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Automatic detection of powdery mildew on grapevine leaves by image analysis: Optimal view-angle range to increase the sensitivity

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ABSTRACT

Powdery mildew is a major fungal disease for grapevine (*Vitis vinifera* L.) as well as for other important specialty crops, causing severe damage, including yield loss and depreciation of wine or produce quality. This disease is thoroughly controlled by uniform spraying of vineyards with agrochemicals according to a calendar, which can easily result in ten to fifteen fungicide applications in several grapevine-growing areas. Since primary infections are localized in discrete foci rather than being uniformly diffused, there are potential benefits linked to the development of systems able to detect initial infection foci and operate targeted treatments instead of the current homogenous and unselective sprayings.

Proximal optical sensing is a major candidate for becoming the preferred technique for identification of foci for powdery mildew in grapevine and other specialty crops, but detection sensitivity of symptoms in the early-middle stage can yield largely limited results due to the combination of small dimensions, low density, and spatial arrangement of thin fungal structures.

This study investigated how the detection sensitivity (i.e., the portion of diseased tissue correctly recognized by the system) can be improved, especially for early-middle symptoms by means of sensing measurements carried out from an angle, rather than perpendicularly to the leaf's surface. To this aim, a multispectral imaging approach was applied to 35 grapevine leaves (10 used as calibration and 25 as validation samples) that were imaged at five different view angles from 0° (camera perpendicular to the leaf surface) up to 75°. Detection sensitivity was evaluated by applying to the validation images an algorithm based on the combination of two spectral indexes. The used algorithm was separately trained basing on the calibration set of images.

Overall results indicate that detection sensitivity generally increases as the view angle is increased, with a peak value obtained for images acquired at 60°. In particular, for tissue with early-middle symptoms, the algorithm's sensitivity exhibits a dramatic improvement, from 9% at 0° up to 73% at 60°.

Provided that the adopted training system results in rather homogenous leaves orientation, these findings suggest that field-sensing systems for detecting initial foci of grapevine powdery mildew can achieve improved results by providing the capability of measuring the canopy from a view angle in the range of 40–60°.

1. Introduction

In current viticulture practice, fungicides are applied uniformly through the vineyard according a spraying calendar, rarely triggered by experts' decisions and weather conditions, but more typically based on regular and frequent fungicide application. For powdery mildew, a major grapevine (*Vitis vinifera* L.) disease caused by the fungus *Erysiphe necator* Schwein (syn. *Uncinula necator* Schwein); this continuous protection approach can easily result in carrying out up to ten or even more treatments per season, in many vineyards of some of the most important wine-producing regions worldwide (Stummer et al., 2003; Calonnec et al., 2004; Crisp et al., 2006a,b; Iriti et al., 2011).

The polycyclic ascomycete *E. necator* is recognized worldwide as a major disease affecting both grape yield and quality. Susceptibility to powdery mildew depends on cultivar and environmental factors. Warm conditions with high relative humidity are favoring the development of the disease. In spring, primary infections can arise very early from overwintering mycelium in the buds, giving rise to the so-called "flag-shoot" or from ascospores overwintering in plant debris, which are then disseminated in the vineyard.

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Secondary infections are propagated by conidia differentiated in conidiophores, eventually evolving in disease patches that in turn produce fresh inoculum for following infection epidemics.

The pathogen colonizes the epidermal tissues of green organs (leaves, shoots, and bunches), causing severe damage to vineyards, including yield loss and depreciation of wine quality. Currently, in the warmer and drier grapevine-growing areas, powdery mildew is thoroughly controlled by agrochemicals, including contact, local, and systemic fungicides sprayed uniformly in vineyards (Stummer et al., 2003; Calonnec et al., 2004; Crisp et al., 2006a,b; Iriti et al., 2011).

Despite this spatially homogenous control approach, primary infection sites are characterized by a localized, discrete distribution rather than being uniformly diffused. Indeed, flag shoots and overwintering ascospores are randomly disseminated throughout the vineyard with an initially very low spatial density.

From this, it can be argued that chemical control of powdery mildew in grapevine would greatly benefit from a system able to target the protection treatments on the primary infection foci in a timely fashion. Indeed, especially during the early stages of disease development, detection of initial symptoms and their selective and targeted treatment would prevent the establishment of the infection and its epidemic spread to wider patches or to whole vineyard. This approach is currently being explored within the EU-funded project CROPS (www.crops-robots.eu).

Among possible solutions for automatic disease symptom detection, proximal optical sensing has specific characteristics particularly relevant in the view of field applications on grapevine and other fruit-tree crops (Oberti, 2003): (i) it performs non-destructive measurements, allowing for repeated acquisitions during the season without interfering with crop growth; (ii) it does not require direct contact with the sample, so it can be operated from almost any desired proximal distance; (iii) it is based on practically instantaneous phenomena, compatible with on-the-go measurements from a vehicle at normal field-operation speed; and (iv) it can inspect the vertical structure of the canopy, with a potential detection capability of early disease symptoms at a centimeter or even sub-centimeter scale.

Sensor technologies for crop diseases have been extensively reviewed by Sankaran et al. (2010) recently, while a more focused discussion on applications of proximal optical sensing for disease detection both in arable and specialty crops can be found in West et al. (2003) and Lee et al. (2010), respectively.

Moshou et al. (2011) developed a tractor-mounted, multi-sensor prototype integrating hyperspectral and multispectral images through real-time data-fusion techniques, demonstrating field functionality in automatic disease detection on wheat, enlarging the site-specific spraying research framework to include autonomous robotic platforms actuation.

However, the research dedicated to proximal optical detection of grapevine diseases is still limited to a few studies. Naidu et al. (2009) investigated the potential of using the leaf reflectance changes induced by grapevine leafroll-associated virus (GLRaV-3) as a diagnostic tool by measuring the VIS–NIR–MIR spectra of detached leaves. They found that the most discriminant wavelength intervals between healthy and virus-infected leaves are in the spectral region of green (near 550 nm), near infrared (near 900 nm), and in mid-infrared (near 1600 nm and 2200 nm). Calcante et al. (2012) evaluated experimentally the capability of two commercial optical devices (GreenSeeker RT100TM, Crop Circle TM) in discriminating different levels of downy mildew (*Plasmopara viticola*) infection on detached grapevine leaves, finding a fairly linear relationship between the disease severity and the processed output data from the tested sensors. Poutaraud et al. (2007) used in vivo fluorescence measurements on leaves inoculated with downy mildew (*P. viticola*) to study the spatial distribution of grapevine stilbenic phytoalexins (protectant secondary metabolites synthetized under stress conditions). They found that the high-intensity blue-violet fluorescence (F390 nm) emitted by stilbenes allowed to detect the stilbenes' local accumulation on the abaxial side of leaves, leading the authors to conclude that stilbenes fluorescence of leaves can be a promising early marker of pathogens attacks. With reference to powdery mildew, Bélanger et al. (2008) also applied fluorescence measurements to detect and quantify infection symptoms on detached grapevine leaves. By investigating different emission/excitation wavelength combinations, finding that the ratio between blue (F440 nm) and green (F520 nm) fluorescence intensity of healthy and diseased areas of leaves showed significantly different results starting from three days after inoculation.

Fungal diseases typically affect leaf reflectance, and for powdery mildew this is particularly due to the proliferation of hyphae filaments of the mycelium on the hosting tissue, leading gradually to a whitish-gray, powdery appearance. Nevertheless, at early-middle stages of mycelium development, these thin filamentous structures are barely detectable since the combination of their small dimensions, low density, and spatial arrangement has low influence on the spectral signature of the leaf surface, which in turn limits the detectability of the disease by optical sensing systems based on reflectance measurements.

During preliminary field measurements on grapevine, however, we have noticed that, thanks to the light back-scattered by hyphae and conidia whitish filaments, the detection of powdery mildew symptoms at early stages can be evidently improved when looking at leaf surface from an angle instead of looking perpendicularly to it. Interestingly enough, when studying proximal optical detection of winter wheat's fungal diseases (including powdery mildew) Zhang et al. (2012) and Yuan et al. (2013) underlined the necessity of investigating the influence of leaf orientations in disease detection by spectral reflectance.

Since in grapevine and other specialty crops, tractor-mounted optical sensing systems are typically used to scout the vegetation from the front of the canopy (i.e., mostly looking perpendicular to leaves faces), in the present study we investigate how the view angle can affect the detection's sensitivity of powdery mildew in grapevine leaves. This study is conducted by applying a multispectral imaging approach based on a relatively simple algorithm designed to this aim and hereafter described. The obtained results are expected to provide valuable information on possible optimization of the configuration of a field system for automatic detection of powdery mildew in grapevine and other specialty crops prone to this disease.

2. Material and methods

2.1. Plant material

Plants of *V. vinifera* L. cv Cabernet Sauvignon were propagated from wood one-bud cuttings, grown in 25 cm diameter pots filled with a mixture of sand and loamy soil (3:1, v/v). They were maintained in a greenhouse under controlled conditions at 25/20 °C day/night temperature, with 75% relative humidity and a 16-h photoperiod (40 μmol quanta m^{-2} s^{-1}). Plants were regularly watered and fertilized (Na/P/K/Mg, 15:10:15:2) and no pesticide treatment was applied. At the age of four months, 25 plants with approx. 15–20 leaves each were selected from the batch and were used in the experiment due to their healthy status of vegetation, as ascertained by visual inspections by plant pathologists. Seven plants were kept isolated under controlled conditions in order to maintain healthy conditions, while 18 plants were separately inoculated with the aim of obtaining a wide range of symptoms.