Plant Soil (2025) 513:2757–2768
https://doi.org/10.1007/s11104-025-07347-0

RESEARCH ARTICLE

Physiological characterization of asparagus decline syndrome

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Received: 19 November 2024 / Accepted: 26 February 2025 / Published online: 12 March 2025
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Abstract

Background and aims Asparagus Decline Syndrome (ADS) threatens the sustainability and productivity of asparagus (*Asparagus officinalis* L.) cultivation. This study aimed to characterize the physiological responses of asparagus plants to ADS, focusing on oxidative metabolism, hormonal regulation, and phenolic compounds profiles to understand the underlying mechanisms and inform management strategies.

Methods A field trial was conducted in the south of Spain comparing asparagus plants grown in soil from a plot previously affected by the ADS with a control soil (not affected). The key parameters assessed included biomass and oxidative stress indicators, phytohormone and phenolic compounds profiles in the root and shoot, and the soil phenolic compounds.

Results ADS-affected plants exhibited lower fresh and dry weight and volume, and elevated oxidative stress, as evidenced by increased malondialdehyde (MDA) and H_2O_2 levels, along with enhanced activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX). Hormonal analysis revealed higher concentrations of abscisic acid (ABA) and jasmonic acid, alongside a concurrent reduction in indoleacetic, suggesting a stress-induced response likely contributing to growth inhibition. Furthermore, the depletion of caffeic acid in roots, alterations in flavonoid profiles in shoot tissues, and increased PPO activity were observed, potentially worsening oxidative stress and depleting antioxidant reserves. Finally, ferulic acid derivatives in the soil were identified as potential allelopathic compounds.

Conclusion These findings highlight the complexity of ADS and underscore the importance of integrated management strategies, including soil health management, resistant varieties selection, and targeted modulation of plant physiological responses to mitigate the impacts of ADS on asparagus production.

Keywords Asparagus decline syndrome · Oxidative stress · Phytohormones · Phenolic compounds · Antioxidant enzymes · *Asparagus officinalis*

Responsible Editor: Beatriz Vazquez-de-Aldana.

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Introduction

Asparagus (*Asparagus officinalis* L.) is a perennial crop of great economic importance in various regions around the world, facing multiple challenges related to its long-term sustainability and productivity (Elmer 2018). Among these challenges, Asparagus Decline Syndrome (ADS) has been identified as one of the most critical issues, severely affecting both yield and crop quality. ADS is a complex physiological and biochemical disorder manifested through symptoms such as premature plant death, reduced vigor, and decreased spear production (Elmer et al. 1996; Knaflewski 1996).

ADS has traditionally been associated with the presence of fungal pathogens, particularly species from the genus *Fusarium* such as F. *oxysporum* f. sp. asparagi, F. *proliferatum*, and F. *redolens* (Elmer 2001; Baayen et al., 2000). However, recent research suggests that imbalances in the physiological and metabolic processes of plants play an equally crucial role in the expression of the disease. Specifically, oxidative metabolism, hormonal regulation, and phenolic compounds accumulation in plant tissues and soil are determining factors that condition the plant's response to the stress associated with ADS (Blok and Bollen 1993).

The overproduction of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) is a common indicator of oxidative stress, which can lead to cellular damage if not properly regulated. Plants possess both enzymatic and non-enzymatic antioxidant systems, including key enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), which play a vital role in the detoxification of ROS. In the case of ADS, studies have shown an increase in malondialdehyde (MDA) levels, a marker of lipid peroxidation, indicating an imbalance in the oxidative metabolism of affected plants. This imbalance not only compromises cellular integrity but also interferes with hormonal signaling and other essential physiological processes (Elmer et al. 1996).

Hormonal regulation is another critical component in the plant's response to ADS. Phytoregulators such as abscisic acid (ABA), ethylene, and auxins are known for their role in mediating stress responses and modulating plant growth and development. It has been observed that in plants affected by ADS, ABA levels are often elevated, suggesting a response to water stress or premature senescence (Blok and Bollen 1993). Similarly, ethylene, a hormone associated with senescence and pathogen response, also shows an increase under severe stress conditions, which may be related to the acceleration of plant death under ADS conditions. On the other hand, auxins, which regulate growth and cell differentiation, may be disrupted, resulting in abnormal growth and loss of vigor in the affected plants.

Phenolic compounds, for their part, play a dual role in the context of ADS. On the one hand, they are defense compounds produced by plants in response to pathogen attack, acting as antioxidants and antimicrobial agents. On the other hand, the accumulation of phenols in the soil can have negative allelopathic effects, inhibiting the growth of new plants and favoring an environment conducive to disease development. The concentration and profile of phenols, both in the root and shoot tissues, are important indicators of the physiological state of asparagus plants and their ability to cope with ADS (Fischer et al. 2011; Kato-Noguchi et al. 2017, 2018).

This study focuses on the physiological characterization of ADS in asparagus by evaluating key parameters related to oxidative metabolism, hormonal, and phenol profiles in the soil and asparagus plants affected by ADS. The research is based on results obtained from a field trial, comparing two main treatments: control soil (soil on which asparagus has never been cultivated) and soil from a plot previously affected by the ADS. Oxidative stress indicators, hormone levels, and phenol profile were analyzed to better understand the physiological mechanisms underlying ADS and develop management strategies to mitigate its effects.

Materials and methods

Experimental setup

The trial was conducted at IFAPA Camino de Purchil (Granada, Spain; 37.1576°N, 3.6343°W), using isolated containers of 6×4 m^2 and 130 cm deep. The containers were lined with continuous plastic on the sides and perforated plastic on the bottom, with a 30 cm layer of gravel at the bottom to facilitate drainage. The soil used in the Control treatment was

taken from a plot at IFAPA that had not been cultivated with asparagus for more than 10 years. On the other hand, the soil used in the ADS treatment was obtained from a plot affected by ADS (El Jau, Spain; 37.2015°N, 3.7477°W), characterized by high plant mortality and a drastic reduction in yield. This experimental trial was established to investigate the physiological and biochemical differences between asparagus plants (*Asparagus officinalis* L.) grown under these two soil conditions.

Asparagus crowns of the "Grande" variety were transplanted on May 21, 2018, at a depth of 20 cm, with a planting frame of 1 × 0.3 m. The experimental design was completely random, with three replications per treatment. Each replication consisted of one container, resulting in a total of six containers. Each container contained six rows of plants, totaling 72 plants per container. Optimal growing conditions were ensured through drip irrigation and fertigation adjusted to the crop's needs.

Two years after the transplantation of the crowns, on June 25, 2020, asparagus plants were sampled. Twelve plants were randomly selected from each container for sampling. Plants from the lateral edges were excluded to avoid edge effects. On the sampling day, plant volume was measured, the shoot and Roots of the plants were separated and weighed, and a soil sample was collected as close as possible to the roots. Subsequently, part of the material was frozen at −40 °C for biochemical analyses, while another portion was lyophilized for the analysis of the phytohormone and phenolic compounds profiles.

Analysis of phytohormones

For the quantification of phytohormones in the shoot, the protocol described by Albacete et al. (2008) was followed, with some modifications. The lyophilized samples (30 mg) were mixed with 1 mL of a cold extraction solution (−20 °C) composed of methanol and water (80/20, v/v). After centrifugation at 20,000 × g for 15 min at 4 °C, the samples were subjected to a second extraction under the same conditions. The combined supernatants were purified using Sep-Pak Plus C18 cartridges (Waters, Milford, MA, USA), and the dry residue was resuspended in 1 mL of a methanol and water solution (20/80, v/v) using an ultrasonic bath. Subsequently, the dissolved samples were filtered through nylon membrane filters of 0.22 µm and 13 mm in diameter (Millipore, Bedford, MA, USA), and 10 µL of the filtered extract was injected into a U-HPLC–MS system for hormone quantification (Albacete et al. 2008).

Evaluation of oxidative metabolism

To evaluate oxidative metabolism in asparagus plants, specific analyses were conducted to determine the concentration of malondialdehyde (MDA) and the activity of antioxidant enzymes such as superoxide dismutase (SOD) and ascorbate peroxidase (APX).

The concentration of MDA, a marker of lipid peroxidation, was measured following the method of Fu and Huang (2001). Briefly, 0.1 g of the shoot was homogenized in 1 mL of a solution of thiobarbituric acid at 0.25% in trichloroacetic acid at 10%. The mixture was heated to 95 °C for 30 min and then cooled in an ice bath. The samples were centrifuged at 9500 rpm for 10 min, and the concentration of MDA in the supernatant was quantified by measuring the absorbance at 532 nm. The turbidity was corrected using the absorbance at 600 nm, and the concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Fu and Huang 2001).

The activity of the superoxide dismutase enzyme (SOD) was measured using the method described by Yu et al. (1998), which is based on the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) (Yu et al. 1998).

The H_2O_2 concentration was determined following the method of Kubiś (2008). The samples were extracted with cold acetone, and the intensity of the yellow color of the supernatant was measured spectrophotometrically at 415 nm. The concentration of H_2O_2 was expressed as µg g^{-1} dry weight (DW) (Kubiś, 2008).

The APX activity in extracts from the shoot was measured by recording the change in absorbance at 290 nm over 3 min (Yu et al. 1998).

Phenolic compounds profile and PPO activity

Phenolic compounds metabolism in the plants was evaluated by quantifying the activity of polyphenol oxidase (PPO) and the detailed composition of phenols present in the roots and the shoots.

PPO activity was measured following the colorimetric method of Chavan and Kamble (2014), based

on the conversion of catechol to o-quinone by PPO. Enzymatic activity was measured at 420 nm over 3 min (Chavan and Kamble 2014).

The phenolic compounds composition of the soil and lyophilized samples from the roots and shoot was determined using HPLC–DAD and HPLC–DAD-ESI–MS/MS. Plant samples were extracted with a solution of methanol:water:formic acid (50:48:2) at a concentration of 100 mg/mL, while soil samples were extracted with a solution containing 80% MeOH and 2% triethylamine. The samples were analyzed using a reversed-phase C18 Kinetex column (5 µm, 250×4.6 mm) with a mobile phase consisting of 1% formic acid in water and acetonitrile in gradient mode. The flow rate was 0.8 mL/min and the injection volume was 20 µL. Spectral data were collected in the range of 200–600 nm, with chromatograms recorded at 330 nm. Phenolic acids (e.g., caffeic acid, p-coumaric acid, and glycosylated derivatives) and flavonoids (mainly flavonol glycosides such as quercetin, kaempferol, isorhamnetin derivatives, and apigenin diglucoside) were identified and quantified by HPLC–DAD using freshly prepared calibration curves with analytical standards. The identified compounds were classified into two main groups phenolic acids and flavonoids. Chlorogenic acid (molecular weight 354) and rutin (molecular weight 628.5) were used as reference standards. The structural characterization of these compounds was performed by HPLC–DAD-ESI–MS/MS using an Agilent 1100 series system coupled to an ion trap mass spectrometer with an electrospray ionization (ESI) interface. The ionization parameters were set at 350 °C and 4 kV, with a nebulizer pressure of 60 psi and a nitrogen flow rate of 11 L/min. The mass spectrometer operated in negative ionization mode, with full scan spectra recorded in the m/z range of 100 to 1000. Collision-induced fragmentation (CID) experiments were performed using helium as the collision gas. Retention times, parent ions, and fragmentation patterns were compared with authentic standards and literature data to confirm compound identities. MS/MS identification was used for compounds that are isomers of the available standards or structurally similar compounds that may cause ambiguities in identification by HPLC–DAD alone. These compounds were: 4-caffeoylquinic acid (4-CQA), feruloylquinic acid (Feruloil-QA), p-coumaric acid derivative, apigenin diglucoside (Vicenin-2), quercetin rutinoside derivative, kaempferol rutinoside, and isorhamnetin rutinoside.

Statistical analysis

To evaluate the differences between treatments, a one-way analysis of variance (ANOVA) was performed with a 95% confidence level. The means and standard errors for each treatment were calculated from the nine individual data points for each analyzed parameter. All statistical analyses were performed using the software Statgraphics Centurion 16.1.03.

Results

Biomass production

The results indicate a significant reduction in biomass accumulation in asparagus plants affected by ADS. Root fresh weight was markedly lower in ADS plants compared to the control and the dry matter content also showed a significant decrease. In addition, in the shoot, fresh and dry weights, and plant volume were reduced in ADS plants, suggesting a negative impact on overall plant growth caused by ADS (Table 1).

Oxidative metabolism

MDA levels were evaluated in the roots and shoots of asparagus plants. The results showed significant

Table 1 Biomass parameters in the root and shoot of asparagus plants grown under control and ADS treatment

	Fresh weight (g/plant)	Dry weight (g/plant)	Plant volume (cm^3)
Root			
Control	524 ± 50	147 ± 31	-
ADS	294 ± 36	76 ± 23	-
P-value	< 0.001	0.019	
Shoot			
Control	517 ± 44	145 ± 29	754 ± 126
ADS	377 ± 40	102 ± 24	476 ± 105
P-value	< 0.001	0.021	0.018

differences in MDA levels between the treatments. In the roots, the ADS treatment exhibited the highest MDA level. In the shoot, no significant differences in MDA levels were found between the treatments. In addition, the SOD enzyme activity in the roots did not reveal significant differences among treatments. However, in the shoot, maximum levels of SOD activity were observed in the ADS treatment. Regarding H_2O_2, significant differences were found in the shoot between treatments, with the ADS treatment presenting higher levels compared to the control. No significant differences were observed in H_2O_2 levels in the roots. Finally, no significant differences were observed for APX activity in the roots among the treatments. Nevertheless, in the shoot, the ADS treatment showed a significant reduction in APX activity compared with the other treatments (Fig. 1).

Phytohormone profile

First, it is important to note that most of the hormones analyzed did not show significant differences between treatments in both plant parts. However, the roots of plants in the ADS treatment showed lower ABA content than in the control. On the other hand, in the shoot, highly significant differences were observed in the levels of 1-aminocyclopropane-1-carboxylic acid (ACC) between the treatments. Plants affected by ADS showed an increase in ACC content by 58% compared with the Control (Table 2).

Phenolic compounds profile and PPO activity

Regarding the phenolic acid profile, the soil from an asparagus culture that previously exhibited ADS showed significantly higher levels of feruloyl

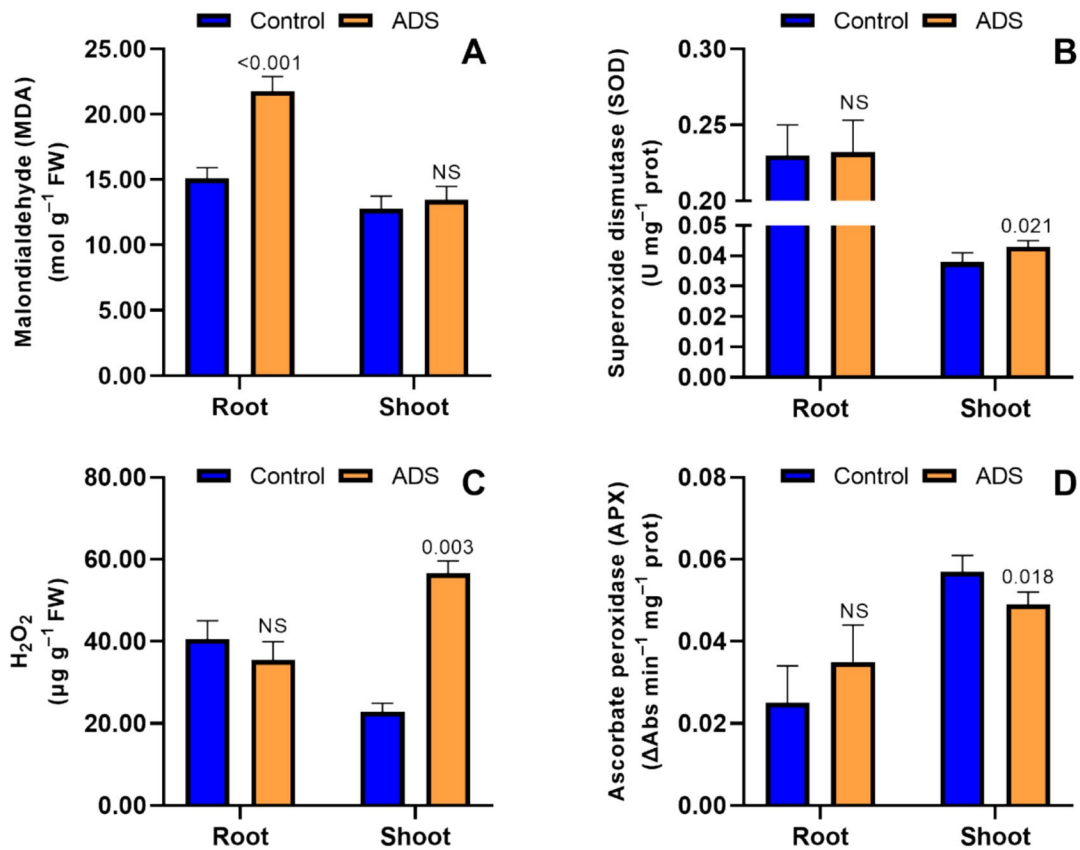


Fig. 1 Oxidative stress parameters in the root and shoot of asparagus plants under control and ADS treatment. MDA concentration (**A**), SOD activity (**B**), H_2O_2 concentration (**C**), and APX activity (**D**). The values above the ADS columns indicate the *p*-values. *NS*: non-significant

Table 2 Hormonal concentrations in the root and shoot of asparagus plants under control and ADS treatment

	Indole Acetic Acid	trans-Zeatin	Zeatin Riboside	Isopentenyl Adenine	GA1	GA3	GA4	ACC	ABA	Jasmonic Acid	Salicylic Acid
Root											
Control	6.13 ± 0.80	69.56 ± 9.90	ND	14.25 ± 3.67	ND	ND	0.41 ± 0.05	229.07 ± 58.50	0.79 ± 0.03	11.14 ± 2.95	289.40 ± 20.12
ADS	7.32 ± 0.95	83.46 ± 14.01	ND	10.86 ± 2.90	ND	ND	0.35 ± 0.04	161.57 ± 51.20	0.84 ± 0.02	8.67 ± 1.44	299.35 ± 18.78
P-value	0.359	0.296	-	0.430	-	-	0.358	0.284	0.012	0.197	0.692
Shoot											
Control	7.74 ± 0.60	9.21 ± 0.90	ND	4.00 ± 0.92	ND	ND	0.30 ± 0.02	13.30 ± 1.11	35.09 ± 2.80	49.03 ± 4.22	143.54 ± 10.54
ADS	8.04 ± 0.65	8.84 ± 1.00	ND	3.13 ± 0.79	ND	ND	0.31 ± 0.03	21.01 ± 1.50	32.26 ± 2.58	52.14 ± 4.57	142.23 ± 9.84
P-value	0.581	0.567	-	0.578	-	-	0.807	< 0.001	0.609	0.601	0.828

ND Not detected; *GA* Gibberellin; *ACC* 1-aminocyclopropane-1-carboxylic acid; *ABA* Abscisic acid. Values are expressed as ng/g FW.

derivatives compared with the soil with no recent history of ADS. In the asparagus roots, plants affected by ADs showed lower levels of caffeic acid compared to control plants. In the shoot, no differences between treatments were observed regarding the detected phenolic acids (Table 3).

The flavonol levels were under the detection limit of the method used in soil and asparagus roots. However, in the shoot, the ADS-affected plants presented lower levels of Apigenin diglucoside (Vicenin-2) and Kaempferol rutinoside (Kaemp Rut.) and showed higher Quercetin rutinoside derivative (Q-rut- deriv.) concentrations in comparison to control plants. No significant differences between treatments were found for Quercetin rutinoside (Q-Rutin) and Isorhamnetin Rutinoside (Isorhamnetin Rut) (Table 4).

PPO enzyme activity in asparagus roots and shoots showed significant differences in response to the treatments. In both shoots and roots, significantly higher PPO activity was observed under ADS compared with the control (Fig. 2).

Discussion

Biomass production

The biomass results confirm the occurrence of ADS in our experiment, as indicated by the significant reduction in both root and shoot biomass. Thus, root biomass decreased by 44% in ADS-affected plants compared to the control, while shoot biomass was reduced by 27%. Similar trends have been reported in previous studies, where ADS has been associated with impaired growth and biomass accumulation (Elmer 2018; Kato-Noguchi et al. 2018). Furthermore, these results align with previously published data on spear yield under the same experimental conditions, reinforcing the negative impact of ADS on asparagus production (López-Moreno et al. 2025).

Oxidative metabolism in asparagus plants under ADS conditions

ADS is a complex phytopathology affecting asparagus plants (Hartung and Stephens 1983; Elmer 2018; Kato-Noguchi et al. 2018; Noperi-Mosqueda et al. 2020; López-Moreno et al. 2021). As in other pathologies, oxidative stress is a key aspect to be

Table 3 Phenolic acid concentrations in the root and shoot of asparagus plants under control and ADS conditions

	3-CQA	4-CQA	Caffeic acid	Feruloil-QA	p-coumaric acid derivative	Ferulic acid derivative
Soil						
Control	ND	ND	ND	ND	ND	0.04 ± 0.01
ADS	ND	ND	ND	ND	ND	0.08 ± 0.01
P-value	-	-	-	-	-	0.004
Root						
Control	ND	ND	3.62 ± 0.13	ND	ND	ND
ADS	ND	ND	2.92 ± 0.09	ND	ND	ND
P-value	-	-	< 0.001	-	-	-
Shoot						
Control	0.36	0.15	ND	ND	ND	0.47 ± 0.21
ADS	0.45	0.19	ND	ND	ND	0.28 ± 0.19
P-value	0.347	0.432	-	-	-	0.385

ND Not detected; *CQA* Caffeoylquinic acid; *Feruloil-QA* Feruloylquinic acid. Values are expressed as mg/g DW.

Table 4 Flavonol concentrations in the root and shoot of asparagus plants under control and ADS conditions (mg/g DW)

	Apigenin digluc (Vicenin2)	Q-Rutin	Kaempferol rutinoside	Quercetin rutinoside derivative	Isorhamnetin Rutinoside
Root					
Control	ND	ND	ND	ND	ND
ADS	ND	ND	ND	ND	ND
P-value	-	-	-	-	-
Shoot					
Control	0.62 ± 0.07	4.59 ± 0.34	0.19 ± 0.02	0.001 ± < 0.000	0.13 ± 0.09
ADS	0.53 ± 0.05	4.60 ± 0.29	0.12 ± 0.01	0.040 ± 0.002	0.11 ± 0.07
P-value	0.036	0.879	0.029	0.008	0.779

ND Not detected; *Digluc* Diglucoside; *Q-Rutin* Quercetin rutinoside (rutin). Values are expressed as mg/g DW.

analyzed as in any syndrome with a negative impact on plants (Saed-Moucheshi et al. 2014). MDA is an end product of lipid peroxidation, and its accumulation is a reliable indicator of the extent of oxidative damage in plant cells (Marnett 1999). The results obtained in this study show a significant increase in MDA concentration in plants subjected to ADS conditions, indicating increased lipid peroxidation and, consequently, an increase in oxidative damage at the cellular membrane level. This finding is consistent with previous studies that associate elevated MDA levels with severe oxidative stress in plants subjected to adverse conditions (Hodges et al. 1999; Marnett 1999; Mittler 2017).

Moreover, the increased activity of antioxidant enzymes, such as SOD and APX, observed in plants under ADS conditions, suggests an adaptive response to counteract ROS accumulation. SOD catalyzes the dismutation of the superoxide anion into oxygen and H_2O_2, while APX uses ascorbate as a substrate to reduce H_2O_2 to water, thus minimizing oxidative damage (Apel and Hirt 2004; Foyer and Noctor 2005). The higher activity of these enzymes in plants under ADS indicates an effort by the plant to mitigate oxidative damage. However, the elevated activity of SOD and APX may also reflect an overload in the plant's antioxidant capacity, suggesting that despite the increased enzymatic activity, the plants may not be fully protected against damage caused by oxidative stress (Mittler 2017).

The significant increase in H_2O_2 concentration in plants affected by ADS evidences the imbalance in oxidative metabolism. Although H_2O_2 acts as a signaling molecule in defense processes, its excessive

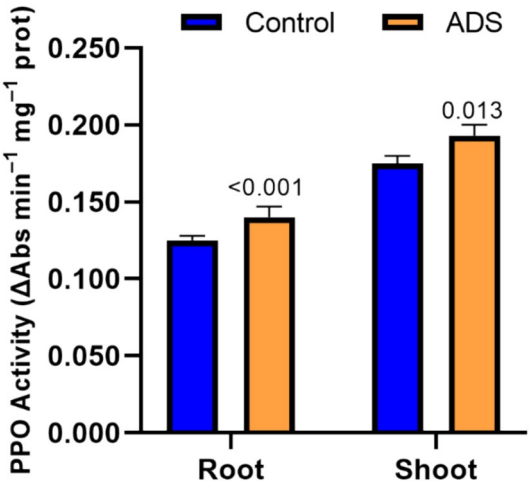


Fig. 2 Comparison of PPO activity in the root and shoot under control and ADS conditions. The values above the ADS columns indicate the *p*-values

accumulation can be harmful to plant cells as it can induce apoptosis or programmed cell death (Gechev et al. 2006). In this context, the high concentration of H_2O_2 observed in plants under ADS could contribute to physiological deterioration, worsening oxidative damage, and compromising cell viability (Ślesak et al. 2007).

The observed differences between the shoot and the roots may be attributed to the more efficient functioning of antioxidant systems, such as SOD, in the shoot tissues, which could prevent a significant increase in MDA levels. In contrast, these mechanisms may either be nonfunctional or overwhelmed in the roots, leading to greater lipid peroxidation and oxidative damage. In the shoot tissues, the higher SOD activity, coupled with a relatively lower APX activity, could account for the significant increase in H_2O_2 levels observed under the ADS conditions. This imbalance suggests that while SOD effectively dismutates superoxide radicals into H_2O_2, the limited APX activity may hinder its subsequent detoxification, producing the H_2O_2 accumulation.

Hormonal response to ADS

The results indicate that most of the phytohormones analyzed did not exhibit significant differences between treatments, implying that the ADS had a limited impact on hormone synthesis, transport, or metabolism. However, notable increases in ABA levels in the roots and ACC levels in the areal parts of ADS plants were observed. These results may indicate a heightened stress response under these conditions. ABA plays a critical role in regulating plant growth and stress adaptation, particularly in adverse conditions such as drought or salinity (Amjad et al. 2014). Thus, elevated ABA levels in the ADS treatment could reflect an intensified activation of these stress response mechanisms in the roots.

The ADS treatment also influenced the ethylene response. For instance, an increase in ACC levels was detected in the shoot of plants, suggesting enhanced ethylene production, as ACC is a precursor to ethylene. Ethylene regulates key stress-related processes, such as ripening, senescence, and pathogen response (Iqbal et al. 2014). The significant increase in ACC under ADS likely indicates the activation of the plant's defense mechanisms in response to this treatment. Additionally, elevated ABA levels in plants under ADS suggest a role in managing abiotic stress through mechanisms like stomatal closure and leaf senescence, potentially reducing water loss but also impacting photosynthetic capacity and accelerating aging (Seo and Koshiba 2002; Zhang et al. 2006).

Phenolic compounds and their role in the response to ADS

Subproducts from asparagus cultivation contain significant quantities of allelopathic compounds, particularly various phenolic compounds (Alcaide et al. 2023). These allelochemicals, accumulated at enough concentration, can induce a ROS burst in target plants, causing oxidative stress and inducing lipid peroxidation (Staszek et al. 2021). These two effects were observed in the present study (Table 1). When the antioxidant defenses are overwhelmed, the cell organelles lose their integrity and functionality. In roots, these effects alter the structure and activity of the apical meristem, impairing root growth and water absorption (Šoln et al. 2022), which could contribute to the ADS syndrome. In our study, the soil from asparagus fields affected by ADS exhibited higher levels of ferulic acid derivatives. Notably, previous research has demonstrated the potentially allelopathic nature of ferulic acid derivatives against plants (dos Santos et al. 2008). The ferulic acid accumulation may be explained by a progressive process: in the

early years of cultivation, healthy plants accumulate higher amounts of ferulic acid, as observed in control plants without ADS. Over time, as cultivation continues, ferulic acid from plant residues might be gradually released into the soil, leading to its accumulation. However, this long-term accumulation appears to be associated with a reduction in ferulic acid content in the aerial parts of plants already affected by ADS. Additionally, stressed plants have been reported to enhance the exudation of phenolic compounds, a phenomenon observed in other plant species as well (Gargallo-Garriga et al. 2018; Clocchiatti et al. 2021). This process could further contribute to the lower phenolic content found in ADS-affected plants while explaining the increased concentration in the soil. On the other hand, while Wu et al. (2010) reported that ferulic acid can inhibit the pathogen *Fusarium*, suggesting a protective effect, it is important to note that the same compound has been shown to reduce hyphal elongation and fungal root colonization (Wacker et al. 1990; Kato-Noguchi 2022), which could have detrimental effects on the symbiotic relationship between asparagus plants and mycorrhizal fungi. The elevated concentration of this phenolic compound in soils affected by ADS implies that it may play a dual role: protecting against certain pathogens while potentially disrupting beneficial plant–microbe interactions, thereby contributing to the reduced plant growth characteristic of ADS. However, further studies are needed to fully elucidate the relative importance of these opposing effects and their contribution to the development of ADS.

Conversely, under different conditions, phenolic compounds within the plant and at physiological concentrations play a beneficial role, as they are integral to defense systems, acting as antioxidants that neutralize ROS and mitigate cellular damage caused by environmental stressors (Rice-Evans et al. 1996; Grace 2005). In asparagus plants, flavonols such as quercetin and kaempferol, along with phenolic acids like caffeic acid, are particularly notable for their antioxidant properties (Michalak 2006). Our data highlights how the phenolic compounds profile and PPO activity are altered under ADS, reflecting a dynamic reprogramming of secondary metabolism and oxidative stress responses.

In roots, the significant reduction in caffeic acid levels under ADS suggests a depletion of this key antioxidant, likely due to elevated ROS activity, as indicated by the high MDA levels observed (Heath and Packer 1968). This reduction could weaken the roots' ability to counteract oxidative stress, worsening the physiological damage. Interestingly, in the shoot, an increase in quercetin rutinoside derivative (Q-rut-deriv.) levels under ADS may represent a compensatory response to oxidative stress, as quercetin and its derivatives are effective in scavenging ROS and stabilizing cellular redox balance (Agati et al. 2012). Conversely, the lower levels of apigenin diglucoside (Vicenin-2) and kaempferol rutinoside in ADS-affected shoot tissues suggest a diminished flavonoid-based defense system, which could further compromise the plant's antioxidant capacity (Santos-Sánchez et al. 2019).

The increase in PPO activity observed in both the roots and shoots under ADS likely exacerbates oxidative stress through the oxidation of phenolic compounds to quinones, generating additional ROS and potentially depleting the phenolic compound pool (Mayer 2006). This enzymatic response could be linked to the hormonal changes detected in the ADS-affected plants. Reduced ABA levels in roots may impair the activation of antioxidant defenses, as ABA regulates stress responses and ROS-scavenging pathways (Finkelstein 2013). Meanwhile, the elevated ACC levels in the shoot, a precursor to ethylene, align with ethylene's role in promoting PPO activity and regulating senescence under stress conditions (Iqbal et al., 2014).

Previous studies have corroborated the importance of phenolic compounds metabolism in asparagus defense systems. For instance, Rosado-Álvarez et al. (2014) reported that caffeic and ferulic acids in asparagus roots are associated with resistance to fungal pathogens, whereas PPO activity is tightly linked to stress-induced phenolic compounds oxidation (Beltagi and Zhang 2023). The elevated PPO activity and altered phenolic compounds profiles observed here mirror findings in other stress contexts, reinforcing the role of oxidative stress in the pathophysiology of ADS.

Conclusions

ADS produced negative symptoms by reducing biomass production and induced a pronounced oxidative imbalance characterized by elevated MDA and H_2O_2

levels, reflecting extensive cellular damage. Concurrently, the upregulation of antioxidant enzymes, such as SOD and APX, suggests a compensatory response that is insufficient to mitigate the accumulated oxidative stress. The hormonal responses, particularly increased ABA levels in roots and elevated ACC in shoot tissues, indicate a complex signaling network aiming to manage stress but potentially contributing to processes such as senescence and reduced photosynthetic efficiency. Moreover, alterations in phenolic compounds metabolism, including depleted caffeic acid in the roots and modified flavonoid profiles in the shoot, coupled with increased PPO activity, underline the systemic oxidative stress and compromised antioxidant capacity in ADS-affected plants. This study suggests that ferulic acid derivatives in soils from ADS-affected asparagus fields may act as potential allelopathic agents. Further experiments are needed to confirm their specific role in ADS, including the effect on mycorrhizal symbiosis. Based on these findings, ADS emerges as a multifaceted syndrome driven by the interplay between oxidative stress, hormonal dysregulation, and phenolic compounds metabolism. Future research should explore targeted interventions, such as enhancing soil health to mitigate allelopathic effects, breeding asparagus varieties with enhanced antioxidant capacities, and developing treatments to modulate redox homeostasis. These approaches could offer sustainable solutions to counter ADS and improve the resilience of asparagus cultivation.

Acknowledgements We would like to express our sincere gratitude to our colleagues from the University of Granada (UGR) and the Institute of Agricultural and Fisheries Research and Training (IFAPA) for their invaluable support and collaboration throughout the development of this project. In particular, we thank Camila Buturi, Angela Grazioso, Araceli Cabello, Pedro Fernandez, Manuel Conejero, Carmelo Caballero, and Emilio Palma for their continuous effort and support, which have been essential for the successful completion of this work.

Author contributions Conceptualization, J.M.R. and T.S.; Methodology, F.J.L.-M., and E.N.-L.; Validation, T.S. and J.M.R.; Formal analysis, F.J.L.-M.; Data curation, F.J.L.-M. and E.N.-L.; Writing— original draft preparation, F.J.L.-M.; Writing—review and editing, J.M.R. and T.S. All authors have read and agreed to the published version of the manuscript.

Funding Funding for open access publishing: Universidad de Granada/CBUA. This work has been developed within the project RTA2015-00008-C02, with INIA and ERDF 2014–2020 funding, in the Intelligent Growth Operational Program, and by a 2017 grant awarded to FJLM for pre-doctoral contracts for the training of doctors contemplated in the State Training Subprogram of the State Program for the Promotion of Talent and its Employability, at the IFAPA. Grant number [BES-2017–080123]. Co-financed by the ESF. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data availability The data presented in this study are available on request from the corresponding author.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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