  | <0.001 ***   | 0.440 ns                 |
| $V_{SHAM-sen}/V_t$ | <0.001 ***| <0.001 ***   | 0.621 ns                 |
| Roots              |           |              |                          |
| 23 °C              |           |              |                          |
| $V_t$              | 0.788 ns  | 0.042 *      | 0.161 ns                 |
| $V_{SHAM-res}$     | 0.902 ns  | 0.022 *      | 0.216 ns                 |
| $V_{SHAM-sen}/V_t$ | 0.405 ns  | 0.108 ns     | 0.621 ns                 |

Asterisks denote significance levels: * $p < 0.05$, *** $p < 0.001$; ns, not significant. $V_t$, total respiration; $V_{SHAM-res}$, SHAM-resistant respiratory pathway; $V_{SHAM-sen}$, SHAM-sensitive respiratory pathway.

Figure 2. The $V_{SHAM-sen}/V_t$ ratio for leaf respiration of onion seedlings, grown on the Umbric Podzols with shungite concentrations of 0 (white bars), 5 (light gray bars), 10 (dark grey bars), and 20 (black bars) g kg$^{-1}$ under drying–wetting (a) or well-watered (b) regimes. Different letters indicate significant differences.
3.2. Ratio of SHAM-Sensitive to Total Respiration

Onion leaf respiration was more sensitive to the inhibitor of the alternative pathway (SHAM) than root respiration, resulting in a decrease of leaf respiration when O$_2$ uptake was measured in the presence of SHAM (Figure 1). However, this decrease was not significant for all combinations of the measurement temperature, soil water regime, and shungite treatment. Therefore, at 13 °C, leaf respiration was more sensitive to SHAM for DW than WW seedlings, but at 23 °C, on the contrary, it was more sensitive for WW than DW seedlings.

The increase of respiration sensitivity to SHAM reflects an increase in the contribution of the Alt respiratory pathway to total respiration. With the increase of the measurement temperature, the $V_{\text{SHAM-sens}}/V_t$ ratio tended to increase (Figure 2). For the 0S leaves, $V_{\text{SHAM-sens}}/V_t$ values were higher in DW than WW seedlings, regardless of the measurement temperature.

In contrast to the roots, for the leaves, the two-way ANOVA revealed a significant effect of both shungite application and soil water availability and their interaction on the $V_{\text{SHAM-sens}}/V_t$ ratio (Table 1). In contrast to the DW cycle, shungite application significantly increased the leaf $V_{\text{SHAM-sens}}/V_t$ values for the seedlings grown under the WW regime at all measurement temperatures, regardless of the soil shungite content (Figure 2).

3.3. Respiratory Coefficient ($Q_{10}$)

The $Q_{10}$ values of leaf $V_t$ and $V_{\text{SHAM-res}}$ did not differ significantly between the 0S seedlings grown under DW and WW regimes, but $Q_{10}$ of $V_{\text{SHAM-sens}}$ strongly decreased following the decrease in soil water availability (Figure 3). Regardless of the shungite treatment and soil water regime, the $Q_{10}$ values were higher for the $V_{\text{SHAM-sens}}$ than $V_t$ and $V_{\text{SHAM-res}}$ rates. The opposite effect of shungite application on the respiratory coefficient was found for seedlings grown under DW and WW regimes. While under the DW condition, shungite application increased $Q_{10}$ of both $V_{\text{SHAM-res}}$ and $V_{\text{SHAM-sens}}$, increasing $V_t$, under the WW regime, shungite significantly decreased the respiratory coefficient of both respiratory pathways.

![Figure 3](image-url)

**Figure 3.** Temperature sensitivity ($Q_{10}$) of the SHAM-resistant (white bars) and SHAM-sensitive (black bars) leaf respiration of onion seedlings growing on the Umbric Podzols, with shungite concentrations of 0 (0S), 5 (5S), 10 (10S), and 20 (20S) g kg$^{-1}$ under drying–wetting (DW) or well-watered (WW) regimes.

4. Discussion

This study was aimed at assessing whether shungite application to soil has the potential to alter the leaf and root respiration rate and temperature sensitivity ($Q_{10}$) of SHAM-resistant respiration, mainly reflecting the Cyt pathway, and SHAM-sensitive respiration, reflecting the Alt pathway, using onion seedlings as a model. Therefore, we quantified the effects of soil water availability on the respiratory $Q_{10}$ value to understand whether its...
response to shungite application depends on the soil water conditions. The onion leaves and roots showed a different response to the soil water deficit (Figure 1). While among all treatments the total root respiration tended to decrease, leaf respiration increased during water limitations. Moreover, in contrast to the roots, onion leaves were sensitive to both shungite application and SHAM.

The positive role of the Alt pathway in plant metabolism under stress conditions has been widely discussed [13–16] due to its ability to stabilize the reduction level of the ubiquinone pool and prevent the production of excessive amounts of reactive oxygen species [24]. Soil water deficit and low temperature are some of the important factors limiting crop yield. We have shown that a soil water deficit can stimulate onion leaf respiration mainly due to an increased rate of SHAM-sensitive respiration (Figure 1). While our results (Figure 2) support previous findings that the contribution of the Alt pathway to total respiration increases following decreased water availability [25], for onion leaves, this was only found at the low and high measurement temperatures. However, at the optimal temperature, the increase of total leaf respiration in response to water limitations was associated with the increase of SHAM-resistant respiration. Thus, different mitochondrial respiratory pathways may be involved in plant respiration acclimation to soil water deficits, with the pathway contribution depending on environmental conditions, particularly temperature.

Our finding (Figure 3) that the temperature sensitivity of SHAM-sensitive respiration is higher than the sensitivity to short-term changes in temperature for SHAM-resistant respiration is consistent with earlier studies [20,21]. It is known that plant respiratory Q_{10} values are affected by the growth environment and may vary significantly [20]. While the temperature sensitivity of respiration has been mainly studied at the total respiration level, the results of this study highlight the variability of both SHAM-resistant and SHAM-sensitive respiratory pathways. The results have shown that the Q_{10} values of the respiratory pathways of onion leaves studied here can be altered by changes in soil water availability, as well as shungite application to the soil. Slot et al. [26] found that the Q_{10} values of total dark respiration of Geum urbanum leaves, as well as the respiration rate, increase in response to the decrease of water availability. For onion leaves, our results have shown the same effect of a water deficit on total respiration, although in our study, the sensitivity of total respiration to short-term temperature changes was not strongly affected by the soil water regime (data not shown). However, the water deficit slightly increased the Q_{10} value of SHAM-resistant respiration and significantly decreased this parameter of the SHAM-sensitive pathway, as was found for seedlings grown on the soil without shungite (Figure 3, 0S treatment).

The effect of shungite application on the temperature sensitivity of SHAM-resistant and SHAM-sensitive respiration of onion leaves was strongly dependent on the soil water availability. While the shungite application increased the Q_{10} values of both SHAM-resistant and SHAM-sensitive respiration during the water deficit, shungite decreased these values under the condition of sufficient water availability. The cause of the variability in respiratory Q_{10} values has not yet been well-established, especially for the respiratory pathways. The temperature sensitivity of respiratory flux has been shown to be variable, depending on the level of ubiquinone reduction, the degree of adenylate control of the Cyt pathway [20], and/or the availability of respiratory substrates [27]. The Q_{10} values of O_{2} consumption can increase following a substrate availability increase [27], an increase of ubiquinone reduction, and an increase of the activation state of the Alt pathway [20]. A recent study [17] showed that the shungite application to the soil can alter nutrient concentrations of onion seedlings. Depending on the soil water regime, shungite can increase the plant content of potassium, manganese, zinc, and nickel, thus affecting some physiological traits of onion seedlings [17]. It can be suggested that the shungite-related change of Q_{10} values of SHAM-resistant and SHAM-sensitive respiration might be partly controlled by the nutrient element contents.
The ability of plants to enhance electron transport through the Alt pathway in cold temperatures [13,14,16,28] could be due to the Alt pathway being less temperature-sensitive than the Cyt pathway [29]. The results of this study are consistent with some earlier reports that have shown that the sensitivity of the Alt pathway to the short-term temperature changes may not be lower than that of the Cyt pathway [20,21,28]. For the 0S onion leaves grown under the condition of sufficient water availability, the higher Q_{10} value of the Alt pathway than the Cyt pathway was connected with the lowest contribution of SHAM-sensitive respiration to total respiration at a low measurement temperature (Figure 2). These data confirm the finding of Armstrong et al. [21] that during the short-term temperature drop, the activity of electron transport through the Alt pathway declines and does not play an important role in maintaining flux through the mitochondrial electron transport. However, both shungite application at sufficient water availability and the soil water deficit may decrease the sensitivity of the SHAM-sensitive pathway to the short-term temperature change. This allows the Alt pathway to be more involved in the electron transport process, and in doing so reduces the production of reactive \text{O}_2 species. Under the condition of the well-watered regime, the shungite application decreased the temperature sensitivity of not only the SHAM-sensitive but also the SHAM-resistant pathway (Figure 3). The decrease of the temperature sensitivity of respiratory pathways can help to maintain mitochondrial electron transport and the cell redox state during temperature drops, and thus the plant resistance to low temperatures.

5. Conclusions

The data demonstrate that both Cyt and Alt respiratory pathways of *A. cepa* leaves and their sensitivity to short-term temperature changes can be dynamic when plants are subjected to the contrasting conditions of soil water availability or shungite content. For plants grown without shungite, the water deficit decreased the Q_{10} values of Cyt but not Alt respiration. The response of the temperature sensitivity of the pathways to shungite application depends on the water availability. The shungite-related decrease of both Cyt and Alt pathways may play an important role in enhancing the resistance of plant respiration to the temperature drop. Although shungite rocks are widely used in various industries, their use in agriculture is still being studied. The obtained results showed that the shungite rocks might have the potential for agricultural application, though further investigations including field studies are required.

**Supplementary Materials:** The poster presentation can be downloaded at: https://www.mdpi.com/article/10.3390/IECAG2021-09688/s1.

**Author Contributions:** E.I. designed and performed the experiments, analyzed the data, and wrote the paper; S.C. wrote the paper; O.B. and V.S. conceived the experiments. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

The following abbreviations are used in this manuscript:

Alt alternative pathway
Cyt cytochrome c oxidase (Cyt) pathway
DW drying-wetting cycle
RAS Russian Academy of Science
SHAM salicylhydroxamic acid
WW well-watered

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