




Review

Precision Agriculture Digital Technologies for Sustainable Fungal Disease Management of Ornamental Plants

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Abstract: Ornamental plant production constitutes an important sector of the horticultural industry worldwide and fungal infections, that dramatically affect the aesthetic quality of plants, can cause serious economic and crop losses. The need to reduce the use of pesticides for controlling fungal outbreaks requires the development of new sustainable strategies for pathogen control. In particular, early and accurate large-scale detection of occurring symptoms is critical to face the ambitious challenge of an effective, energy-saving, and precise disease management. Here, the new trends in digital-based detection and available tools to treat fungal infections are presented in comparison with conventional practices. Recent advances in molecular biology tools, spectroscopic and imaging technologies and fungal risk models based on microclimate trends are examined. The revised spectroscopic and imaging technologies were tested through a case study on rose plants showing important fungal diseases (i.e., spot spectroscopy, hyperspectral, multispectral, and thermal imaging, fluorescence sensors). The final aim was the examination of conventional practices and current e-tools to gain the early detection of plant diseases, the identification of timing and spacing for their proper management, reduction in crop losses through environmentally friendly and sustainable production systems. Moreover, future perspectives for enhancing the integration of all these approaches are discussed.

Keywords: agronomic practices; fungal risk models; fluorescence sensors; fungicides; hyperspectral; multispectral; thermal imaging sensors; ornamentals; molecular biology; spectroscopy



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1. Introduction

1.1. The Ornamental Plant Sector

The ornamental plant sector, or green industry, represents one of the most diversified branches within horticulture. It consists of two main specialised subsectors: (i) floricultural crops, meaning cut flowers and foliage, bulbs, indoor and bedding pot plants and perennials; (ii) nursery stocks, meaning trees and shrubs, perennial hardy plants as aromatics, young plants and transplants for the amenity and landscaping market, rootstocks and liners, turfgrass seed and turf rolls [1]. The reference market for these subsectors is quite diversified as well. The floriculture industry is mainly pointed toward domestic consumption since its products are especially purchased for expression of love or friendship, thankfulness or appreciation, aesthetical purposes, gifts for events [2]. Nursery stock is oriented toward landscaping and lawn services, turning to designers, garden centres, gardeners, and maintenance firms among others [3].

Worldwide the main markets are Europe, the USA, and Japan [2], and, among EU countries, Germany is the most important market for the ornamental sector, considering a volume of around 8.9 billion euros in terms of retail prices [4]. On the other hand, the

more relevant producer regions/countries are Europe (around 31%), China (around 19%), the USA (around 12%), and Japan (8%) on a value scale [5]. Among EU countries, The Netherlands (30%), Italy (13%), Germany (13%), France (12%), and Spain (10%) are at the top of rankings in terms of current prices [6]. Europe, China, Japan, and the USA are characterised by both high production and consumer demand resulting in them being self-sufficient in satisfying their internal requests, with only a few exceptions for specific or seasonal products. Other important countries with ornamental industries are Colombia, Ecuador, and Kenya, especially as mature exporting producers of cut flowers, while other emerging domestic producers are Brazil, Mexico, and some Asian countries as Thailand, Malaysia, and the Philippines [7].

The most globally appreciated and marketed cut flowers are the rose, carnation, gerbera, chrysanthemum, liliun, gladiolus, and orchids [6,7], while the most popular indoor pot plants belong to the genera *Anthurium*, *Chrysanthemum*, *Dracaena*, *Ficus*, *Hedera*, *Hydrangea*, *Kalanchoë*, *Phalaenopsis*, *Rose*, and *Spathiphyllum* [8]. Other important productions are represented by bedding and/or balcony potted plants, such as poinsettia, petunia, wax begonia, pelargonium, and cyclamen [4].

This review aims to examine the application of conventional practices and current e-tools for fungal disease management and trace future perspectives in the ornamental plant sector, identifying research lines to further develop interconnected systems as an upgrade of the integrated ones. Specifically, we first discuss the main fungal diseases and their incidence in the ornamental plant sector, focusing on the significant symptoms and signs of pathogens on the plant canopy, detectable by non-imaging- and imaging-based sensors, as well as the optimal climatic conditions for pathogen spread, usable for the implementation of fungal risk models. We move forward the conventional practices used for fungal disease management. Afterwards, we describe the digital and molecular tools currently used for the recognition and monitoring of diseases, focusing on their importance for early pathogen detection. Finally, we propose an integration of several approaches based on conventional (i.e., agronomic and breeding) and innovative (i.e., spectroscopy and imaging sensors, fungal risk models) practices to improve the sustainable and effective fungal disease management in ornamental plant productions.

Roses are the most famous and cherished plants because of their wide range of uses, including as flowering landscape shrubs and formal garden specimens, blooming potted plants, cut flower production as well as a source of essence and vitamin C [9]. Thus, the application of some digital tools described along the review is presented for this important ornamental species.

1.1.1. Open-Field and Protected Crops: The Current Agronomic Practices

The ornamental plant sector is characterised by a wide range of different cultivation systems according to the very diversified typologies of products. A first important subdivision is between open-field and protected crops and a further division is in soil or soilless culture. Floriculture crops are mainly grown in a protected environment, both as soil and soilless cultivations, while many seasonal products, especially cut flowers and foliage, are usually grown directly in soil in open-field conditions, using very simple and low-cost production systems. Nursery stocks are mainly grown in open-field conditions, both in soil or containers, with a few exceptions, such as rootstocks and young plants that need a protected environment, or shade plants, usually cultivated under shade nets.

The ornamental sector is increasingly moving towards a highly sustainable production, with attention to some critical issues, such as water and energy saving, reduction in chemical inputs, or circular economy concepts. Many actual changes in production practices are driven by both market requirements and a greater awareness of growers regarding the environment, promoting the transition to a fair, healthy and resilient horticulture. A relevant role is covered by the Internet of Things (IoT) technologies and devices, which are finding an increasing application in greenhouse crop management, but also in nursery production. The availability of a wide range of sensors for monitoring

both the environment, soil/growing media, and eco-physiological parameters allows the implementation of a predictive and real-time decision support system (DSS), improving the efficient use of resources, such as water and nutrients, and directly controlling plant growth, health, and yield [10]. Among the novel and widespread strategies, technologies for saving water and enhancing the water use efficiency are not only among the most studied but have also found great application at an operational level since they impact the disease susceptibility. Attention has been pointed towards irrigation systems providing drip irrigation and management tools based on real crop needs, instead of a timer-based scheduling [11]. Technologies and DSSs for improving the nutrient use efficiency are among the most studied as well. These systems are often based on vegetation indexes and are also applied on protected crops in the ornamental sector [12]. Other technologies that have found success in protected crops concern the use of light for controlling photoperiod regulation, flowering time, or improving yield and ornamental traits [13].

1.1.2. The Use of Pesticides for Ornamental Plant Productions

Despite the increasing attention about safety of consumers, workers, and the environment and the increasing use of Integrated Pest Management (IPM) practices, that are mandatory in EU countries [14], pesticides are still widely used. In ornamental crops, the quality and loss of yield are closely correlated and, thus, fungicides are still perceived as the affordable remedy for counteracting fungal diseases [15]. Pretty and Bharucha [16] recently pointed out the global market value of pesticides amounts to USD 45 billion per year, where fungicides represent 22%. China, the USA, and Argentina account for 70% of world pesticide use in agriculture, with the Asian colossus representing half of this value. European countries, thanks to the EU Directive, have been showing significant decreases in pesticide consumption in last few years (around –32% between 1995 and 2015 [17]). Integrated Pest Management programs are spreading among EU members and the USA (see the United States Department of Agriculture (USDA) IPM program—<https://nifa.usda.gov/program/integrated-pest-management-program-ipm>, accessed date 25 March 2021), while they have experienced some difficulties in developing countries, especially in Africa and Asia [18]. Surveying 20 greenhouse growing systems, Wei et al. [19] estimated that the direct costs due to the fungicide use account on average for 1.3% of total production cost for a selection of greenhouse annual and perennial plants, while costs referring to insecticides and other chemicals represent 1.2%. Therefore, even if the use of fungicides does not substantially impact on production costs, it must be reduced to overcome the limitations imposed by the regulation of agrochemical registration and application, as well as to promote the transition toward a more sustainable fungal disease management [17].

The main fungicides used on ornamental crops are boscalid, prochloraz, pyraclostrobin, carbendazim, and iprodione, as shown by a survey on residues from plants on sale [20]. In particular, fungicides belonging to strobilurin and phenylamide groups are widely applied for the control of soilborne diseases [15], while to prevent grey mould both multi-site fungicides, such as captan, and site-specific ones, such as methylbenzimidazole carbamates, dicarboximides, succinate dehydrogenase inhibitors, anilinopyrimidines, quinone outside inhibitors, phenylpyrroles, and sterol biosynthesis inhibitors class III fungicides are conventionally used [21]. These fungicides are also used for the control of wilt diseases, together with copper oxychloride [22]. Fungicide effectiveness is closely related to environmental conditions, flower developmental stage, application method, selectivity of active molecules and level of fungicide resistance by the pathogens [17,21]. The most used application methods in the nursery sector are air-blast sprayers and boom vertical or horizontal sprayers (Figure 1A,C), while the handled gun sprayer or knapsack are the most used for protected crops (Figure 1B,D), although the latter is considered as the least effective. Many new technologies have been developed for open-field crops, such as intelligent variable-rate sprayers or other systems based on Geographic Information System (GIS) data, while the greenhouse sector is still predominantly based on traditional methods [17,21].

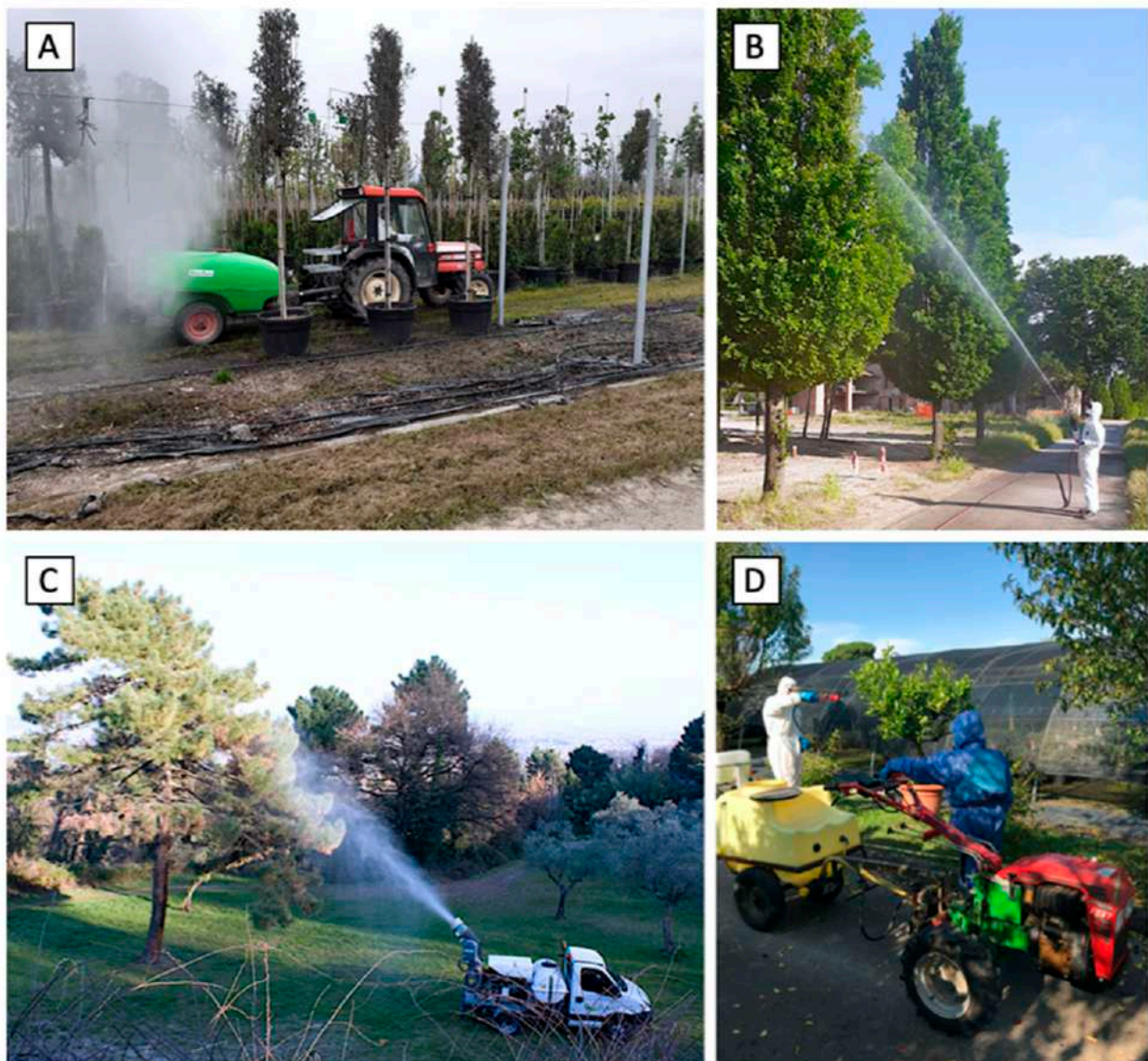


Figure 1. Conventional methods for pesticide distribution used in the ornamental plant sector. (A) A trailed agricultural atomiser (air-blast sprayer) used for potted or in soil trees or big shrubs; (B) a long-range spray gun commonly used for single trees or shrubs; (C) a centrifugal fan with long-range and wide-angle spray treatments commonly used for high trees; (D) a boom sprayer used for intra- and inter-row treatments, both in greenhouses and open-fields.

1.2. Fungal Disease Incidence in Ornamental Sector and Digital Tool Implementation for Their Early Detection

Among horticultural productions, ornamental plants are severely affected by fungal diseases since they cannot present even small imperfections. Plant pathogens can be divided in two main groups based on where they live and the pathogenesis starts: (i) soil-borne phytopathogens, including several fungi and oomycetes such as *Fusarium*, *Phytophthora*, *Rhizoctonia*, *Sclerotinia*, *Sclerotium*, as the main pathogens; (ii) air-borne phytopathogens, including agents of downy mildews, powdery mildews, leaf blights, black spots, canker, and anthracnosis [23]. Some examples of fungal diseases on ornamental plants are reported in Table 1, highlighting the typical signs and symptoms on the canopy and the optimal climatic conditions for the pathogen cycle. This information could be really useful for the implementation of digital tools based on spectroscopic and imaging technologies as well as fungal risk models.

Table 1. Some examples of fungi affecting ornamentals with their typical signs and symptoms on the canopy, detectable by digital tools, and the optimal climatic conditions for their cycle, useful for the implementation of fungal risk models.

Fungus	Host	Visual Signs and Symptoms	Optimal Climatic Parameters	References
<i>Soilborne Fungal and Oomycete Diseases</i>				
<i>Fusarium</i> spp.	All ornamentals	Vascular wilt	T: 25 °C T: 15–25 °C	[24]
<i>Verticillium</i> spp.	All ornamentals	Vascular wilt	High water activity T: 23 °C	[25]
<i>Ceratocystis fimbriata</i>	Forest ornamentals	Wilt, canker stain, and tissue rot	Relative humidity 85–97%	[26,27]
<i>Rhizoctonia solani</i>	Woody ornamentals	Quick decline resulting in total losses	T: 20–25 °C High water activity	[28,29]
<i>Thielaviopsis basicola</i>	Several ornamentals	Black root rot	Relative humidity > 85%	[30,31]
<i>Sclerotinia</i> spp.	Herbaceous ornamentals	Stem and crown rot/wilt	T: 15–27 °C Relative humidity > 85%	[32,33]
<i>Phytophthora</i> spp.	Woody ornamentals	Root, collar and crown rot till plant decline	Wide range of optimal temperatures Presence of free water	[34,35]
<i>Airborne Fungal and Oomycete Diseases</i>				
Leaf blight (e.g., <i>Alternata alternata</i>)	Gerbera and other ornamentals	Leaf blights, pathogenic spots on leaves, twigs, flowers	T: 23–29 °C Relative humidity ≈ 80%	[36,37]
Powdery mildew (e.g., <i>Sphaerotheca</i> spp.)	All ornamentals	White powder on aerial organs, buds fail to open, tips desiccation	Moderate temperatures Relative humidity 75–98% Not too high light intensity	[38,39]
Downy mildew (e.g., <i>Peronospora sparsa</i>)	Rose and other ornamentals	Purplish-red, brown or black leaf spots, square or angular	T: 15–25 °C Relative humidity > 85%	[40]
Grey mould (e.g., <i>Botrytis cinerea</i>)	All ornamentals	Dark spots developing in soft rotting, grey spore carpet on tissues, blights in dry condition	Presence of free water T: >21 °C Relative humidity > 99%	[41,42]
Canker (e.g., <i>Seiridium cardinale</i>)	All ornamentals	Dark brown discoloration and necrotic lesions	Wide range of optimal temperatures High relative humidity	[43]
Anthraxnose (e.g., <i>Colletotrichum</i> spp.)	Several ornamentals	Irregular, desiccated brown leaf spots	T: 25 °C Relative humidity ≈ 100%	[44,45]
Rust (e.g., <i>Puccinia</i> spp.)	Several ornamentals	Orange/yellow to brown/black pustules	T: 12–20 °C	[46,47]

Since the most common source of fungal disease spreading is the trading of living plants, the detection of potential risks for pest introduction is important as it is done in the sentinel nursery planting of Beijing, China, where the most common ornamental woody species are checked before shipment to Europe [48]. Fungal species constitute 3.6% of harmful organisms intercepted in 2018 in ornamental crops imported from non-EU countries to Europe [49].

Generally, soil-borne pathogens such as *Rhizoctonia* spp., *Phytophthora* spp., *Sclerotinia* spp., *Pythium* spp., *Verticillium* spp., and *Fusarium* spp. produce 50 to 75% of economic losses in several woody ornamentals [50]. In the USA, soil-borne pathogens cause about 90% of the main crop diseases due to their ability to survive in the soil for long periods and the complexity of a reliable diagnosis because of the similarity in symptoms with other fungal diseases [51]. *Phytophthora* spp. strongly affect woody ornamental production, having approximately about 130 host species, including many important shrubs and trees, and having been detected in almost every nursery of Europe, the USA, and Canada [52]. In California, *Phytophthora* spp. have been detected on 37% of native plant nursery stocks presenting symptoms of root and crown rot, collected among 22 host plant families from 26 different nurseries [53]. Several *Fusarium* spp. are responsible for various diseases in orchids causing severe economic losses in all countries involved in their production including the USA, Britain, Japan, China, Taiwan, Thailand, Australia, and Singapore. As an example, orchids represent a total wholesale value of USD 288 million in USA and are mainly produced for national trade [54]. *Fusarium oxysporum* f. sp. *tulipae* causes up to 50% losses in tulip production worldwide [55] while *F. oxysporum* f. sp. *gladioli* affects gladiolus with an incidence up to 100% causing a 60–70% mortality in several countries [22].

As reported for soil-borne pathogens, air-borne pathogens cause important crop losses in ornamental production as well. For instance, during 2017 in Georgia (USA), economic losses due to fungal leaf spots, stem cankers, and needle blights accounted for USD 30.7 million, root and crown rots for USD 34.1 million, powdery mildew for USD 6.4 million, downy mildew for USD 5.4 million, grey mould for USD 3.3 million [56]. The main economic loss was achieved in the floriculture sector (64% of total). In North Carolina and Virginia, the flower crops most affected by powdery mildew belong to *Zinnia* spp., followed by *Dahlia* spp., while the most infected by grey mould belong to *Ranunculus* spp., followed by *Anemone* spp. and *Dahlia* spp. [57]. Ornamental trees and shrubs are damaged by fungal diseases as well. Severe epidemics of cypress canker caused by *Seiridium cardinale* have been reported in California and the Mediterranean regions affecting different cypress species with a disease incidence up to 53% [43]. In Europe, *Ceratocystis platani* is responsible for the most destructive disease of ornamental plane trees causing epidemics and severe ecosystem degradation thanks to its rapid diffusion in both urban and rural ecosystems also through ambrosia beetles [58]. The pathogen responsible of boxwood blight disease has been found to infect one of the most important woody ornamental plants worldwide causing serious economic impacts on nursery productions. Among broadleaf evergreens, boxwood represents 15% of sales in the USA, with an estimated total annual value of USD 126 million [59]. The nursery industry in Connecticut lost USD 5.5 million in the first five years after the boxwood blight discovery [60]. Several pathogens are deeply feared for their high fungicide resistance and difficult management. As an example, reliable information about *Botrytis* spp.'s economic impact in ornamental crops is lacking in the literature, despite their worldwide incidence due to broad host range, endemic nature, ability to cause quiescent infections and incredible adaptability to different environments and fungicide resistance [41]. Fungal diseases affect the production of all most common flowers. A study carried out in 2017–18 in India investigated the occurrence of pathogenic diseases of economically important floricultural crops, highlighting an incidence of fungal infections of 57% in chrysanthemum, 56% in gerbera, 47% in rose, 46% in gladiolus, and 46% in marigold [61]. The early detection of pathogens, also through digital tools, is the only option to tackle these significant losses and achieve a sustainable ornamental production.

2. Materials and Methods of Case Studies

The topic has been reviewed on recent literature data principally referring to the research period 2017–2021. Imaging and non-imaging case studies on rose ornamental plants are presented here as special part to show some applicative examples and combine experimental data with review data. For this purpose, the application of some non-imaging and imaging sensor-based methods for the detection of the main fungal diseases affecting rose plants were tested.

Rose plants were maintained at CREA Research Centre for Vegetable and Ornamental Crops in Pescia, Tuscany, Italy (lat. 43°54' N, long. 10°42' E) and imaging and non-imaging case studies were acquired in spring–summer 2020.

For the application of non-imaging methods, rose healthy leaves or those affected by rust (*Phragmidium* sp.) were collected under natural pathogen pressure and detected through an ASD FieldSpec® 4 Hi-Res portable spectroradiometer (ASD Inc., Boulder, CO, USA) attached to an external ASD single-leaf clip and contact probe device, with an integrated 100 W halogen lamp and a spot size of reflectance measurement of c. 10 mm (Figure 2).

For the application of hyperspectral imaging methods, firstly, a description of some important vegetation indexes was done (Figure 3); the image was captured on a landscaping rose by using a Specim IQ hyperspectral camera (Specim Ltd., Oulu, Finland) working in the VIS-NIR spectral range (400–1000 nm). Together with the red–green–blue (RGB) image acquisition, the following vegetation indices, commonly used for fungal disease detection, were elaborated upon using R software: the Normalised Difference Vegetation Index (NDVI), Leaf Rust Disease Severity Index (LRDSI), and Photochemical Reflectance

Index (PRI). In addition, the NDVI index was also elaborated upon with the Analyst 2020 software (Analyst Group, Avellino, Italy) on images acquired by a Sony Ilce QX-1 multispectral camera modified with an Agrowing System and equipped with an NDVI objective (Agrowing Ltd., Israel) (Figure 4). The images were acquired on floribunda shrubs in open-field conditions on healthy leaves or those affected by black spot (*Diplocarpon rosae*).

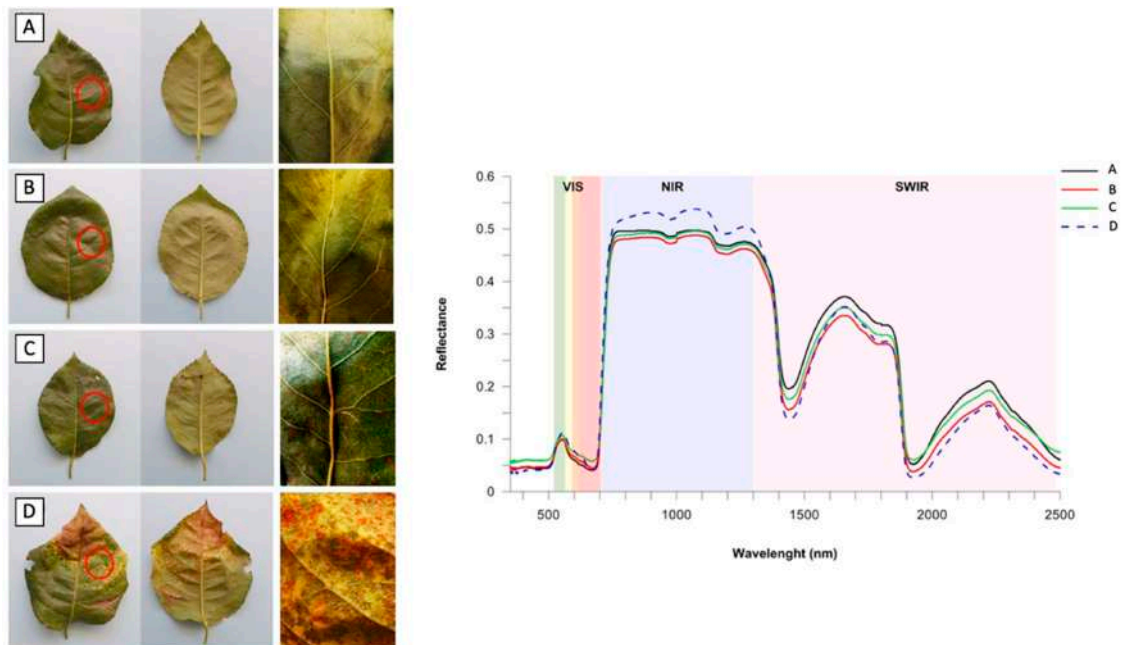


Figure 2. Spectral reflectance signature (350–2500 nm) of healthy (A) and rust-affected rose leaves at low (B), medium (C), and high (D) severity of attack. Measurements were performed using the ASD FieldSpec® 4 Hi-Res portable spectroradiometer.

The thermal imaging was tested on rose plants used for cut flower production maintained in a protected environment and affected by powdery mildew (*Sphaerotheca pannosa*) (Figure 5). The images were acquired with HD FLIR T1030sc thermal camera (FLIR® Systems, Inc., Wilsonville, OR, USA).

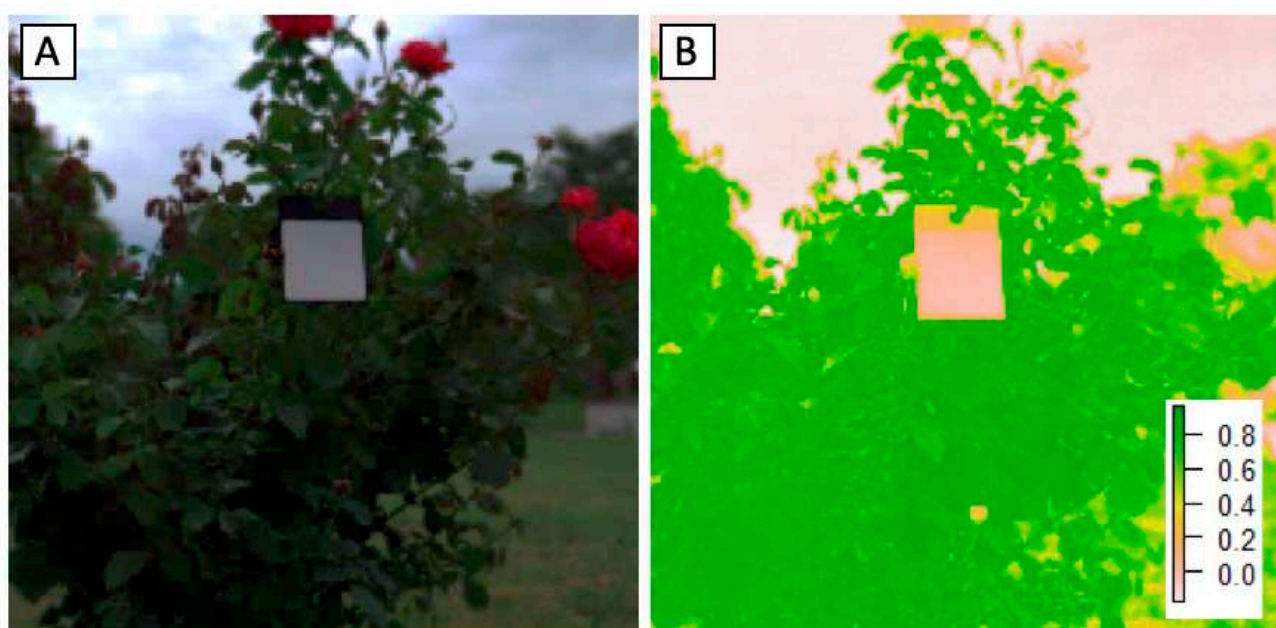


Figure 3. Cont.

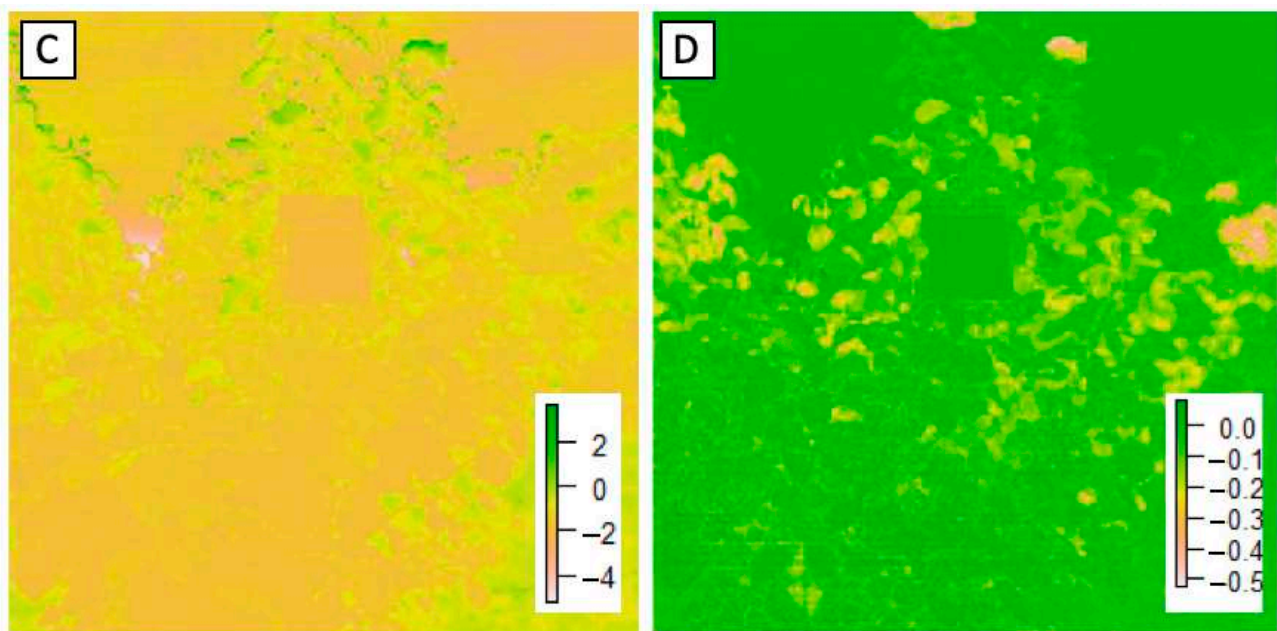


Figure 3. Red–green–blue (RGB) and vegetative indexes images of an ornamental rose plant acquired by a Specim IQ hyperspectral camera. (A) RGB image; (B) Normalised Difference Vegetation Index (NDVI) image; (C) Leaf Rust Disease Severity Index (LRDSI) image; (D) Photochemical Reflectance Index (PRI) image.

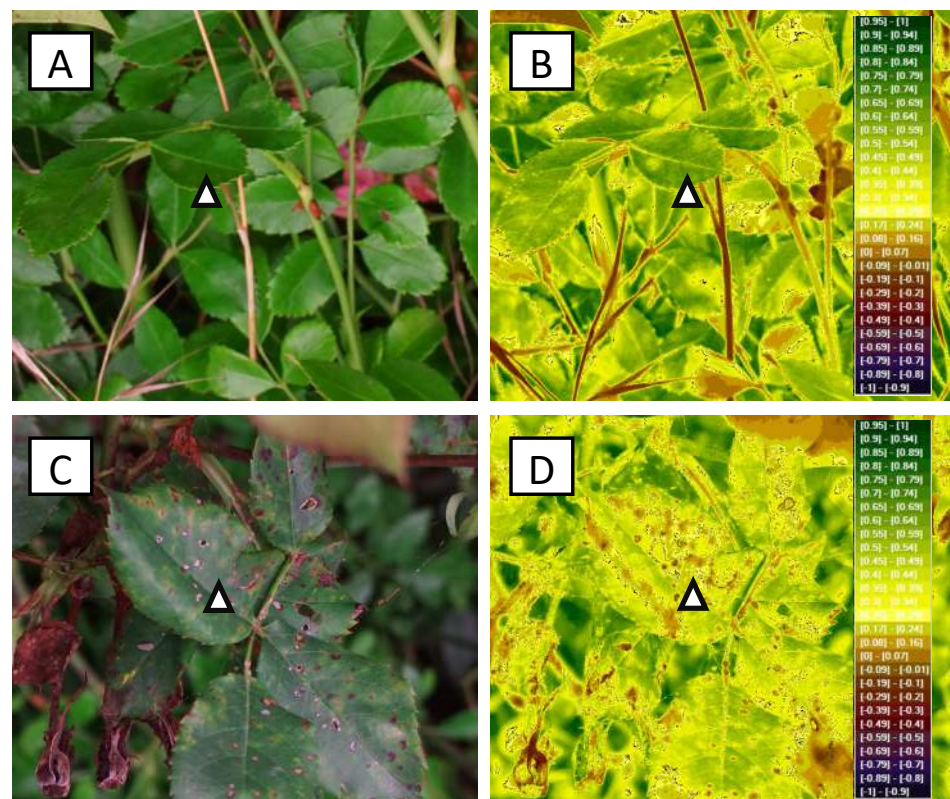


Figure 4. Fungal disease detection through multispectral imaging on rose. A and C are red–green–blue (RGB) images while B and D are elaborations of the Normalised Difference Vegetation Index (NDVI). White arrows point out a healthy leaf (A,B) or a leaf affected by black spot (C,D). Images were acquired by the Agrowing System and elaborated upon with the Analyst 2020 software.

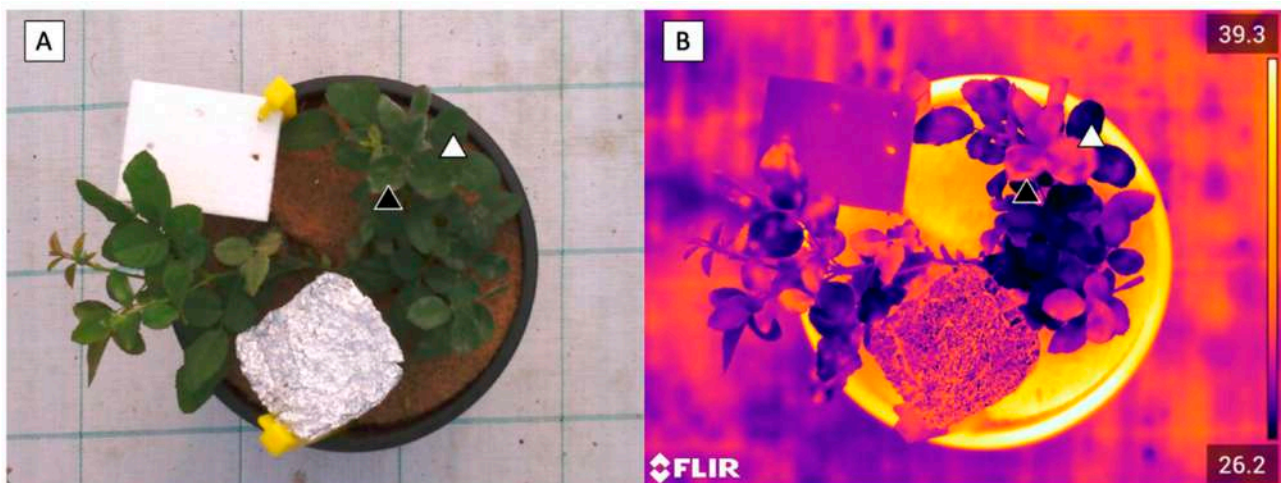


Figure 5. RGB (A) and thermal (B) images of rose plants in greenhouse affected by powdery mildew acquired with an HD FLIR T1030sc thermal camera. White arrows point out a healthy leaf while black arrows a leaf covered by powdery mildew.

Finally, the use of fluorescence sensors was tested on plants artificially inoculated with *Botrytis cinerea* and maintained in a growth chamber (23 ± 1 °C, 65% humidity). Specifically, plants were sprayed with a suspension of 1×10^5 conidia, obtained by recovering conidia from potato dextrose agar medium (Oxoid, Hampshire, UK) plates. After the spraying, plants were kept in a transparent plastic bag to promote the pathogen infection. Photochemical (qP) and non-photochemical (NPQ) quenching values were measured after 14 days from the inoculum during an induction curve using a MINI-PAM fluorimeter (Heinz Walz GmbH, Effeltrich, Germany). Plants were dark-adapted for 30 min before the measurements.

3. Conventional Disease Management in Ornamental Plant Productions

Agronomic practices include a wide range of methods directly and indirectly contributing to disease control [62], starting with common management techniques (e.g., crop density, pruning, fertilisation), moving to disease control actions (e.g., sanitation, ionising irradiation, resistance induction, use of biocontrol agents), and finally to practices with both agronomic and disease control significance (breeding, choice of variety/cultivar, grafting, rotation, etc.). Among greenhouse techniques, the opportunity of controlling the greenhouse environments by aerating, heating, and ventilating, as well as the carbon dioxide fertilisation, can significantly reduce the appearance and spread of many airborne fungal diseases on ornamentals [21]. Moreover, light management, strongly influenced by covering with plastic films and the use of next generation lamps, can influence sporulation and pathogen spreading [62]. Climate monitoring can implement models on pathogen cycle allowing the setting-up of alert systems in both protected and open-field crops.

The maintenance of clean cultivation surroundings is determinant as well. This means adopting sanitation practices of cultivation structures and all adopted equipment, like irrigation and soilless systems and utensils (especially those used for cutting and pruning). Sanitation includes the removal of fallen leaves, pruned branches, debris, infected plants and weeds [40,62]. Another fundamental action is soil and substrate disinfestation, especially for controlling soil-borne agents. As worldwide the use of chemical fumigants has been forbidden or strongly reduced, nowadays steam disinfestation, solarisation, and anaerobic soil disinfestation can be considered the most used methods [62], both alone and in combination. The use of amendments, such as compost or other products like *Brassica carinata* pellets, can be effective in reducing some important soil-borne agents [15]. Compost, especially from green waste, can enhance the plant growth function as a fertiliser improver, growth regulator [63], and soil-borne disease suppressor [64]. Lastly, sanitation by disinfection or chemical treatment of seeds and starting planting material ensures an

effective control of diseases, especially those caused by soil-borne agents [22]. Sanitation by chemical and non-chemical treatments (e.g., ionising irradiation) is finally employed to enhance the post-harvest shelf-life of cut flowers [21,65]. Chemical fertilisation and the macronutrient and microelement balancing as well as the choice of their different forms also have a role in disease control [62]. Calcium, both supplied through root-zone nutrient solution or foliar spray, has been reported as effective against grey mould on rose or scab on apple tree [21,66]. Phosphate and phosphite, bicarbonate, chloride, and silicate salts are effective in both air- and soil-borne pathogen control as host resistance inducers [67]. The irrigation management can influence pathogen spread and disease severity. The choice of drip irrigation or sub-irrigation, supplying water according to the real plant needs, guarantees a healthier condition for both root and aerial plant parts [21,62].

Biocontrol agents (BCAs), including a lot of different microorganisms, are finding an increasing use in disease control, exploiting different modes of action to prevent the occurrence of disease, for example by establishing a complex tree-way antagonistic-plant-pathogen interaction [68]. The biocontrol effects can be attributed to: (i) antibiosis mechanism due to antifungal secondary metabolites delivered towards the surrounding environment [69]; (ii) hyperparasitism due to the production of cell wall lithic enzymes responsible of entering hosts and killing them [70]; (iii) competition for space and nutritive elements [71]; (iv) induction of systemic resistance through jasmonic acid/ethylene signalling [72]. Even if their use is spreading in ornamental sectors, the major critical issue concerns the need for care at an operational level, especially considering the need for optimal microclimate conditions [68]. Nowadays, many commercial products, also known as biofungicides, are available both for soil- and air-borne fungi. Among bacteria, *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp. have found success in the control of principal fungal diseases [21,22]. Plant growth promoting rhizobacteria (PGPR), including those previously mentioned, also through the addition of organic amendments, can stimulate plant yield and protect from diseases through several mechanisms [73]. On the other hand, many fungi also show effectiveness in disease management, mostly belonging to the genus *Clonostachys*, *Gliocladium*, *Ulocladium*, *Cladosporium*, and *Trichoderma* [68,74]. In particular, *Trichoderma* spp. have a dual function as soil-borne disease protectants and plant growth promoters [75].

A final mention concerns the increasing spread of plant growth regulators, plant extracts, essential oils, as well as of biostimulant compounds that appear as substitutes for chemical products and/or for an organic crop management [21,76].

In the ornamental sector, plant breeding for achieving disease resistance is also widely used. The traditional breeding for disease resistance is based on the screening of existing germplasms and the identification of interesting sources carrying natural disease resistance genes to be used for the plant improvement [77]. A roundup of examples of breeding on florists' crops, such as anthurium, gladiolus, hydrangea, lily, daffodil, and tulip, to obtain plant resistant to diseases has been reported by [78]. In the last few years, transgenic technologies have also enhanced ornamental plant features by modifying or engineering the plant genomes [79].

4. Traditional and Novel Approaches for Fungal Disease Detection and Monitoring

4.1. Molecular Biology Methods

Direct diagnostic methods for fungal detection have widely changed over the last few decades, moving from the conventional methods, based on pathogen isolation and observation by microscopes, through the biochemical assay, until the use of molecular biology methods [80]. Indeed, the nucleic acid-based techniques, based on Polymerase Chain Reaction (PCR), are the most widely used tools for the direct detection, differentiation, and quantification of fungal pathogens in both symptomatic and asymptomatic plants due to their precision, sensitivity, speed, and reliability [81]. PCR methods help in predicting the outbreak risk of airborne diseases through both the detection of an increase in pathogen presence and the genetic changes in the pathogen genome that can make the

control ineffective [82]. Thanks to the development of specific primers, the conventional PCR assay can be used for the detection of specific fungi in plant tissues, as examples of *Colletotrichum capsici*, the causal agent of anthracnose in 121 host plant genera [83], *F. oxysporum* in Paris daisy [84], or *Corynespora cassiicola* and *Cercospora* sp. among other leaf-spot pathogens of hydrangea [85]. Moreover, a pool of different fungal species can be revealed by a multiplex PCR using more pairs of primers in the same analysis as reported for *Verticillium* spp. [86] or *Sclerotinia* spp. [87]. Another molecular technique frequently used for pathogen detection is quantitative PCR (qPCR) that allows an accurate, sensitive, and high-throughput detection of air-borne, soil-borne, and water-borne fungi and oomycetes in plant tissues, air, soil, as well as water [88]. The qPCR technique permits a precise measure of fungal DNA content giving information not only about pathogen presence but also on pathogen quantity [89]. A main advantage of this method is the detection of really low amounts of fungal DNA as reported for *P. cryptogea* on gerbera where the detection limit has been shown to be 100 times lower than using conventional PCR [90]. The sensitivity and specificity of pathogen detection through both simple and multiplex PCR and qPCR can be increased using a nested PCR, a method based on two rounds of amplification using two different sets of primers, the second of which targets the first run amplicon [91]. Recent advances in molecular biology tools also include droplet digital PCR, surface enhanced Raman spectroscopy, and isothermal amplification such as the loop mediated amplification (LAMP) method [92]. In particular, LAMP is a rapid and highly efficient method for the detection of plant pathogens with a sensitivity at least tenfold higher than conventional PCR; it is based on the use of four or six primers targeted on one unique nucleic sequence [93]. To facilitate fungal detection in the field, real-time microchip PCR systems have been developed for a portable, rapid, and accurate plant disease diagnosis [94]. LAMP-based portable tools have been recently tested on-site for the rapid detection of fungal pathogens in trees and ornamental plants [95]. Finally, high-throughput sequencing technologies, also known as next-generation sequencing, are receiving increasing interest for plant pathogen detection as well [96]. Outbreak predictions using molecular biology tools can provide useful guidelines for preventing a severe epidemic risk [82]. Thanks to the possibility of obtaining quantitative information from these tools, they could be used to calibrate other prediction methods such as imaging and models on microclimate.

4.2. Non-Imaging and Imaging Sensor-Based Methods

In the last few years, indirect methods concerning non-imaging and imaging sensor-based applications have provided new insights into rapid, objective, time-repeated, and non-destructive identification and quantification of plant diseases, supporting both precision crop protection and plant phenotyping. These sensors seize on the interaction between electromagnetic radiation and plants, measuring the plant/leaf optical properties within different regions of the electromagnetic spectrum. Since diseases can induce the alteration of a wide range of physiological processes, the spectral signature of stressed plants substantially differs from the signature of the healthy, unstressed ones [97]. The most promising tools in disease detection include those that measure reflectance, temperature, and fluorescence [98]. Sensors can be distinguished into non-imaging (spot spectroscopy) and imaging ones, which produce a multi-dimensional image with spatial and spectral dimensions. Sensors for non-imaging are mostly radiometers–spectroradiometers and fluorescence radiometers. Imaging sensors are usually cameras for red–green–blue (RGB), multispectral (broadband), hyperspectral (narrowband), thermal infrared, or fluorescence detection [99]. Both non-imaging and imaging hyperspectral sensors measure the radiation reflected by leaves/canopy in the visible (VIS, 0.4–0.7 μm), near-infrared (NIR, 0.7–1.3 μm), and short-wave infrared (SWIR, 1.3–2.5 μm) regions [100]. These sensors can identify the responsible pathogen by linking spectral signatures to changes in plant physiological processes through the evaluation of: (i) leaf pigments (500 to 680 nm); (ii) cellular structure of leaves or damaged organs (700–1000 nm); (iii) leaf/plant water content (1400 and 1930 nm);

(iv) specific stress-related metabolites [100]. However, these methods can be easily used under laboratory conditions, in field the discriminatory ability of these sensors could be influenced or compromised by light conditions and abiotic stress occurrence [101]. The main limitation of non-imaging hyperspectral sensors lies in their output as a mean of spectral reflectance over their area of view, without any spatial information. Therefore, early pathogen detection and the symptom measures could be compromised, especially at low disease severity, since the signal includes information from both infected and healthy areas [102]. Nevertheless, many studies have pointed out on the spectral responses of plants to fungal pathogens using hyperspectral non-imaging sensors in the horticultural sector. For example, in tomato, the VIS/NIR spectroscopy showed a great potential for relating the concentration of conidia of *F. oxysporum* with the spectral response of leaves [103], as well as for discriminating the plants inoculated with *F. oxysporum* from those subjected to water stress [104]. However, to the best of our knowledge, very few studies using reflectance hyperspectroscopy have been performed on ornamental plants. Poona and Ismail [105] selected an optimal subset of wavebands able to discriminate healthy and infected pine seedlings inoculated with *F. circinatum*. The spectral information can be considered using the whole spectrum as a plant “fingerprint”. In Figure 2 is shown the typical output of a non-imaging hyperspectral sensor. The use of this technique highlighted the possibility to discriminate the spectral reflectance signature (350–2500 nm) of healthy and rust affected leaves of rose shrubs. Another common method is to use vegetation indices, as a combination of reflectance on few wavelengths (2–6) of interest. Many vegetation indices have been defined over years, and some of the most commonly used in disease detection are summarised in Figure 3, in which they were tested on rose shrubs. Although many vegetation indices have found wide application for untargeted vegetation studies [106], others have been developed in specific plant–disease combinations. For example, to model *F. circinatum* stress in pine seedlings [107], to detect *Austropuccinia psidii* on lemon myrtle plants [108], or to group necrotic and non-necrotic leaves of eucalyptus inoculated with *Teratosphaeria* spp. [109]. Significant results have been obtained on many agricultural species, even to conceive a pre-symptomatic method for fungal detection [110]. Given the high cost and complexity that characterise hyperspectral cameras, research is oriented towards the building of cost-effective and accurate multispectral imaging systems, calibrated on specific wavebands, potentially dedicated to peculiar pathogens, and more suitable for the on-field applications [100,111]. For this purpose, starting from a laboratory-based hyperspectral system, the study in [112] selected eight bands suitable for the detection of grey mould in cyclamen plants, with the aim to develop a fast-multispectral camera. Multispectral sensors are comparable to hyperspectral ones, since both measure the up-coming reflected light from the leaf and/or canopy; however, a multispectral system often covers only three wavebands in the VIS (i.e., R, G, and B) in addition to NIR, allowing the calculation of some common vegetation indices, such as the NDVI. Multispectral sensors have been already used on ornamental species for the detection of biotic stress, such as the relationships between ray blight disease and pyrethrin and flower yields in pyrethrum [113] and the development of an automatic machine-vision-based system to screen healthy and infected tulip plants [114]. The application of a multispectral sensor, specifically modified with a NDVI objective, was tested on rose shrubs affected by black spot (Figure 4), highlighting the possibility of detecting the disease presence through the use of VIS and NIR wavebands.

Thermal sensors measure the emitted radiation by the leaves/canopy in the thermal infrared (7.0–30.0 μm , TIR) region and display the temperature information as a visual colour or greyscale intensity image. They are based on the relationship between leaf temperature and transpiration, finding application in the study of the water–crops relations [115]. However, the suitability of thermal imaging in fungal diseases detection is far from negligible and scarcely applied. Fungal infections generally affect leaf structure, cuticular and stomatal conductance and, thus, leaf transpiration and water loss [116], resulting in a progressive increase in leaf temperature and allowing detection of the disease [117]. Thermal imaging has allowed the early detection of powdery mildew and grey mould

in rose [118,119]. Indeed, the application of thermal imaging was tested for the detection of powdery mildew on rose plants used for cut flower production and maintained in a protected environment (Figure 5). The thermal imaging highlighted modifications of leaf temperature also in the surrounding of the visible fungal mycelium, highlighting a higher diffusion of disease.

Lastly, fluorescence sensors can be successfully applied for the study of plant–pathogen interactions and early fungal detection, due to their sensitivity to modifications of photosynthetic activity. Sensors rely on the measurement of the emitted light from fluorescent molecules in the regions of (blue–green fluorescence (BGF), 0.40–0.60 μm range), and red to near-infrared (chlorophyll *a* fluorescence (ChlF), 0.65–0.80 μm). In particular, BGF signals are related to components of cell walls [120] and are particularly prone to monitor early fungal attacks [121]. Fluorescence parameters have been applied for the study of the pathogenicity of some fungi on chrysanthemum [122]. Here, an example of the qP and NPQ values obtained through an induction curve on rose leaves inoculated with grey mould conidia is presented (Figure 6), highlighting different patterns for both values over time in infected plants in comparison with healthy ones. The rapid technological advances have made it possible to combine fluorescence principles with charge-coupled device (CCD) cameras (fluorescence imaging). In ornamentals, this technique was applied to detect *Puccinia horiana* in chrysanthemum [123].

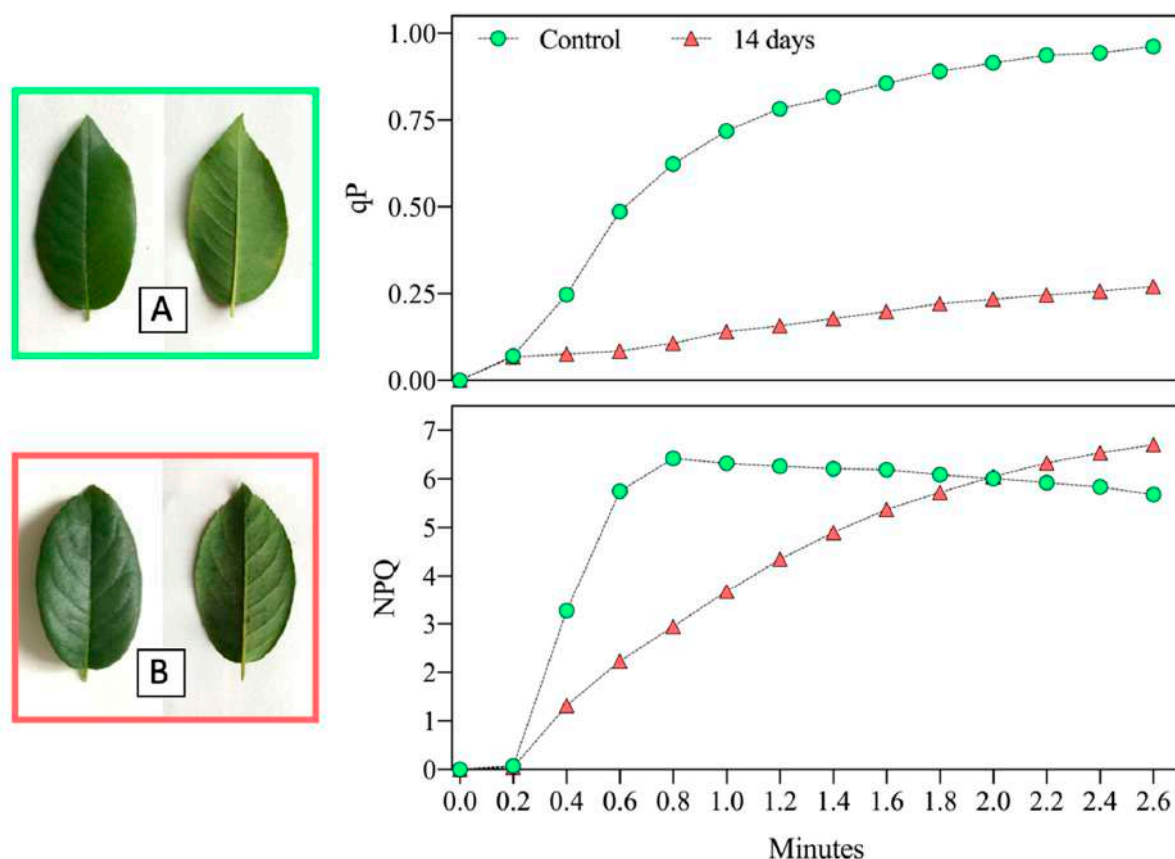


Figure 6. An example of photochemical (qP) and non-photochemical (NPQ) quenching values measured during an induction curve using a MINI-PAM fluorimeter on rose plants maintained in a growth chamber. Values were measured on leaves of control plants (A) and plants inoculated with grey mould after 14 days from the inoculum (B).

All the above-mentioned non-destructive techniques could represent a real strength to support integrated disease management strategies in the ornamental sector [92]. An integration between sensing techniques should be assured starting from simple RGB cam-

eras, which have been successfully used in the detection of tulip breaking virus [124] and powdery mildew in rose [119,125], to VNIR, SWIR, and ChlF sensors, to 3-D sensors [100].

4.3. Fungal Risk Models Based on Microclimate Trends

The goal of epidemiological models on fungal plant diseases is to determine if an infection will develop over time and space through the evaluation of relationships between a specific pathogen, its host plant, and the surrounding environment [126]. The models on pathogen infection evaluate the combination of favourable conditions, through hourly or daily environmental inputs, making previsions for disease risk [127], helping farmers to schedule a precise and effective use of fungicides [128]. The main steps needed for the development of a model on plant pathogens have been reviewed by the authors of [129] and consider inoculum pressure, disease progress, impacts on plants, and agricultural management practices (e.g., the use of fungicide). Each model must be validated using ad hoc experiments or through historical comparisons [130]. In the current climate change scenario, the development of models on infection risk is more important than ever to understand how the future pathogen dynamics will be modified [131]. Bergot et al. [132] modelled the effect of climate warming on *P. cinnamomi* infections on oak predicting a potential expansion of this pathogen in France in the near future. Given a pathogen and its host plant, the best environmental input to run a model should be quantitative, with a well-known maximum value, simply measurable, and reproducible [130]. Among climate indicators, models on air-borne fungal diseases usually consider the temperature, air moisture, and precipitation that are crucial for infection and strongly depend on pathogen type [133] while models on soil-borne fungal diseases usually evaluate the soil temperature [134] but also the soil moisture [135]. The leaf wetness duration is a key factor for air-borne fungal infection and can be included within a model considering both the humidity measured by a standard weather station and the wetness measured by sensors located within the canopy [130]. An example of monitoring networks for climate indicators related to fungal infections in protected or open-field environments is reported in Figure 7. Climate indicators are also related to other parts of the disease cycle such as the sporulation and dispersion that are the cause of secondary infections [127]. Models have been developed considering the effect of wind or rain splash on spore dispersion as for *Venturia inaequalis* [136]. For floricultural crops usually grown in protected cultivation, models have to consider the microclimate greenhouse parameters such as the solar radiation, temperature distribution, and relative humidity [137] as well as the greenhouse type [138]. For example, the Dynamic Greenhouse Climate Model has been applied on rose protected crops for the prediction of outbreaks of downy mildew, powdery mildew, and grey mould [139]. Moreover, also spore dispersion models can consider the microclimate greenhouse parameters, as reported for grey mould in rose crops [140], as well as the greenhouse type [141].

Despite their importance, not so many models have been developed for fungal infections in ornamental plants in both protected and open-field conditions. Models developed in protected cultivations include those for *S. pannosa* in rose [142], *B. cinerea* in gerbera [143], and *E. polygoni* in clematis [144]. Models developed in open-field cultivations includes those for *Rhizoctonia* spp. in azalea [145], *B. cinerea* in strawberry flowers [146], *S. homoeocarpa* in turfgrass [147], and *P. sparsa* in the *Rosaceae* family [148]. General models for foliar fungal pathogens in open-field conditions have been developed as well by Magarey et al. [127] and Launay et al. [131]. In addition, some models describe the fungal spore diffusion in relation to the weather conditions without any relation with the plant species [149].

Fungal risk models can be implemented considering other parameters indirectly related to climate variables, such as the plant phenological susceptibility [150], or independent of the weather conditions, such as the plant density, the genotypic resistance, the rate of pathogen reproduction, the fungicide management [129,151] or the commercial trade [152]. Indeed, models considering the use of biological control agents have been developed for grey mould in flowering species [153].

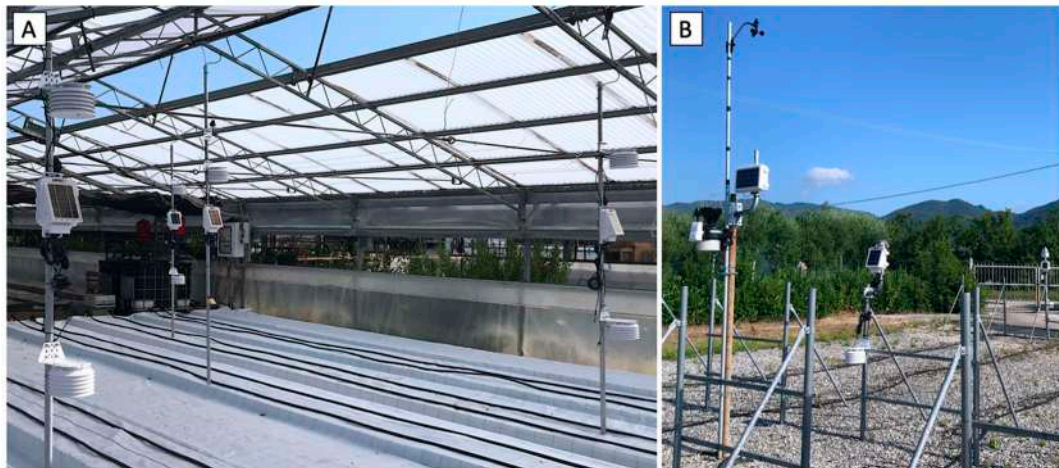


Figure 7. Monitoring networks for climate variables developed for the elaboration of fungal risk models of air-borne fungal infections on ornamental plants in both protected (A) and open-field (B) cultivation systems. The network is currently used at CREA—Research Centre for Vegetable and Ornamental Crops of Pescia (PT), Italy, for the monitoring of fungal disease development on rose plants.

5. Integration of Multidisciplinary Approaches for a Sustainable Management of Ornamentals

The main aim of an integrated disease management is the containment of fungal infections through a combination of protective means with different and/or complementary modes of action that can come together for the elimination or reduction of potential inoculum, the reduction of infection rate and interactions between the pathogen and its host plant [130]. The final objective of this sustainable strategy is to reduce the indiscriminate use of chemicals and prevent the yield losses applying the required specific treatments scheduled following the real plant needs. For example, in unheated greenhouses, the integrated biological and chemical control of grey mould allowed a drastic reduction in fungicides for vegetable production thanks to the monitoring of favourable environmental conditions for disease outbreak [154]. Recently, the integrated management for plant pathogen control has been already tested in several agronomical crops. The combined use of fungicides, biocontrol through *Trichoderma* spp., hot water treatment, and molecular identification of fungal pathogens has been tested in ginger [155]. The integration of chemicals, BCAs, an organic amendment, and microclimate parameters has been evaluated in tomato [156]. The effect of cultural practices, field sanitation, BCAs, bio-stimulants, and fungicides in legumes has been revised by Vandana et al. [157]. Nevertheless, the integration of multidisciplinary approaches has not yet been adopted for ornamental plants. Indeed, here we proposed a point of reflections and new perspectives for the integration of multidisciplinary approaches to increase the control effectiveness by also taking advantages from the early detection of plant diseases. In particular, the integration of information obtained through the spectroscopic and imaging techniques and models on climate parameters might be a successful strategy for obtaining early detection of fungal diseases. In particular, the information obtained through imaging techniques, such as the progressive measurements of plant canopy and architecture and the detection of plant physiological alterations, have the required realism to be included into the mathematical epidemiological models and therefore correct their output on the effective disease severity also taking advantage of the pre-symptomatic detection of plant alterations that are not visually apparent [158]. These interconnected systems can count on molecular tools for the validation and calibration to increase their robustness and reliability. These innovative tools must be used in combination with conventional practices, as agronomic inputs or a precise phytochemical use in time and space, to increase both the efficiency and the sustainability of ornamental plant productions. Indeed, the use of proper agronomic practices, and their

integration in epidemiological models, has been already showed to be a successful strategy for fungal disease control in horticultural trees [159].

6. Conclusions and Future Perspectives

A very wide range of symptomatic manifestations, distinctive pathogen signs, and microclimatic factors are strictly linked to the fungal disease evolution. These factors may be noticed by digital sensors both through high-throughput monitoring and IoT systems developed to sustain decisions and elaborate processes that are propaedeutic for a sustainable management. In particular, the use of non-imaging and imaging sensors-based methods constitutes a promising strategy for the early and large-scale detection of fungal diseases on ornamental plants. Indeed, the case studies highlighted the suitability of all the revised digital technologies for the detection of the most common diseases on rose plants in both open-field and protected environments. These sensors could be also calibrated using molecular tools to offer quantitative information on pathogen presence; moreover, they might be integrated directly into the monitoring networks for the microclimatic variables used for the elaboration of fungal risk models. Therefore, the development of an interconnected system that advises the producers that the favourable climatic conditions for pathogen spread are present in combination with alterations on plant canopy, detected with a remote imaging or spectroscopic system calibrated using molecular methods, might be a successful strategy to adopt in combination with proper and even innovative up-to-date agronomic and/or biological (e.g., BCAs) practices, thus reducing pesticide use as much as possible and enhancing the adoption of IPM strategies. Although the ornamental plant sector is still bound to traditional, high input based agricultural systems, these innovations should advance the current strategies for crop protection both in open-field and protected conditions, promoting a transition toward a sustainable production of ornamental plants.

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