



Biotic and Abiotic Factors Affecting *Cistus ladanifer* Production in Cultivated Plots from Mainland Spain [†]

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Abstract

Interest in the cultivation of rockrose (*Cistus ladanifer* L.) is focused not only on its commercial products but also on its role in maintaining ecosystems. Very few pests and diseases are known to affect it. However, in cultivated plots in mainland Spain, a high number of plants with symptoms of decline and death were observed. This damage occurred after a period of rain of 21 days in late spring, in tilled soil, and with plants grown from cuttings. Laboratory analysis revealed the presence of the phytopathogenic fungal species *Macrophomina phaseolina*, *Fusarium acuminatum*, *F. equiseti*, and *F. tricinctum*, which were identified by ITS sequencing. These fungi, along with the agronomic and edaphic–climatic conditions, suggest an association of factors involved in the damage observed in the rockroses, pointing out the need to develop an integrated management strategy for this type of agroecosystem.

Keywords: rockrose; symptoms; disease; phytopathogen; environmental conditions; sustainability



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1. Introduction

The sustainable production of rockroses is being actively encouraged due to their contribution to rural development and circular economy. Particularly, rockrose (*Cistus ladanifer* L.) exploitation has significant socioeconomic and environmental potential, since it is not only a source of essential oils and labdanum gum, but also of residual products involved in biomass generation [1,2]. The advantages of rockrose cultivation lie in the possibility of using it to obtain products of pharmacological interest, antioxidant products for use in the food and cosmetic industries, phytochemical agents, antifungal products for agricultural use, as well as applications in soil phytostabilization and even in the production of bioethanol [2–5]. The management of rockroses has beneficial effects on the preservation of traditional landscapes and their biodiversity, favouring the development of areas affected by rural depopulation and the abandonment of farmland [3,4].

It is considered that the cultivation of rockrose does not have high resource requirements, and *C. ladanifer* can be considered ecologically well-adapted to the conditions of mainland Spain. With respect to diseases, very few are known to affect it, and reports on pathogenic biological agents threatening this crop under cultivation are scarce. Sticky rockrose canker, caused by the fungus *Botryosphaeria dothidea* as the primary pathogen, was

the first description of a disease of this kind in this shrub species [6] and is considered the only serious disease that can have an impact on it. This canker is characterized by leaf wilting and branch dieback, leading to the death of the entire plant. Rockrose may also be susceptible to fungal species of the genus *Armillaria*, which spread below the surface, altering the roots and decomposing dead wood. Regarding other *Cistus* species, *C. incanus* and *C. monspeliensis* have been associated with RNA viruses [7]. On the other hand, with respect to *in vitro* conditions, plant extracts and essential oils of *C. ladanifer* have been described to have antimicrobial properties [8–10]. Thus, the essential oil of this crop, which is high in oxygenated sesquiterpenes, was able to inhibit the growth of a wide variety of bacterial and fungal strains [8–10], and the hydrolate also proved to have fungicide activity [10].

However, in cultivated rockrose plots from mainland Spain, a high number of plants was observed with symptoms of general yellowing and decline, eventually resulting in death and affecting crop production. Based on the established knowledge about *C. ladanifer* cultivation and the extreme adaptation of this crop to stressful conditions [1–5], it is highly unlikely that this is due to a single cause. The objective of this study was to address the different biotic and abiotic factors involved in the decline in order to determine the origin.

2. Materials and Methods

Data were collected from soil and environmental conditions in the area, and plant samples were taken for pathogen diagnosis.

2.1. Rockrose Cultivation

The crop was planted using rooted and potted nodal cuttings from 24 selected clones. At the onset of symptoms, plants were about one year old. Preliminary tillage was carried out using a rototiller for over 20 years, 3–4 times a year. Row cultivation was in a $1.60 \times 0.30 \text{ m}^2$ frame (20,800 plants·ha⁻¹), with two foot-operated irrigations with a total dose of approximately 6000 L and no fertilizers, phytosanitary products, or biostimulants. Weed control was carried out manually and with a rototiller between rows. The 1300 m² plot had a tillage sole at 25 cm and, in some areas, 15 cm. No subsoiling or chisel work was ever carried out to break up the tillage sole.

2.2. Abiotic Factors

Soil analyses were performed on composite samples taken at about 25 cm. Foliar analyses were performed on 200 g of leaves taken from plants randomly distributed throughout the area. Soil and foliar analyses were performed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) according to [11]. Infiltration measurements were made either with a double- or single-ring infiltrometer. Test pits were dug in duplicate with a mini-excavator to a depth of 1.40 m, and the profile was conditioned manually. Soil samples in the test pit for bulk density were taken every 5 cm, using a 5 cm diameter and depth cylinder. The samples were kept in an oven at 105 °C until constant weight.

Rainfall and temperature data for the period were consulted (www.aemet.es, accessed on 19 December 2025). The official weather meteorological stations were less than 13 km from the cultivation site.

2.3. Biotic Factors

For the phytopathological analyses, representative plants from the field were selected. Isolations were performed from crowns and stems, previously decontaminated with 2% sodium hypochlorite for 2 min, washed with sterile water, and cut into fragments, which were placed on PDA (Potato Dextrose Agar) medium [potato starch 4 g/L (~200 g/L

of infusion from potatoes), dextrose 20 g/L, agar 15 g/L] supplemented with streptomycin at 0.5 g/L. Plates were incubated at 25 °C and monitored every 2–3 days.

Mycelium preparations were made from purified cultures for microscopic observation after staining with 0.1% (*w/v*) methylene blue and visualized using a ZEISS brightfield microscope equipped with an Axiocam digital camera ZEN blue v3.0+ (Carl Zeiss Iberia S.L., Madrid, Spain).

To identify fungal species at the molecular level, genomic DNA was first extracted from the mycelia using the commercial Plant/Fungi DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada), following the manufacturer's instructions.

The DNA was subjected to semi-nested PCR, as described in [12], based on the amplification of a fragment of the ITS (Internal Transcribed Spacer) region. Thus, a first PCR was performed with the primers ITS 1 (5' TCC GTA GGT GAA CCT GCG G 3') and ITS 4 (5' TCC TCC GCT TAT TGA TAT GC 3') at a final concentration of 0.625 µM, and, subsequently, from 1 µL of the amplicon, a second PCR was performed with the primers ITS 4 and ITS 86 (5' GTG AAT CAT CGA ATC TTT GAA C 3') at concentrations of 1 µM and 2 µM, respectively. The annealing temperature in both PCRs was 55 °C. After partial sequencing of the resulting amplicons by the Sanger method, the fungal species were identified molecularly by comparing the obtained sequences with those deposited in the NCBI (National Center for Biotechnology Information) GenBank database using the BLAST (Basic Local Alignment Search Tool) program in its BLASTN mode (BLAST+ 2.17.0).

3. Results

3.1. Abiotic Factors

Soil was sandy loam, pH 7. From the analysis, values were high for phosphorus (385 mg·kg⁻¹), iron (324 mg·kg⁻¹), and zinc (10.0 mg·kg⁻¹), and low for calcium (279 mg·kg⁻¹), according to [13]. With respect to the foliar analysis, although there is no reference data for *C. ladanifer*, the values for macronutrients did not appear to indicate any deficiencies or excess toxicities that could explain the symptoms of decline. However, for micronutrients, an excess of iron (>300 mg·kg⁻¹ according to [13] for herbaceous perennials) was observed. Data are shown in Table 1. In the soil and foliar analyses, Fe data refer to available Fe.

Table 1. Foliar analysis for the main macro- and micronutrients.

Parameter (% <i>w/w</i>)		Parameter (mg·kg ⁻¹)	
Nitrogen	2.17	Iron	456.6
Phosphorus	0.40	Manganese	86.6
Potassium	1.00	Copper	6.10
Calcium	1.47	Zinc	94.3
Magnesium	0.21	Boron	96.9

Concerning soil infiltration, with the double-ring infiltrometer, the rate was 5.64 cm·h⁻¹ (moderate), whereas with the single-ring infiltrometer, the rates were 8.02 cm·h⁻¹ (moderate), 18.37 cm·h⁻¹ (rapid), 24.97 cm·h⁻¹ (rapid), and 15.95 cm·h⁻¹ (moderate). The soil depth was 70 cm, with 40 cm of decomposed bedrock. Data on the variation in density in the soil up to the parent rock are presented in Table 2.

Rainfall at the stations close to the cultivated field was several times more than the average for the same period in previous years. It began on late spring and lasted for 21 days practically without interruption. Temperatures were >20 °C.

Table 2. Apparent density of the soil according to depth in each test pit.

Depth (cm)	Apparent Density (g·cm ⁻³)											
	0–6	12	18	24	30	36	42	48	54	60	66	70
C-1	1.14	1.43	1.41	1.38	1.51	1.36	1.46	1.56	1.40	1.49	1.52	1.46
C-2	1.18	1.20	1.56	1.39	1.42	1.39	1.61	1.61	1.71	1.62	1.66	1.60

Standard deviation of the data ranged from 0.01 to 0.11 g·cm⁻³.

3.2. Biotic Factors

Molecular identification of the fungal isolates of interest revealed the presence of the species *Macrophomina phaseolina*, *Fusarium acuminatum*, *F. equiseti*, and *F. tricinctum* with ≥99% similarity, all isolated from crowns of the plant material. Colonies on PDA medium are displayed in Figure 1. Microscopic observations confirmed the presence of microsclerotia in *M. phaseolina* colonies, and macroconidia in the case of *Fusarium* cultures (Figure 1). The fact that these pathogens were detected does not imply that they constitute the cause of the decline, as many other factors can be involved.

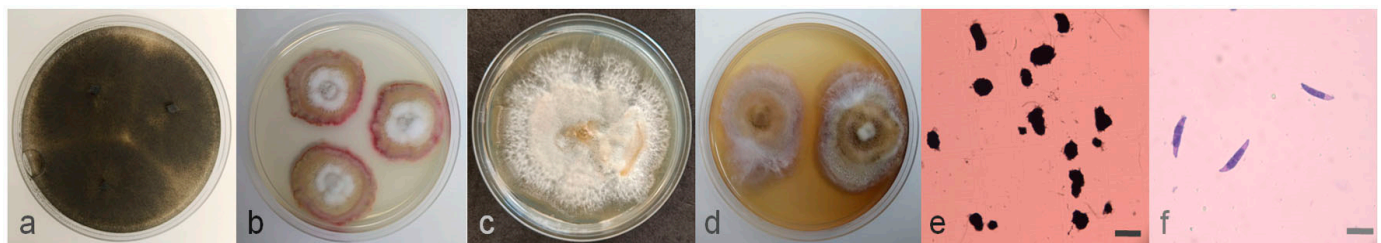


Figure 1. Fungi identified in symptomatic rockrose. Colonies of (a) *Macrophomina phaseolina* at day 15, (b) *Fusarium acuminatum* at day 22, (c) *F. equiseti* at day 42, and (d) *F. tricinctum* at day 22. Microsclerotia of *M. phaseolina* at ×100 (e), and (f) macroconidia of *Fusarium* sp. at ×1000. Scale bars: 50 µm (e) and 5 µm (f).

4. Discussion

C. ladanifer is typical of climates with hot, dry summers and grows in shallow, degraded soils [1,5]. Cultivation from cuttings can limit the plant's ability to adapt to the soil [14], since this species develops a taproot that penetrates deep into it. The fact that the crop was grown from cuttings in sandyloam soil with a tillage sole for more than 20 days of rainfall and temperatures above 20 °C, probably resulted in atypical high vegetative activity. Variations in soil density did not affect root development, and infiltration rates did not seem to justify waterlogging and plant damage. However, upon removal of the infiltrometer, a horizontal flow of water was observed. It can be hypothesized that, if this flow is significant and the vertical is insufficient, under conditions of high metabolic activity, an oxygen deficit could occur in the root zone that could be associated with the symptoms. Fruit trees waterlogged in winter do not suffer damage due to low cellular activity and, therefore, low oxygen demand [15]. Also, the excess of iron in the soil and plant could be a contributor. In plants, toxicity due to iron excess can cause several symptoms, among which is generalized yellowing, notably occurring in poorly drained soils [16]. All these soil conditions, which are stressful for the plant, could favour root infections that would not take place otherwise. In this way, the fungal species identified from symptomatic plant material are all described as disease-causing agents in other hosts.

M. phaseolina is a soilborne fungus that can cause stem and root rot, as well as charcoal rot, in numerous plant hosts [17], while *F. acuminatum*, *F. equiseti*, and *F. tricinctum* are pathogenic to both woody and herbaceous plant species, including horticultural crops, producing symptoms such as wilting [18]. Similarly, the species *M. phaseolina*, *F. eumartii*,

F. oxysporum, and *F. solani* have been described as causal agents of root rot and wilt in chickpeas [19]. In the case of the *C. ladanifer* decline described in this work, the identified fungal species would be acting more opportunistically rather than as primary pathogens.

Globally, the data seem to suggest the involvement of the identified fungal species, along with the agronomic, edaphic, and climatological conditions of the area, in the decline observed in *C. ladanifer* plants in mainland Spain, indicating the advisability of implementing sustainable integrated management strategies in these agroecosystems. Regarding the measures to prevent future plant problems within an integrated programme, apart from planting selected material and monitoring symptoms, cross subsoiling and cross chisel ploughing would be recommended to prevent drainage problems and reduce soil bulk density.

5. Conclusions

The time of year, the type of soil, and the climate factors, as well as the management and handling and the species-specific nature, most likely influenced the development of symptoms, with the isolation of various microorganisms identified as causing damage to many plant species. In this case, all the factors seem to be decisive: the key abiotic drivers were soil, rain and temperature; the key biotic agents were the isolated fungal species; the practical implications indicate the need to address holistic approaches; and the study limitations imply the impossibility of simulating the whole conditions to reproduce *in vitro* the *C. ladanifer* decline symptoms observed in the field.

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