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Root and crown rot caused by *Fusarium pseudograminearum* in the euhalophyte *Salicornia europaea*: pathogenicity and mycotoxin production in plants grown in soilless culture

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Summary. *Salicornia europaea* L. is a euhalophyte increasingly cultivated as a high-value green vegetable. In July 2021, root and crown rot occurred on 6-month-old *S. europaea* plants grown in peat-filled pots under a greenhouse, affecting 25% of plants. The causal agent was identified as *Fusarium pseudograminearum* O'Donnell & T. Aoki using morphological and molecular analyses. An experiment to assess the pathogenicity of this fungus to *S. europaea* was conducted with 96 seedlings in hydroponic culture. Half of these plants were inoculated with a conidial suspension of *F. pseudograminearum*. At 24 days post inoculation (dpi), half of the plants were transferred into a new hydroponic system, while the other plants were transplanted into pots. At 80 dpi, all inoculated plants grown in pots had shoot browning and desiccation symptoms, while these symptoms developed more slowly in 70% of the hydroponically grown inoculated plants. A qualitative symptom severity assessment scale showed that disease severity was greater (63%) in pot-grown plants than in hydroponically grown plants (46%). *Fusarium pseudograminearum* was consistently reisolated from diseased plants in both cultivation systems (62% from pots and 83% from hydroponics) fulfilling Koch's postulates. Production of deoxynivalenol (DON) and zearalenone (ZEA) was investigated *in vitro* and *in planta*. Traces of DON (0.029 ± 0.012 mg kg^{-1}) were found in severely damaged plants grown in hydroponics. In the *in vitro* test, *F. pseudograminearum* isolates from wheat crops in Spain (isolate ColPat-351) and Italy (isolate PVS Fu-7) were also assessed, and all tested isolates produced considerable amounts of ZEA. *Fusarium pseudograminearum* isolates obtained from *S. europaea* produced more DON (6.81 ± 0.24 mg kg^{-1}, on average) than the Italian isolate PVS Fu-7 (0.37 ± 0.06 mg kg^{-1}), while DON production by the Spanish isolate ColPat-351 was less than the limit of detection (< 0.25 mg kg^{-1}). This is the first report of root and crown rot caused by *F. pseudograminearum* on *S. europaea*.

Keywords. Deoxynivalenol, fungi, glasswort, hydroponic system, plant disease, zearalenone.

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INTRODUCTION

Salicornia europaea L. (syn. *Salicornia perennans* Willd. subsp. *perennans*, *Amaranthaceae*), commonly known as glasswort or sea asparagus, is a euhalophyte that is widespread in many regions of the Northern Hemisphere, especially in the Mediterranean region (Lombardi *et al*., 2022). This plant thrives in saline ecosystems and can tolerate up to 1000 mM NaCl, with optimal growth at 200–400 mM NaCl (Cárdenas-Pérez *et al*., 2021). For this reason, it has received considerable interest as an alternative crop in saline agriculture, especially in desert areas and marginal lands (Araus *et al*., 2021). *Salicornia europaea* has a wide range of applications as a food and non-food crop. This plant is a good candidate for biodiesel production because of its high seed oil contents, for the remediation of saline soils, and for pharmaceutical purposes (Ventura and Sagi, 2013; Cárdenas-Pérez *et al*., 2021). It has edible shoots, raw or cooked, and is increasingly cultivated as a high-priced green vegetable (Araus *et al*., 2021; Cárdenas-Pérez *et al*., 2021). Despite the broad range of agronomic usages of *S. europaea*, little attention has been paid to phytopathological issues for this plant.

In July 2021, at the University of Pisa, severe root and crown rot symptoms were observed in pot-grown *S. europaea* plants purchased from a nursery in northern Italy (Figure 1, a, b, and c). Symptoms appeared on 25% of 6-month-old seed-propagated plants. The causal agent of the disease was identified as *Fusarium pseudograminearum* O'Donnell & T. Aoki. This fungus was previously described as *F. graminearum* Schwabe Group 1 and was identified for the first time in Australia in 1983 (Burgess *et al*., 1987). Subsequently, Aoki and O'Donnell (1999a) described the novel species *F. pseudograminearum*, based on morphological and molecular observations.

Fusarium pseudograminearum is a heterothallic Ascomycete, although the formation of perithecia by the teleomorph *Gibberella coronicola* T. Aoki & O'Donnell has been rarely observed (Aoki and O'Donnell, 1999b). This fungus is a soil-borne pathogen, primarily causing Fusarium crown rot (FCR) on small grain cereals, which is a common disease in warm, dry regions (Chakraborty *et al*., 2006; Poole *et al*., 2013; Sabburg *et al*., 2015). The fungus is widespread in Asia, especially China where it is becoming the predominant cause of FCR (Xu *et al*., 2017; Zhou *et al*., 2019; Deng *et al*., 2020), and in Australia (Obanor *et al*., 2013; Kazan and Gardiner, 2018). In Europe, the occurrence of *F. pseudograminearum* is unclear, as the pathogen has only been reported a few times. It was first identified as the cause of root rot on durum wheat in Foggia, southern Italy, by Balmas (1994), when it was still designated as *F. graminearum* Group 1. In 2016, *F. pseudograminearum* was found in Cordoba, southern Spain, causing FCR on *Triticum aestivum* (Agustí-Brisach *et al*., 2018). The pathogen has been described on wheat in the Mediterranean countries Algeria (Abdallah-Nekache *et al*., 2019), Syria (Alkadri *et al*., 2013), Tunisia (Kammoun *et al*., 2009), and Turkey (Tunali *et al*., 2008). However, Akinsanmi *et al.* (2007) noted that some alternative host plants (monocots and dicots) were infected by *F. pseudograminearum*, indicating that the pathogen is not host-specific. In Croatia, this fungus was first recorded on naturally infected rotted apples (Sever *et al*., 2012), and seeds of the wild legume *Vicia cracca* (Miličević *et al*., 2013). An endophytic *F. pseudograminearum* was also isolated from coastal dunegrass (*Leymus mollis*), improving plant growth and salinity resistance (Shan *et al*., 2021). Due to the uncertainty of the occurrence of the pathogen, the European Food Safety Authority (EFSA) has recently proposed to designate *F. pseudograminearum* as a potential quarantine pathogen for the European Union (EFSA Panel Health, 2022).

A key feature in several *Fusarium* species (including *F. culmorum*, *F. graminearum*, and *F. pseudograminearum*) is their ability to produce trichothecene B mycotoxins. This class of mycotoxins includes deoxynivalenol (DON), nivalenol (NIV), and acetylated derivatives (3-acetyldeoxynivalenol, 3ADON; 15-acetyldeoxynivalenol, 15ADON). Based on the type of trichothecene produced, each isolate can be assigned to a specific chemotype (3ADON, 15ADON, NIV). Each chemotype is determined by the trichothecene gene cluster (*tri*), composed of ten to 12 contiguous genes. The number of functional genes in this cluster varies depending on the species and chemotype (Pasquali and Migheli, 2014). Zearalenone (ZEA) is another mycotoxin of interest that exhibits strong estrogenic effects in mammals. ZEA is a polyketide mycotoxin synthesized from the acetate-polymalonate pathway, in which four genes are involved: *pks4*, *pks13*, *zeb1*, and *zeb2* (Nahle et al. 2021). Determining the mycotoxins produced by an isolate is essential for studying the dynamics of a fungal population in a specific agricultural area, and to evaluate toxigenic risks of specific chemotypes in food and feed.

Root and crown rot caused by *F. pseudograminearum* on cultivated *S. europaea* plants has not been previously described. Therefore, the aims of the present study were: (i) to assess the pathogenicity and evaluate disease severity caused by a selected isolate of *F. pseudograminearum* towards *S. europaea* plants grown in two different growing systems (pots and hydroponics); and (ii) to determine the mycotoxins produced by three isolates of

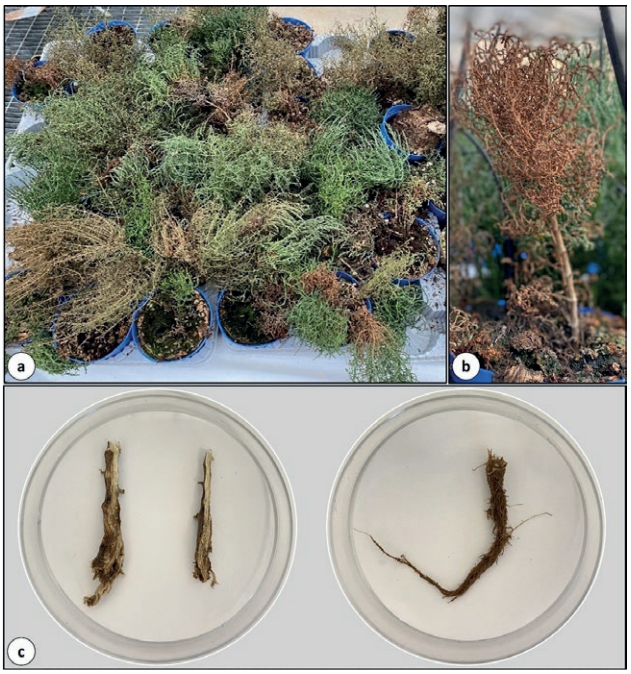

Figure 1. Diseased *Salicornia europaea* plants grown in pots. a) Overview of the pots on a greenhouse bench. b) A completely desiccated plant. c) Diseased plant crowns and roots.

F. pseudograminearum from *S. europaea* compared to two other European isolates of *F. pseudograminearum*.

MATERIALS AND METHODS

Origin of infected samples and isolation of fungal isolates

Severe root and crown rot symptoms were observed on seed-propagated *S. europaea* plants (approx. 6 months old) that had been purchased from a commercial nursery in northern Italy. The plants were grown in pots filled with peat (Figure 1, a and b). Symptoms affected 25% of the plants.

To isolate the putative causal agent, the roots and crowns of affected plants were gently washed under running tap water. Symptomatic tissues (Figure 1 c) were then cut into small pieces and surface sterilized with sodium hypochlorite (NaOCl; 1% available chlorine) for 2 min, rinsed twice for 2 min in sterile distilled water, and then plated onto Potato Dextrose Agar plates (PDA, 42 g L^{-1}, BioLife, Milan, Italy) amended with streptomycin (0.3 g L^{-1}, Sigma-Aldrich, Saint Louis, MO, USA). The plates were then incubated at 25 ± 1°C under fluorescent light and checked daily for mycelium development. After 4 days of incubation, hyphae emerging from infected plant tissues were transferred onto new PDA plates.

Monoconidial cultures and storage conditions

Colony morphology, and macroconidia shapes observed under an optical microscope indicated that the putative causal agent of the disease belonged to the genus *Fusarium*. Further analyses, both morphological and molecular, were carried out by generating monoconidial cultures of three selected isolates (designated 3B, PD-A, and PD-B), each obtained from a different diseased plant. An isolate of *F. oxysporum* (DAFE SP21-23), a fungus often associated with *S. europaea* plant tissues but not pathogenic, was included in the study as a negative control. For preservation of the isolates, PDA plugs derived from the above-described monoconidial cultures were transferred onto Synthetic Nutrient-poor Agar (SNA), which contained: 1 g L^{-1} KH_2PO_4; 1 g L^{-1} KNO_3; 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$; 0.5 g L^{-1} KCl; 0.2 g L^{-1} glucose; 0.2 g L^{-1} sucrose; and 20 g L^{-1} agar (Nirenberg, 1981). The cultures were stored at 4°C. All the chemicals used in the culture medium were obtained from Sigma-Aldrich (Saint Louis, MO, USA).

Species identification

Morphological observations

The three monoconidial isolates, derived from three different plants, were identified at species level by combining a morphological and molecular approach. Colony morphology, pigmentation, and macroconidia sizes were compared to the published descriptions of Aoki and O'Donnell (1999a). Suspensions of macroconidia were obtained by rinsing 2-week-old PDA plates, which had been incubated at 25 ± 1°C in complete darkness, with 10 mL of sterile distilled water and gently scraping the mycelium with a sterile glass Drigalski spatula. The suspensions were then filtered through a layer of sterile Miracloth (Calbiochem, San Diego, CA, USA) and were observed using an optical microscope (model Dialux 22, Leitz, Wetzlar, Germany). Micrograph images were captured using a Leica DFC 450C digital camera, and fungal structures were measured using the software Leica Application Suite X Version 3.1.1.17751 (Leica Microsystems Ltd., Heerbrugg, Switzerland). For each isolate, at least 50 macroconidia with different numbers of septa (1- 3- and 5-septa) were measured.

DNA extraction and molecular identification of isolates

Five agar discs (diam. 6 mm) were cut from 1-week-old monoconidial PDA plates and were inoculated into 50 mL tubes each containing 25 mL of Yeast Malt

Broth: 3 g L^{-1} yeast extract; 3 g L^{-1} malt extract; 5 g L^{-1} peptone; 10 g L^{-1} glucose. The tubes were then kept for 5 days at room temperature (22–25°C) under constant stirring at 60 rpm on the Multi-RS 60 programmable rotator (Biosan, Riga, Latvia), with a rotation angle of 75°. Mycelium was then collected by filtering each liquid culture through a layer of sterile Miracloth (Calbiochem, San Diego, CA, USA), washed twice with sterile distilled water, dried on sterile filter paper, and then stored at -20°C for DNA extraction.

Genomic DNA was extracted with the Genesig® Easy DNA/RNA extraction kit (Primer Design Ltd, UK), using the method described by Spada *et al.* (2023). DNA was quantified using the Qubit™ DNA BR Assay Kit in a Qubit™ 4 Fluorometer (Invitrogen by Thermo Fisher Scientific Inc., Eugene, OR, USA). A fragment of the *ef-1α* gene was amplified according to O'Donnell *et al.* (1998), using the EF-1/EF-2 primer pair. PCR was carried out in a 25 µL reaction mix with GoTaq Green Master Mix 2X (Promega Corporation, Madison, WI, USA), 0.1 µM of each primer, 30-50 ng of DNA, and adding nuclease-free water to the volume. PCR conditions were: initial denaturation at 95°C for 8 min, followed by 35 cycles of 95°C for 30 sec, 53°C for 1 min, and 72°C for 1 min, and final extension at 72°C for 5 min. A negative control (no DNA) was included in the reaction. PCR products were analyzed by electrophoresis in 0.5× Tris-Borate-EDTA (TBE) buffer with 1% (w/v) agarose gels and detected by UV fluorescence after GelRed™ staining (Biotium Inc., CA, USA) according to the manufacturer's instructions. The 100 bp DNA ladder (Promega, Madison, WI, USA) was used as a molecular size marker. Amplicons were purified with the QIAquick PCR purification kit (QIAGEN, Milan, Italy), following the manufacturer's instructions. The purified amplicons were sent to BMR Genomic (Padua, Italy) for Sanger sequencing in both directions, with the same set of primers used for amplification. Consensus sequences were edited with BioEdit v 7.7 and used as queries in BLASTn searches of the GenBank database hosted by NCBI. To further confirm the species, the species-specific primers Fp1-1 and Fp1-2 were used, targeting a 523 bp fragment in the *ef-1α* gene (Aoki and O'Donnell, 1999a). As described above, each PCR was conducted in a 25 µL reaction mix using 0.24 µM of each primer and setting the thermal conditions described by the authors.

Artificial inoculations of Salicornia europaea *seedlings grown in pots and in hydroponics*

To assess the pathogenicity of *F. pseudograminearum*, inoculations of *S. europaea* plants were carried out. The plants were grown in pots and hydroponics (floating raft system). One isolate (*F. pseudograminearum* 3B) was used in this experiment. Seeds of *S. europaea* were purchased from Alsagarden (Niederhaslach, France), and were sown into polystyrene trays containing stone wool plugs (Grodan, Roermond, The Netherlands), which were kept under a greenhouse for germination (Figure 2 a). Plants were selected for phenotypic uniformity based on height and number of shoots. At 46 days after sowing (das), seedlings (n = 96) were removed from stone wool plugs and transferred to a small-scale floating raft system (Figure 2 b). This consisted of polystyrene rafts (7 x 10 cm, each containing eight plants) placed in a 200 mL dark-colored polypropylene tank filled with nutrient solution. The solution contained 14.0 mM NO_3, 2.0 mM NH_4, 2.0 mM H_2PO_4, 10.0 mM K, 4.5 mM Ca, 2.0 m M Mg, 5.0 mM SO_4, 40.0 µM Fe, 40.0 µM B, 3.0 µM Cu, 10.0 µM Zn, 10.0 µM Mn, and 1.0 µM Mo. The nutrient solution in each hydroponic tank was continuously aerated to supply oxygen to the plants and maintain the inoculum in a homogeneous suspension. The plants were maintained in a growth chamber (25.0 ± 1.5°C, PAR 150 µmol m^2 s^{-2} from LED tubes) for 11 days to promote root development. Subsequently, at 57 das, plant (n = 48) roots were inoculated with a suspension of *F. pseudograminearum* macroconidia (final concentration 10^5 macroconidia mL^{-1}) (Figure 2 c1), and the other half with an equal volume of sterile distilled water (Figure 2 c2). Macroconidia suspensions were obtained as described above. At 24 days post inoculation (dpi; 81 das), half of the inoculated plants (n = 24) were transferred into 1 L pots filled with sterile peat, and the other half were transferred into 50 L tanks filled with 25 L of nutrient solution. The same method was used for control plants (those treated with sterile distilled water), and all plants were moved under a greenhouse (Figure 2 d). The plants had been randomly selected for transplanting in pots or hydroponics. The pots were sub-irrigated daily with nutrient solution, while in the hydroponic tanks, the nutrient solution was periodically added to maintain the initial volume.

The trial lasted until 80 dpi (corresponding to 137 das), when disease symptoms (shoot browning) were assessed using a six-point empirical qualitative ordinal scale (0 to 5): 0 = no symptoms; 1 = browning on the main stem; 2 = browning on the basal lateral shoots; 3 = complete browning of the main stem and primary lateral shoots; 4 = almost complete browning, pale-green secondary shoots; and 5 = completely desiccated (dead) plant (Figure 3, a to e). A score was assigned to each plant and, for each treatment (i.e. potted-inoculated plants, hydroponics-inoculated plants, potted-non-inoc-

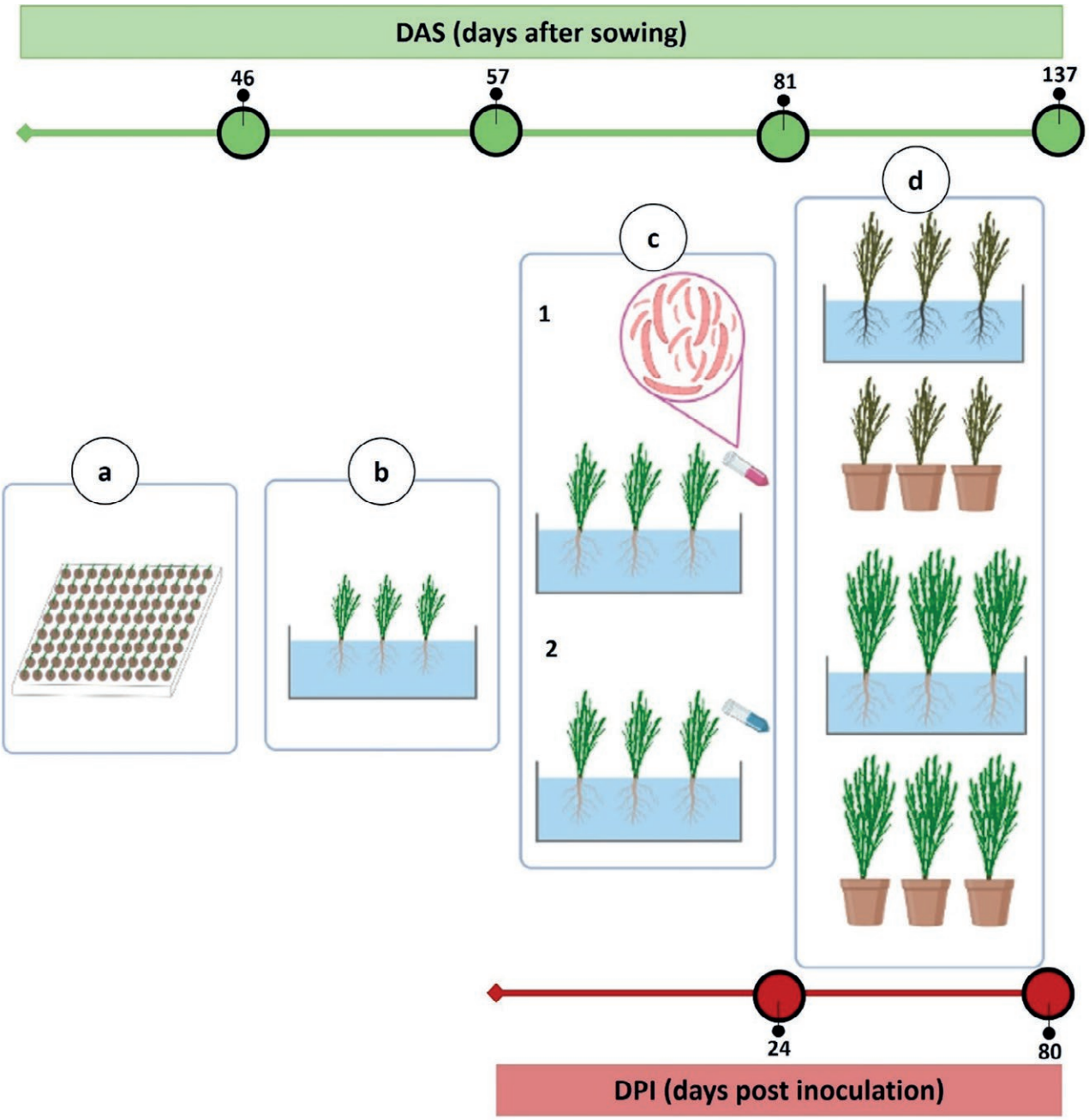

Figure 2. Diagram describing the protocol used for artificial inoculations of *Salicornia europaea* plants with *Fusarium pseudograminearum* isolate 3B. a) Sowing of *S. europaea* seeds in a seedling tray. b) Hydroponic cultivation of plants for root development. c) Inoculation of plants with *F. pseudograminearum* macroconidia (c 1) or sterile distilled water (control plants) (c 2). d) Greenhouse cultivation in pots or hydroponics.

ulated plants, and hydroponic-non-inoculated plants), the plants (n = 24) were randomly divided into three groups of eight plants each. The McKinney Index (MKI; McKinney, 1923), which incorporates disease incidence and severity, was calculated for each group using the following formula:

$$MKI = \frac{\sum(d \times f)}{N \times D} \times 100$$

where d = disease score, f = disease frequency (for a given group of plants), N = total number of observed plants, and D = the greatest level of disease infection on the empirical qualitative ordinal scale. Disease incidence

was determined by counting the plants showing symptoms of at least 1 on the empirical scale.

At 80 dpi, re-isolations were carried out (as previously described) from plant tissues for the fulfillment of Koch's postulates. In addition to plant roots and crowns, the first nine segments of the main stem and the two basal lateral shoots were plated, and the development of resulting organisms was assessed.

At 80 dpi, control and inoculated plants were also assessed for shoot fresh weight (SFW), shoot dry weight (SDW), and shoot succulence. For the inoculated plants, samples were collected from asymptomatic plants grown in hydroponics and from plants grown in pots with a disease severity score of 1. Shoot dry weights were measured after drying the fresh samples in a ventilated oven at 70°C until constant weight. Shoot succulence was calculated as the ratio of shoot water content to SDW.

Mycotoxins production: fungal isolates and culture conditions

The following isolates were used in the experiment on mycotoxin production: the three *F. pseudograminearum* isolates (3B, PD-A, and PD-B) from *S. europaea*, *F. pseudograminearum* ColPat-351 from wheat (Agustí-Brisach *et al.*, 2018), *F. pseudograminearum* PVS Fu-7 from wheat (Balmas, 1994), and the non-pathogenic *F. oxysporum* DAFE SP21-23 isolate, obtained from *S. europaea* plant tissues in the present study, used as a negative control since this species does not produce DON or ZEA. The isolate DAFE SP21-23 was also used as further confirmation that only *F. pseudograminearum* isolates were DON and ZEA producers. All fungi were grown on PDA plates and maintained on SNA, as described before.

Amplification of mycotoxin-related genes

To determine whether *F. pseudograminearum* isolates were potentially mycotoxigenic, we first investigated if they had the gene pool required for mycotoxin biosynthesis. The *tri5* gene and the *pks4* gene were assessed in each isolate, as *tri5* is involved in the biosynthesis of DON (Proctor *et al.*, 1995; Desjardins *et al.*, 1996), and *pks4* is involved in the biosynthesis of ZEA (Lysøe *et al.*, 2006).

A 650 bp fragment of the *tri5* gene was amplified using the Tox5-1/Tox5-2 primer pair (Niessen and Vogel, 1998), and a 280 bp fragment of the *pks4* gene using the PKS4F/PKS4R primers (Meng *et al.*, 2010). PCR was carried out in a 25 µL reaction mix with GoTaq Green Master Mix 2X (Promega Corporation, Madison, WI, USA), 0.4 µM of each primer for *tri5* and 0.5 µM for *pks4*, 30–50 ng of DNA, and adding nuclease-free water to the volume. Negative controls (no DNA) were included. The amplification conditions used were those described by the respective authors (cited above). Amplicons were purified and sequenced, and consensus sequences were generated as previously described. Amplification of fragments of the *tri3* and *tri12* genes (Starkey *et al.*, 2007) was carried out in a multiplex PCR to find out whether the isolates belonged to a specific DON-chemotype (i.e. nivalenol, NIV; and acetylated forms of DON: 3-acetyldeoxynivalenol, 3ADON; and 15-acetyldeoxynivalenol, 15ADON). Primers 3CON, 3NA, 3D15A, and 3D3A were used to target the *tri3* gene. Primers 12CON, 12NF, 12-15F, and 12-3F were used for the *tri12* gene. Multiplex PCRs were carried out following the methods described by the authors.

In vitro *production of mycotoxins*

Production of DON and ZEA was evaluated *in vitro* in a model system that consisted of growing the *F. pseudograminearum* isolates on moistened barley grains. This system method was chosen so that the fungi were grown in optimal conditions for mycotoxin production (Blaney and Dodman, 2002; Clear *et al.,* 2006; Kokkonen *et al.,* 2010). Thus, 30 g of dried barley grains, obtained from a local organic farm, were rinsed overnight in 25 mL of sterile distilled water in 500 mL flasks and sterilized twice at 121°C for 20 min with 24 hours between the two heat treatments. Each flask was then inoculated with a PDA disk (diam. 10 mm) cut from an actively growing culture of each isolate. A mycelium-free PDA disk was used as a control. An experiment was established consisting of five biological replicates for each isolate. The fungi were grown for 3 weeks at room temperature, and the cultures were manually shaken to avoid grain clumps. After incubation, the grains were dried under a laminar flow cabinet for 48 hours and were ground with a coffee grinder.

Mycotoxin production was evaluated for DON and ZEA with the immunochromatographic RIDA® QUICK RQA ECO tests (R-Biopharm AG, Milan, Italy), according to the manufacturer's instructions. The limits of detection (LOD) were 0.25 mg kg^{-1} for DON and 0.05 for ZEA, and the maximum measurable amounts were 50 mg kg^{-1} for DON and 1 mg kg^{-1} for ZEA. Sequential 10-fold dilutions were made, with the appropriate solvent, for samples outside these ranges until measurable amounts of mycotoxin were obtained. The DON test does not discriminate between acetylated forms of DON.

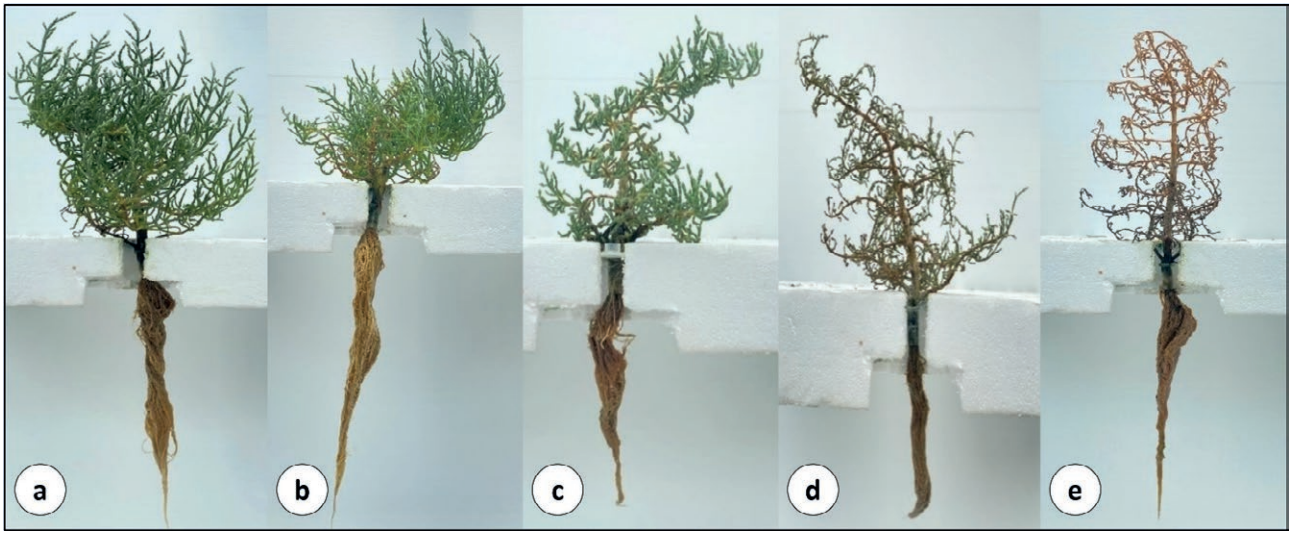

Figure 3. The empirical qualitative ordinal scale of disease symptoms (1 to 5) in *Salicornia europaea* plants grown hydroponically (floating raft system) and inoculated with *Fusarium pseudograminearum* isolate 3B. The photographs were taken 80 days post inoculation. a) Browning on the main stem (score 1). b) Browning on basal lateral shoots (score 2). c) Complete browning of the main stem and primary lateral shoots (score 3). d) Almost complete browning, pale-green secondary shoots (score 4). e) Completely desiccated (dead) plant (score 5).

In planta *production of mycotoxins*

Mycotoxin production in *S. europaea* plants was assessed using LC-MS/MS, which is a commonly used multi-mycotoxin method to determine levels in feeds and foods to overcome specific matrix effects (De Santis *et al*., 2017; Iqbal, 2021). The method used (protocol: MP 213, rev. 3, 2022) detects 17 mycotoxins, including NIV, ZEA, DON, and the acetylated forms 3- and 15-acetyldeoxynivalenol.

According to the empirical scale of host plant symptoms (Figure 3), the plants were pooled and divided into three groups: no symptoms (score 0); mild symptoms (scores 1 to 2), and severe symptoms (scores 3 to 5). The plants were also divided based on the growing methods (i.e. in pots or hydroponics). For each group of plants, the analyses were carried out on three biological replicates. Therefore, at 80 dpi, the above-ground parts of each plant were cut and freeze-dried. Samples were sent to Biochemie Lab s.r.l. (Campi Bisenzio, Florence, Italy; Accredia No. 0195; https://www.accredia.it/) for LC-MS/MS analyses.

Statistical analysis

Data were subjected to 1-way ANOVA using CoStat 6.4 statistical software (Cohort Software, Monterey, CA, USA). Percentage data (disease incidence and MKI) were $arcsin\sqrt{\%}$ transformed before ANOVA. In the artificial inoculation experiment, for each cultivation method, plants (n = 24) were randomly divided into 3 groups (eight plants each), and disease incidence and MKI values were calculated for each group. The normality of the data was assessed using the Shapiro–Wilk test and homoscedasticity was tested using Bartlett's test. Means were separated using Tukey's honestly significant difference post-hoc (HSD) test ($P \leq 0.05$).

Artificial inoculations of *F. pseudograminearum* on *S. europaea* plants were repeated twice in pots, using the same protocol, and both inoculations gave similar results. In addition, in the first experiment carried out in pots, the *F. oxysporum* isolate DAFE SP21-23 was also inoculated, but the inoculation did not cause any symptoms (data not shown). Only the results of the experiment conducted to compare the two growing methods (i.e. pots and hydroponics) are presented in this paper.

RESULTS

Identification

Fusarium pseudograminearum colonies were isolated from 64% of the total specimens of diseased stems, roots, and crowns collected from *S. europaea* plants. *Fusarium oxysporum* colonies were also frequently observed, representing 45% of the total plated samples. On PDA, colonies appeared whitish to brownish-yellow on the upper surface, especially during the first few days of growth. After

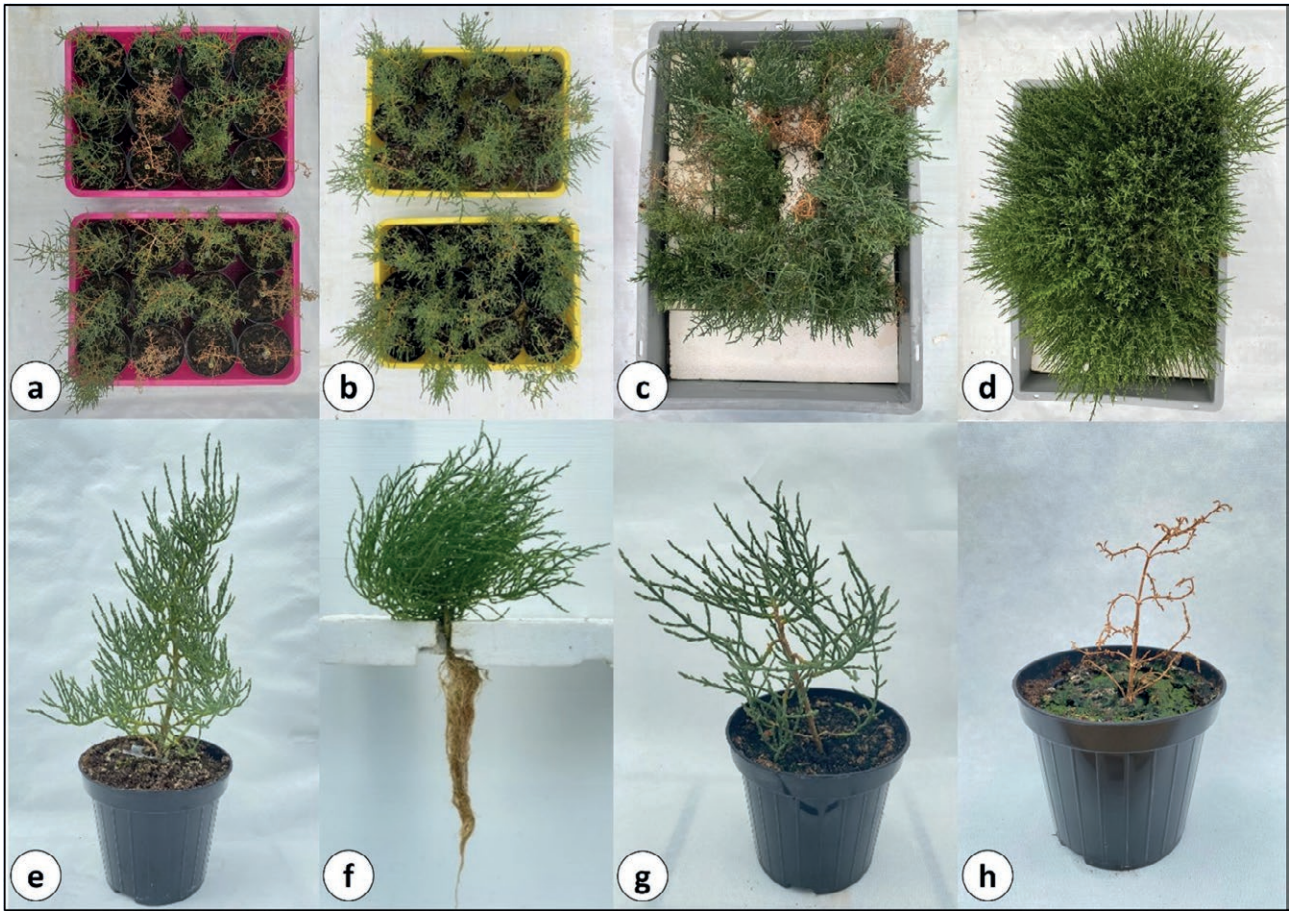

Figure 4. *Salicornia europaea* plants grown in pots (a and b) and hydroponics (c and d), and inoculated with *Fusarium pseudograminearum* isolate 3B, at 80 days post inoculation (a and c). Control plant in a pot (e) and hydroponics (f). Plants with disease severity score 1 (g) and score 5 (h), grown in pots.

one week, reddish to dull-red pigmentation was visible. The reverse colony pigmentation ranged from pale brown to brownish yellow, becoming red, reddish-brown, and ruby after 2 weeks. Abundant and floccose aerial mycelium was typically observed during colony growth. The size of macroconidia was comparable to that described by Aoki and O'Donnell (1999a) (Table S1), although no 7-septate macroconidia were observed. Microconidia were absent. Three isolates (3B, PD-A, and PD-B) were selected for morphological and molecular studies.

The consensus sequences of the partial translation elongation factor 1 alpha (*ef-1α*) gene were 100% similar, in whole length, to those of *F. pseudograminearum* [GenBank accession numbers (AN) MG670539 to MG670541 and OM746828 to OM746837]. These sequences were deposited in GenBank under the AN PQ045864 to PQ045866. A species-specific PCR, with the Fp1-1/Fp1-2 primer pair, confirmed that the three isolates belonged to *F. pseudograminearum* (Figure S1).

Artificial inoculations and plant disease assessments

No symptoms were observed in control plants (Figure 4, b, d, e, and f). The plants cultivated in pots and hydroponics had different growth patterns (Table 1). The pot-cultivated plants tended to be woodier than those in hydroponics, especially at the base of the stem. In these cases, desiccation of at least 50% of the main stem of each affected plant was considered as score 1 on the empirical qualitative ordinal scale of disease symptoms (Figure 4 g). In addition to the control plants, asymptomatic inoculated plants (i.e. score 0), grown in hydroponics, and potted plants with a score of 1, were collected for the determination of shoot fresh weight (SFW), shoot dry weight (SDW), and succulence. Differences between inoculated and non-inoculated plants, within the same cultivation method, were statistically significant ($P \leq 0.05$) for potted plants, but not for plants grown hydroponically (Table 1).

Table 1. Mean shoot fresh weight, dry weight, and succulence of *Salicornia europaea* plants grown in pots or hydroponics, sampled at 80 days post inoculation with *Fusarium pseudograminearum* isolate 3B. Shoot succulence was calculated as the ratio between shoot water content and shoot dry weight. For inoculated plants, measurements were carried out on asymptomatic plants in hydroponics and on pot-grown plants with a score of 1 on the empirical qualitative scale of disease symptoms (browning on the main stems). Within each growing system, means (n = 4; ± SE) accompanied by the same letters are not significantly different according to Tukey's HSD post-hoc test ($P \leq 0.05$).

Parameter	Hydroponics		Pots	
	Inoculated	Non-inoculated	Inoculated	Non-inoculated
Shoot fresh weight (g/plant)	56.3 ± 6.4 a	62.9 ± 4.9 a	7.3 ± 0.8 a	16.7 ± 0.5 b
Shoot dry weight (g/plant)	6.4 ± 0.6 a	6.8 ± 1.1 a	1.4 ± 0.2 a	2.6 ± 0.2 b
Succulence (g/g)	7.8 ± 0.4 a	7.7 ± 1.2 a	4.2 ± 0.3 a	5.4 ± 0.3 b

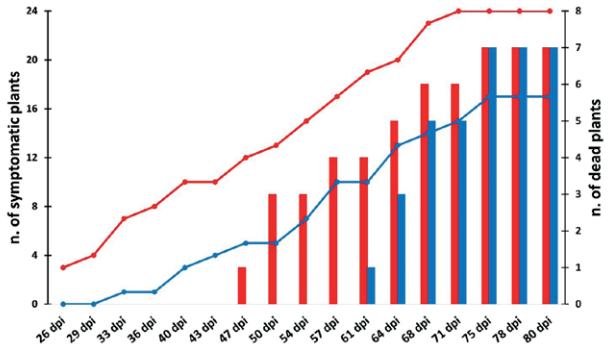

Figure 5. Progression of symptom emergence (lines) and plant death (histograms) in *Salicornia europaea* plants inoculated with *Fusarium pseudograminearum* isolate 3B, in pots (red) or hydroponics (blue), at different days post inoculation (dpi).

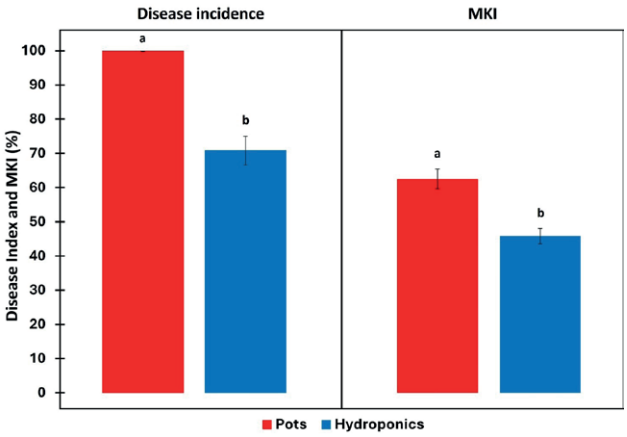

Figure 6. Disease incidence and McKinney Index for potted (red bars) and hydroponically grown (blue bars) *Salicornia europaea* plants that were inoculated with *Fusarium pseudograminearum* isolate 3B. Different letters above the bars indicate significant differences between the mean values (n = 3; ± SE), according to Tukey's HSD post-hoc test ($P \leq 0.05$). Statistical analyses were conducted separately for each disease measurement.

Symptoms of aerial parts of plants were characterized by acropetal patterns, initially affecting the stems and basal lateral shoots (Figure 4, a and c), following damage to the roots. The disease had a long asymptomatic phase. Initial wilting symptoms were observable at 24 dpi, when the plants were transplanted into pots or in hydroponics. There was no clear evidence of root or crown rot before transplanting. Clear signs of disease in the aboveground portions (i.e. desiccation of the main stems) were observed in pots 2 days after transplanting (26 dpi). The plants in hydroponics showed evidence of desiccation at 1 week after transplanting (31 dpi). These different rates of symptom appearance were very obvious at 80 dpi. First plant death in pots was recorded at 47 dpi (Figure 4 h) and at 61 dpi in hydroponics (Figure 5).

In pots, 100% (24/24) of the inoculated plants showed symptoms attributable to at least class 1 in the empirical qualitative ordinal scale of disease symptoms. In hydroponics, disease only affected 70.8% (17/24) of the plants, and seven out of 24 plants were asymptomatic and were assigned scores of zero. The number of dead plants (score 5) was the same at 80 dpi (seven) in pots and in hydroponics. Accordingly, disease incidence and McKinney Index were significantly greater in pot-grown plants than in those grown hydroponically (Figure 6).

Koch's postulates were fulfilled by reisolating *F. pseudograminearum* from below- and above-ground tissues collected from both groups of plants, regardless of disease severity. The pathogen was mainly present in the roots and crowns. In pot-grown plants, *F. pseudograminearum* was re-isolated from 96% of sampled roots and 100% of sampled crown tissues, and 87% of root and 82% of crown samples of hydroponically grown plants. Pathogen re-isolation from above-ground tissues of potted plants was 41% from stems, 91% from first lateral branches, and 59% from second lateral branches. In hydroponics, re-isolations of *F. pseudograminearum* in above-ground plant tissues were higher, 85% from stems, 91% from first lateral branches, and 64% from second

lateral branches (Table S2). Overall, re-isolations of *F. pseudograminearum* were greater from plants grown in the hydroponic system (83% of total plated tissues) than those grown in pots (62%). *Fusarium pseudograminearum* was also re-isolated from asymptomatic hydroponically grown plants.

Production of mycotoxins in vitro *and* in planta

In this experiment, in addition to the three isolates of *F. pseudograminearum* (3B, PD-A, and PD-B), two further isolates of *F. pseudograminearum*, PVS-Fu 7 (Balmas, 1994) and ColPat-351 (Agustí-Brisach *et al*., 2018) and one isolate of *F. oxysporum* (DAFE SP-21-23) isolated from *S. europaea* plants were included.

First, we tested whether the isolates had the gene pool required for the production of deoxynivalenol (DON) and zearalenone (ZEA) by amplifying a fragment of the *tri5* (Niessen and Vogel, 1998) and *pks4* (Meng *et al*., 2010) genes. All the isolates, except for *F. oxysporum* DAFE SP21-23, were positive for the 650 bp fragment of the *tri5* gene (Figure S2), and the 280 bp fragment of the *pks4* gene (Figure S3). Consensus sequences were used for BLASTn search to verify sequence identity and were deposited under the GenBank AN PQ045872 to PQ045876 for *tri5* and PQ045867 to PQ045871 for *pks4*. For *tri5*, the sequences exhibited 98.65% (isolates 3B, PD-A, and PD-B) and 98.80% (isolates ColPat-351 and PVS-Fu 7) identity with the *tri5* gene of *F. pseudograminearum* strains NRRL 28334, NRRL 28338, and 28062 (respectively, AN AY102583, AY102585, and AY102580). Lower sequence similarity was found for *pks4*. The sequences showed 95.02% (isolates 3B, PD-A, and PD-B), 95.37% (isolate ColPat-351), and 94.66% (isolate PVS-Fu 7) similarity with the *pks4* gene of *F. pseudograminearum* CS3096 (AN: XM_009259983). The amplification of *tri3* and *tri12* genes (Starkey *et al*., 2007), with a multiplex PCR developed to determine the DON-chemotype, failed to produce any amplicons. Although several attempts were made, such as modifying the annealing temperature, concentration of DNA, primers combinations, and $MgCl_2$ in the reaction mixture, the multiplex PCR yielded no results. Therefore, no specific DON-chemotype (i.e. NIV, 3ADON, 15ADON) could be assigned to the isolates.

The mycotoxigenic ability of the *F. pseudograminearum* isolates was assessed by growing them on barley grains. Table 2 shows the results for DON and ZEA production, assessed using the respective immunochromatographic test kits (Figures S4 and S5). Zearalenone was detected in all the samples with considerable yields. The PD-B isolate produced the least amount of toxin (40.01 ± 1.05 mg kg^{-1}) while isolate ColPat-351 produced the greatest amount (69.1 ± 5.45 mg kg^{-1}). In contrast, in the latter isolate the production of DON was below the LOD (0.25 mg kg^{-1}), while isolate PVS-Fu 7 produced only a small amount of this mycotoxin (0.37 ± 0.06 mg kg^{-1}). Interestingly, the *F. pseudograminearum* isolates obtained from *S. europaea* were higher DON producers, each with comparable yields. As expected, the *F. oxysporum* isolate DAFE SP21-23 did not produce the tested mycotoxins.

Mycotoxin production *in planta* was low. Deoxynivalenol was detected by LC-MS/MS only at 0.029 ± 0.012 mg kg^{-1} in severely damaged *S. europaea* plants (disease scores 3 to 5) grown in hydroponics, while all samples were negative for ZEA, NIV, 3ADON, and 15ADON. Since *S. europaea* plants were inoculated with the *F. pseudograminearum* 3B isolate, the results were consistent with those obtained from the molecular analyses, which did not identify any specific DON chemotype.

Table 2. Mean amounts of deoxynivalenol (DON) and zearalenone (ZEA) produced by five isolates of *Fusarium pseudograminearum* on barley grains after 21 days. Data are shown as mg of toxin per kg of substrate. Mycotoxin production was evaluated with immunochromatographic RIDA® QUICK RQA ECO kits. The limit of detection (LOD) was 0.25 mg kg^{-1} for DON and 0.05 mg kg^{-1} for ZEA. Means (n = 5; ± SE) accompanied by the same lowercase letters are not significantly different, according to Tukey's HSD post hoc test ($P \leq 0.05$).

Isolate	DON (mg kg^{-1})	ZEA (mg kg^{-1})
F. pseudograminearum 3B	6.93 ± 0.43 a	61.10 ± 2.17 ab
F. pseudograminearum PD-A	6.72 ± 0.11 a	52.94 ± 2.93 abc
F. pseudograminearum PD-B	6.79 ± 0.19 a	40.01 ± 1.05 c
F. pseudograminearum ColPat-351	< LOD c	69.10 ± 5.45 a
F. pseudograminearum PVS Fu-7	0.37 ± 0.06 b	47.48 ± 4.49 bc
F. oxysporum DAFE SP21-23	< LOD c	< LOD d
Control	< LOD c	< LOD d

DISCUSSION

This is the first report of root and crown rot caused by *F. pseudograminearum* on cultivated *S. europaea* plants. There are very few records of diseases affecting *Salicornia* species (Delli Compagni *et al*., 2024). The only report regarding cultivated species is for *S. bigelovii,* which was found to be susceptible to *Botrytis cinerea* in Mexico (Rueda Puente *et al*., 2014). Pathogenicity of this fungus was confirmed by spraying a conidial suspension on detached branches. There are no documented artificial inoculation experiments on *S. europaea*, since the pathogenic species found on this halophyte have only

been described in wild plants (Delli Compagni *et al*., 2024). In the present study, artificial inoculations were carried out on seed-propagated plants of *S. europaea*.

The pathogenicity test showed that root and crown rot caused by *F. pseudograminearum* was less severe when test plants were grown hydroponically (MKI = 46%) than when grown in potting soil (MKI = 63%) and all the pot-grown plants developed browning symptoms. Disease incidence was less in the hydroponic system, with seven out of 24 plants remaining asymptomatic (Figure 6). These results indicate that disease severity depended on the cultivation method. At 80 dpi, SFW, SDW, and shoot succulence of inoculated, asymptomatic plants (i.e. score 0) grown in the hydroponic system resembled parameters measured for the control plants, whereas the same parameters in score 1 potted plants were significantly different from the respective controls (Table 1). Moreover, the potted plants also had disease symptoms soon after transplanting, while in hydroponics symptom development was delayed (Figure 5). However, mortality rates were the same between the two groups of plants (each with seven out of 24 plants), although in hydroponics the first plant death occurred 2 weeks later than in pots (Figure 5).

Hydroponic cultivation can reduce plant susceptibility to infectious diseases compared to soil cultivation. In a similar experiment, Maurer *et al*., (2023) found that sweet basil plants grown in a static hydroponic solution were much less sensitive to downy mildew caused by *Peronospora belbahrii* than those grown in soil. They stated that the higher antioxidant content in hydroponically grown basil plants could alleviate symptom development. Moulin *et al*. (1994) showed that several *Pythium* species (*P. aphanidermatum*, *P. intermedium*, *P. irregulare*, *P. sylvaticum*, and *P. ultimum* var. *ultimum*) caused damping-off of cucumber when grown in sand-peat soil, but only *P. aphanidermatum* caused disease in substrate (stone wool) and in hydroponic culture. These authors reported that symptoms were milder in hydroponics than in substrate. Moreover, inoculations with *Streptomyces scabies* resulted in less infection of potato plants grown hydroponically compared to pot-grown plants (Khatri *et al*., 2011). Similarly, rice artificially inoculated with *Xanthomonas oryzae* pv. *oryzae*, the causative agent of bacterial leaf blight, had lower disease severity in hydroponic culture than in soil (Song *et al*., 2016).

In the present study, the greater growth rate of hydroponically grown plants than those in potting soil, was likely due to the better supply of water and minerals, and oxygenation of roots compared to soil cultivation, which may have resulted in the reduced rate of development of *F. pseudograminearum* root and crown rot on *S. europaea*. In addition, *F. pseudograminearum* is a soil-borne pathogen and causes disease in warm and dry climates (Poole *et al*., 2013), with increased virulence in drought conditions (Kazan and Gardiner, 2018). In agricultural areas characterized by low moisture and high temperatures, *F. pseudograminearum* was found to be predominant compared to other *Fusarium* species (e.g. *F. culmorum*), which prefer cool and humid regions (Poole *et al*., 2013).

Little information is available on mycotoxins on *Salicornia* species. Lopes *et al*. (2020) reported that several *S. ramosissima* and *Salicornia* sp. samples were contaminated by aflatoxins in Portugal. These compounds were detected in plants collected in the wild, and also in those cultivated with conventional and organic methods, but no mycotoxins were detected from greenhouse and hydroponically cultivated plants.

In the present study, DON was detected in low amounts from inoculated plants. Traces of DON (0.029 ± 0.012 mg kg^{-1}) were detected only in severely damaged (score 3–5) plants grown hydroponically. This result could be due to the greater water content of these plants compared to those grown in potting soil since high levels of water activity (a_w) have been shown to be necessary for optimal production of trichothecenes by *Fusarium* spp. (Hope *et al*., 2005; Han *et al*., 2018; Rybecky *et al*., 2018; Belizán *et al*., 2019). Therefore, one hypothesis is that the inoculated *F. pseudograminearum* isolate produced the mycotoxin only in suitable conditions (such as high plant water content) and during an advanced stage of disease development. In wheat kernels, the most favorable conditions for DON and ZEA production by *F. pseudograminearum* were an a_w of 0.97 and a temperature of 25°C (Cui *et al*., 2022).

When inoculated on barley grains, all the tested *F. pseudograminearum* isolates produced ZEA with considerable yields, similar to those documented by other authors (Blaney and Dodman, 2002; Clear *et al*., 2006; Alkadri *et al*., 2013). Furthermore, only the three *F. pseudograminearum* isolates obtained from *S. europaea* were high DON producers, each with similar production (Table 2). The other two European isolates had different mycotoxigenic activities. Low production of DON was detected in the Italian isolate *F. pseudograminearum* (PVS Fu-7), while production of DON by the Spanish isolate (ColPat-351), was below the LOD (Table 2). In addition, PCR targeting the *tri3* and *tri12* genes to discriminate between NIV and acetylated derivatives of DON producers (i.e. 3ADON and 15ADON) did not identify any specific DON chemotype. Deng et al. (2020), in a molecular analysis to determine the DON chemotypes of 372 isolates of *F. pseudograminearum*,

showed that 196 isolates did not produce any fragments in *tri3* and *tri12* PCR assays, with the same primers used in this study. After LC-MS/MS analysis, isolates without a chemotype marker were assigned to the putative 15-ADON chemotype (Deng *et al*., 2020).

The present study is the first to record root and crown rot of *S. europaea* caused by *F. pseudograminearum*. This study also provides an important contribution to the current distribution and host range of this pathogenic fungus in Europe. In addition, the mycotoxin deoxynivalenol was detected in diseased plants, although only in small quantities and under specific conditions. Further studies are ongoing to assess the environmental conditions (e.g. salinity and temperature) that may promote mycotoxigenic activity in *F. pseudograminearum* and to determine potential mycotoxin exposure risk in *S. europaea*.

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LITERATURE CITED

Abdallah-Nekache N., Laraba I., Ducos C., Barreau C., Bouznad Z., Boureghda H., 2019. Occurrence of *Fusarium* head blight and *Fusarium* crown rot in Algerian wheat: identification of associated species and assessment of aggressiveness. *European Journal of Plant Pathology* 154(3): 499–512. https://doi.org/10.1007/s10658-019-01673-7

Agustí-Brisach C., Raya-Ortega M.C., Trapero C., Roca L.F., Luque F., … Trapero A., 2018. First report of *Fusarium pseudograminearum* causing crown rot of wheat in Europe. *Plant Disease* 102(8): 1670. https://doi.org/10.1094/PDIS-11-17-1840-PDN

Akinsanmi O.A., Chakraborty S., Backhouse D., Simpfendorfer S., 2007. Passage through alternative hosts changes the fitness of *Fusarium graminearum* and *Fusarium pseudograminearum*. *Environmental Microbiology* 9(2): 512–520. https://doi.org/10.1111/j.1462-2920.2006.01168.x

Alkadri D., Nipoti P., Döll K., Karlovsky P., Prodi A., Pisi A., 2013. Study of fungal colonization of wheat kernels in Syria with a focus on *Fusarium* species. *International Journal of Molecular Science* 14(3): 5938–5951. https://doi.org/10.3390/ijms14035938

Aoki T., O'Donnell K., 1999a. Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the Group 1 population of *F. graminearum*. *Mycologia* 91(4): 597–609. https://doi.org/10.1080/00275514.1999.12061058

Aoki T., O'Donnell K., 1999b Morphological characterization of *Gibberella coronicola* sp. nov., obtained through mating experiments of *Fusarium pseudograminearum*. *Mycoscience* 40(6): 443–453. https://doi.org/10.1007/BF02461021

Araus J.L., Rezzouk F.Z., Thushar S., Shahid M., Elouafi I.A., … Serret M.D., 2021. Effect of irrigation salinity and ecotype on the growth, physiological indicators and seed yield and quality of *Salicornia europaea*. *Plant Science* 304: 110819. https://doi.org/10.1016/j.plantsci.2021.110819

Balmas V., 1994. Root rot of wheat in Italy caused by *Fusarium graminearum* Group I. *Plant Disease* 78: 317A. https://doi.org/10.1094/PD-78-0317A

Belizán M.M.E., Gomez A. de los, Terán Baptista Z.P., Jimenez C.M., Sánchez Matías M. del H., … Sampietro D.A., 2019. Influence of water activity and temperature on growth and production of trichothecenes by *Fusarium graminearum sensu stricto* and related species in maize grains. *International Journal of Food Microbiology* 305: 108242. https://doi.org/10.1016/j.ijfoodmicro.2019.108242

Blaney B.J., Dodman R.L., 2002. Production of zearalenone, deoxynivalenol, nivalenol, and acetylated derivatives by Australian isolates of *Fusarium graminearum* and *F. pseudograminearum* in relation to source and culturing conditions. *Australian Journal of Agricultural Research* 53(12): 1317–1326. https://doi.org/10.1071/AR02041

Burgess L.W., Klein T.A., Bryden W.L., Tobin N.F., 1987. Head blight of wheat caused by *Fusarium graminearum* Group 1 in New South Wales in 1983. *Australasian Plant Pathology* 16: 72–78. https://doi.org/10.1071/APP9870072

Cárdenas-Pérez S., Piernik A., Chanona-Pérez J.J., Grigore M.N., Perea-Flores M.J., 2021. An overview of the emerging trends of the *Salicornia* L. genus as a sustainable crop. *Environmental and Experimental Botany* 191: 104606. https://doi.org/10.1016/j.envexpbot.2021.104606

Chakraborty S., Liu C.J., Mitter V., Scott J.B., Akinsanmi O.A., … Simpfendorfer S., 2006. Pathogen population

structure and epidemiology are keys to wheat crown rot and *Fusarium* head blight management. *Australasian Plant Pathology* 35: 643–655. https://doi.org/10.1071/AP06068

Clear R.M., Patrick S.K., Gaba D., Roscoe M., Demeke T., ... Turkington T.K., 2006. Trichothecene and zearalenone production, in culture, by isolates of *Fusarium pseudograminearum* from western Canada. *Canadian Journal of Plant Pathology* 28(1): 131–136. https://doi.org/10.1080/07060660609507279

Cui H., Wang S., Yang X., Zhang W., Chen M., ... Wang S., 2022. Predictive models for assessing the risk of *Fusarium pseudograminearum* mycotoxin contamination in post-harvest wheat with multi-parameter integrated sensors. *Food Chemistry: X* 16:100472. https://doi.org/10.1016/j.fochx.2022.100472

De Santis B., Debegnach F., Gregori E., Russo S., Marchegiani F., ... Brera C., 2017. Development of a LC-MS/MS method for the multi-mycotoxin determination in composite cereal-based samples. *Toxins* 9(5): 169. https://doi.org/10.3390/toxins9050169

Delli Compagni E., Pardossi A., Pecchia S., 2024. Fungal and fungal-like diseases of halophytes in the Mediterranean basin: a state-of-the-art review. *Horticulturae* 10(4): 313. https://doi.org/10.3390/horticulturae10040313

Deng Y.Y., Li W., Zhang P., Sun H.Y., Zhang X.X., ... Chen H.G., 2020 *Fusarium pseudograminearum* as an emerging pathogen of crown rot of wheat in eastern China. *Plant Pathology* 69(2):240–248. https://doi.org/10.1111/ppa.13122

Desjardins A.E., Proctor R., Bai G., McCormick S., Shaner G., ... Hohn T.M., 1996 Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. *Molecular Plant-Microbe Interactions*. 9(9): 775. http://doi.org/10.1094/MPMI-9-0775

EFSA Panel on Plant Health (PLH), Bragard C, Baptista P, Chatzivassiliou E, Di Serio F, Gonthier P, ... Reignault P.L., 2022 Pest categorisation of *Fusarium pseudograminearum*. *EFSA Journal* 20:e07399. https://doi.org/10.2903/j.efsa.2022.7399

Han Z., Shen Y., Di Mavungu J.D., Zhang D., Nie D., Jiang K., ... Zhao Z., 2018. Relationship between environmental conditions, TRI5 gene expression and deoxynivalenol production in stored *Lentinula edodes* infected with *Fusarium graminearum*. *World Mycotoxin Journal* 11(2): 177–186. https://doi.org/10.3920/WMJ2017.2245

Hope R., Aldred D., Magan N., 2005. Comparison of environmental profiles for growth and deoxynivalenol production by *Fusarium culmorum* and *F. graminearum* on wheat grain. *Letters in Applied Microbiology* 40(4): 295–300. https://doi.org/10.1111/j.1472-765X.2005.01674.x

Iqbal S.Z., 2021. Mycotoxins in food, recent development in food analysis and future challenges; a review. *Current Opinion in Food Science* 42: 237–247. https://doi.org/10.1016/j.cofs.2021.07.003

Kammoun L.G., Gargouri S., Hajlaoui M.R., Marrakchi M., 2009. Occurrence and distribution of *Microdochium* and *Fusarium* species isolated from durum wheat in northern Tunisia and detection of mycotoxins in naturally infested grain. *Journal of Phytopathology* 157(9): 546–551. https://doi.org/10.1111/j.1439-0434.2008.01522.x

Kazan K., Gardiner D.M., 2018. *Fusarium* crown rot caused by *Fusarium pseudograminearum* in cereal crops: recent progress and future prospects. *Molecular Plant Pathology* 19(7): 1547–1562. https://doi.org/10.1111/mpp.12639

Khatri B.B., Tegg R.S., Brown P.H., Wilson C.R., 2011. Temporal association of potato tuber development with susceptibility to common scab and *Streptomyces scabiei*-induced responses in the potato periderm. *Plant Pathology* 60(4): 776–786. https://doi.org/10.1111/j.1365-3059.2011.02435.x

Kokkonen M., Ojala L., Parikka P., Jestoi M., 2010. Mycotoxin production of selected *Fusarium* species at different culture conditions. *International Journal of Food Microbiology* 143(2): 17–25. https://doi.org/10.1016/j.ijfoodmicro.2010.07.015

Lombardi T., Bertacchi A., Pistelli L., Pardossi A., Pecchia S., ... Sanmartin C., 2022. Biological and agronomic traits of the main halophytes widespread in the Mediterranean region as potential new vegetable crops. *Horticulturae* 8(3): 195. https://doi.org/10.3390/horticulturae8030195

Lopes M., Castilho M., Sanches-Silva A., Freitas A., Barbosa J., ... Ramos F., 2020. Evaluation of the mycotoxins content of *Salicornia* spp.: a gourmet plant alternative to salt. *Food Additives & Contaminants: Part B* 13(3): 162–170. https://doi.org/10.1080/19393210.2020.1741692

Lysøe E., Klemsdal S.S., Bone K.R., Frandsen R.J.N., Johansen T., ... Giese H., 2006. The PKS4 gene of *Fusarium graminearum* is essential for zearalenone production. *Applied Environmental Microbiology* 72(6): 3924–3932. https://doi.org/10.1128/AEM.00963-05

Maurer D., Sadeh A., Chalupowicz D., Barel S., Shimshoni J.A., Kenigsbuch D., 2023. Hydroponic versus soil-based cultivation of sweet basil: impact on plants' susceptibility to downy mildew and heat stress, storability and total antioxidant capacity. *Journal of the*

Science of Food and Agriculture 103(15): 7809–7815. https://doi.org/10.1002/jsfa.12860

McKinney H.H., 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *Journal of Agricultural Research* 26: 195–218.

Meng K., Wang Y., Yang P., Luo H., Bai Y., Yao B., 2010. Rapid detection and quantification of zearalenone-producing *Fusarium* species by targeting the zearalenone synthase gene PKS4. *Food Control* 21(2): 207–11. https://doi.org/10.1016/j.foodcont.2009.05.014

Miličević T., Kaliterna J., Ivić D., Stričak A., 2013. Identification and occurrence of *Fusarium* species on seeds of common vetch, white lupin and some wild legumes. *Poljoprivreda* 19(1): 25–32.

Moulin F., Lemanceau P., Alabouvette C., 1994. Pathogenicity of *Pythium* species on cucumber in peat-sand, rockwool and hydroponics. *European Journal of Plant Pathology* 100: 3–17. https://doi.org/10.1007/BF01871963

Nahle S., El Khoury A., Atoui A., 2021. Current status on the molecular biology of zearalenone: its biosynthesis and molecular detection of zearalenone producing *Fusarium* species. *European Journal of Plant Pathology* 159: 247–258. https://doi.org/10.1007/s10658-020-02173-9

Niessen M.L., Vogel R.F., 1998. Group specific PCR-detection of potential trichothecene-producing *Fusarium*-species in pure cultures and cereal samples. *Systematic and Applied Microbiology* 21(4): 618–631. https://doi.org/10.1016/S0723-2020(98)80075-1

Nirenberg H.I., 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. *Canadian Journal of Botany* 59(9): 1599–1609. https://doi.org/10.1139/b81-217

O'Donnell K., Kistler H.C., Cigelnik E., Ploetz R.C., 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Science* 95(5): 2044–2049. https://doi.org/10.1073/pnas.95.5.2044

Obanor F., Neate S., Simpfendorfer S., Sabburg R., Wilson P., Chakraborty S., 2013. *Fusarium graminearum* and *Fusarium pseudograminearum* caused the 2010 head blight epidemics in Australia. *Plant Pathology* 62(1): 79–91. https://doi.org/10.1111/j.1365-3059.2012.02615.x

Pasquali M., Migheli Q., 2014. Genetic approaches to chemotype determination in type B-trichothecene producing *Fusaria*. *International Journal of Food Microbiology*. 189: 164-182. https://doi.org/10.1016/j.ijfoodmicro.2014.08.011

Poole G.J., Smiley R.W., Walker C., Huggins D., Rupp R., ... Paulitz T.C., 2013. Effect of climate on the distribution of *Fusarium* spp. causing crown rot of wheat in the Pacific Northwest of the United States. *Phytopathology* 103(11): 1130–1140. https://doi.org/10.1094/PHYTO-07-12-0181-R

Proctor R.H., Hohn T.M., McCormick S.P., 1995. Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. *Molecular Plant-Microbe Interactions* 8(4): 593–601. https://doi.org/10.1094/mpmi-8-0593

Rueda Puente E.O., Hernandez Montiel L.G., Holguin Peña J., Murillo Amador B., Rivas Santoyo F.J., 2014. First report of *Botrytis cinerea* Pers. on *Salicornia bigelovii* Torr. in North-West México. *Journal of Phytopathology* 162(7-8): 513–515. https://doi.org/10.1111/jph.12205

Rybecky A.I., Chulze S.N., Chiotta M.L., 2018. Effect of water activity and temperature on growth and trichothecene production by *Fusarium meridionale*. *International Journal of Food Microbiology* 285: 69–73. https://doi.org/10.1016/j.ijfoodmicro.2018.07.028

Sabburg R., Obanor F., Aitken E., Chakraborty S., 2015. Changing fitness of a necrotrophic plant pathogen under increasing temperature. *Global Change Biology* 21(8): 3126–3137. https://doi.org/10.1111/gcb.12927

Sever Z., Ivić D., Kos T., Miličević T., 2012. Identification of *Fusarium* species isolated from stored apple fruit in Croatia. *Archives of Industrial Hygiene and Toxicology* 63(4): 463–470. https://doi.org/10.2478/10004-1254-63-2012-2227

Shan X., Zhu Y., Redman R., Rodriguez R.J., Yuan Z., 2021. The chromosome-scale genome resource for two endophytic *Fusarium* species, *F. culmorum* and *F. pseudograminearum*. *Molecular Plant-Microbe Interactions* 34(6): 703–706. https://doi.org/10.1094/MPMI-07-20-0205-A

Song A., Xue G., Cui P., Fan F., Liu H., ... Liang Y., 2016. The role of silicon in enhancing resistance to bacterial blight of hydroponic- and soil-cultured rice. *Scientific Reports* 6: 24640. https://doi.org/10.1038/srep24640

Spada M., Pugliesi C., Fambrini M., Palpacelli D., Pecchia S., 2023. Knockdown of BMP1 and PLS1 virulence genes by exogenous application of RNAi-Inducing dsRNA in *Botrytis cinerea*. *International Journal of Molecular Sciences* 24(5): 4869. https://doi.org/10.3390/ijms24054869

Starkey D.E., Ward T.J., Aoki T., Gale L.R., Kistler H.C., O'Donnell K., 2007. Global molecular surveillance

reveals novel *Fusarium* head blight species and trichothecene toxin diversity. *Fungal Genetics and Biology* 44(11): 1191–1204. https://doi.org/10.1016/j.fgb.2007.03.001

Tunali B., Nicol J.M., Hodson D., Uçkun Z., Büyük O., ... Bağci H., 2008. Root and crown rot fungi associated with spring, facultative, and winter wheat in Turkey. *Plant Disease* 92(9): 1299–1306. https://doi.org/10.1094/PDIS-92-9-1299

Ventura Y., Sagi M., 2013. Halophyte crop cultivation: the case for *Salicornia* and *Sarcocornia*. *Environmental and Experimental Botany* 92: 144–153. https://doi.org/10.1016/j.envexpbot.2012.07.010

Xu F., Song Y.L., Wang J.M., Liu L.L., Zhao K., 2017. Occurrence of *Fusarium* crown rot caused by *Fusarium pseudograminearum* on barley in China. *Plant Disease* 101(5): 837. https://doi.org/10.1094/PDIS-10-16-1436-PDN

Zhou H., He X., Wang S., Ma Q., Sun B., Ding S., ... Li H., 2019. Diversity of the *Fusarium* pathogens associated with crown rot in the Huanghuai wheat-growing region of China. *Environmental Microbiology* 21(8): 2740–2754. https://doi.org/10.1111/1462-2920.14602