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# Effects of pyraclostrobin on leaf diseases, leaf physiology, yield and quality of durum wheat under Mediterranean conditions

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## ABSTRACT

A two-year field experiment was conducted to study the putative “health” effects of pyraclostrobin along with its fungicidal action on two durum wheat (*Triticum turgidum* L. subsp. *durum*) cultivars under rainfed, Mediterranean conditions. Five foliar treatments were applied: single applications [100 g active ingredient (ai) ha$^{-1}$] at the end of tillering (BBCH 31, $T_{31}$) and the flag leaf stage (BBCH 39, $T_{39}$), application (100 g ai ha$^{-1}$) at both growth stages (a total of 200 g ai ha$^{-1}$; $T_{31+39}$), chemical control [$T_{CC}$; a tank mixture of myclobutanil (60 g ai ha$^{-1}$) and fenpropimorph (750 g ai ha$^{-1}$) at BBCH 31, 39 and 69 (end of flowering) growth stages] and untreated control ($T_{UC}$). The two predominant foliar diseases [powdery mildew (PM) caused by *Blumeria graminis* f. sp. *tritici*, and Septoria tritici blotch caused by *Zymoseptoria tritici*] were assessed five times, initiating at BBCH 30, and the area under disease progress curve (AUDPC) was calculated. At the end of flowering (BBCH 69), physiological traits were determined on flag leaf [nitrogen concentration (Leaf N), chlorophyll content as assessed by SPAD-502 (SPAD), specific leaf area (SLA)] and canopy [canopy temperature depression (CTD)]. Also, carbon and nitrogen isotopes were measured on flag leaf [carbon isotope discrimination ($\Delta_{FL}$), $^{15}$N natural abundance ($\delta^{15}N_{FL}$)] and mature grains ($\Delta_G$ and $\delta^{15}N_G$) at BBCH 69 and 99 (harvest maturity), respectively.

Pyraclostrobin applications suppressed both foliar diseases as AUDPC revealed. The $T_{31+39}$ was the most effective treatment leading to highest grain and protein yields (GY and PY) while $T_{31}$ ranked second in yields indicating that early suppression of foliar diseases ($T_{31}$, $T_{31+39}$) was the most beneficial. The negative correlations between yields and $\Delta_{FL\text{-}G}$, a measure of flag leaf isotopic signature on grains, signified the importance of the prolongation of flag leaf photosynthesis as a result of foliar diseases suppression. On the other hand, foliar treatments had no significant effect on grain quality. The high-yielding cv. Elpida was tolerant to powdery mildew, water conservative during grain filling and more dependent on carbohydrates translocation to filling grains (higher $\Delta_{FL\text{-}G}$). The high-yielding growth season also showed higher grain protein concentration and vitreousness as a result of the lower diseases pressure, which was mirrored in higher flag leaf N, SLA and CTD. Concluding, the positive yield response of durum wheat to pyraclostrobin was not due to a “plant health” effect but owing to foliar diseases suppression, which allowed prolonged flag leaf photosynthesis.

## 1. Introduction

Winter cereals are the dominant crops in Mediterranean agriculture and in Greece. Among them, durum wheat (*Triticum turgidum* L. subsp. *durum*) is well-adapted under the semiarid, Mediterranean conditions producing high-quality grains (high vitreousness and protein) for pasta making mainly (Ross and Bettge, 2009; Stoskopf, 1985). In Greece, yearly, approximately 25% of the arable land is cultivated with durum wheat, which is the major crop (Hellenic Statistical Authority, 2016).

Under Mediterranean conditions, precipitation and temperatures are highly variable within and across growth seasons causing yield and quality to fluctuate intensively from season to season (Symeonidis et al., 2012). This fluctuation can partly be ascribed to the variability in diseases incidence and severity, which is caused by the season's climatic parameters (Chakraborty and Newton, 2011; Jevtić et al., 2017). An array of foliar diseases [Septoria tritici blotch [*Mycosphaerella graminicola* (anamorph *Zymoseptoria tritici*)], Stagonospora nodorum blotch (*Parastagonospora nodorum*), tan spot (*Pyrenophora tritici-repentis*), powdery mildew (*Blumeria graminis* f. sp. *tritici*) and rusts (*Puccinia triticina*, *Puccinia graminis* f. sp. *tritici*, *Puccinia striiformis* f. sp. *tritici*)] has been reported to infest *Triticum* species in Greece with devastating effects on yield and quality (Koutsika-Sotiriou et al., 2011). For this

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reason, among other traits, selection for high tolerance to diseases is a constant target for wheat breeders in Greece.

Growing tolerant cultivars is the most cost-effective way to encounter diseases. However, field-grown wheat is usually attacked by a complex of fungi and the commercial cultivars are usually bequeathed with tolerance, if any, to a specific fungus. Thus, an integrated approach including agronomic measures (N fertilization, rotation, tillage system, etc) and tolerant cultivars emerges as the most effective way to contain fungal diseases in wheat; in this approach, fungicides are the last-ditch defense against fungi (Jørgensen and Olsen, 2007; Poole and Arnaudin, 2014). Moreover, tolerant cultivars compared to sensitive are more benefited by fungicide application in terms of leaf health, photosynthetic performance and finally yield (Rios et al., 2016). The main goal of fungicide application in wheat is to expand the leaf life span and slow down the senescence of flag leaf, the last leaf to senesce, which has a high contribution of photosynthetic assimilates to grain filling, especially under semiarid Mediterranean conditions (Blandino and Reyneri, 2009; Pepler et al., 2005).

In Greece, no fungicide application against foliar diseases of durum wheat was occurring till 2010 when pyraclostrobin was introduced in wheat production. Pyraclostrobin, as a member of strobilurins (quinone outside inhibitors-QoIs), is a wide-spectrum fungicide, which disrupts the energy cycle of fungus via halting the ATP production (Bartlett et al., 2002). Apart from the fungicidal action, strobilurins were attributed with a “greening” or “plant health” effect as of their interventions in leaf $CO_2$ assimilation, water and nitrogen (N) consumption, and abscisic acid and other plant hormones (Bartlett et al., 2002). Fungicides causing a “health” effect are considered to delay leaf senescence due to an enhanced enzyme activity which protects the plants from harmful active oxygen species (Zhang et al., 2010). This way, a prolongation of crop green area occurs maximizing the grain filling period and thus grain yield (Bartlett et al., 2002; Dimmock and Gooding, 2002; Nason et al., 2007).

The rationale behind the health effect is that where foliar diseases are not yield-limiting, the crop is still availed. At field trials, the pertinent reports were not much supportive of the plant health effect in economic terms. In corn (*Zea mays* L.), a preventive application of strobilurin had an economic return in roughly 39% of the cases but an analogous application was unjustified in winter wheat (Weisz et al., 2011), soybean (Mahoney et al., 2015; Swoboda and Pedersen, 2009), and cotton (Woodward et al., 2016).

The physiological effects designated to strobilurins are associated with gas exchange functions thus conferring changes in photosynthesis and plant water economy in terms of leaf water use efficiency (WUE, the ratio of $CO_2$ assimilated per unit of water transpired via stomata). However, findings hitherto are disputable; both increases and decreases have been reported for photosynthesis in cereals (wheat, barley) and the soybean (Fagan et al., 2010; Nason et al., 2007). In a pot experiment, pyraclostrobin application delayed water uptake by wheat roots resulting in slowing down of soil drying; these findings, however, were not confirmed at a field experiment (Inagaki et al., 2009).

The above-mentioned dictate that the putative physiological actions of pyraclostrobin should be further tested especially under field conditions. To this direction, it would be helpful to employ indirect, long-term assessments instead of easily biased, instantaneous measurements (Tsialtas et al., 2017). In this line, SPAD-502, measuring leaf greenness, has been proved a reliable non-destructive assessment of leaf chlorophyll and N in wheat and it was related to grain yield, protein concentration and quality in both bread and durum wheat (Poblaciones et al., 2009; Spaner et al., 2005; Yildirim et al., 2011). Moreover, SPAD was used as a reliable, highly-heritable and discriminative tool for cultivar selection with concurrent foliar disease and heat stress tolerance (Rosyara et al., 2010a; b). Apart from long green leaf duration, high yielding in wheat was found to relate with lower specific leaf area (SLA, the ratio of leaf area to dry leaf mass) of flag leaf (Wang et al., 2008), which is indicative of lower photosynthetic rate but higher leaf longevity (Wright et al., 2004). A healthy and functional leaf canopy transpires water seamlessly comforting plants via cooling; this function is recorded as canopy temperature depression (CTD, the difference between canopy temperature and the ambient), which was found to relate with higher wheat grain yield under stress conditions like high temperatures, drought or leaf senescence by diseases (Balota et al., 2008; Rosyara et al., 2010b).

An indicator integrating both leaf transpiration and $CO_2$ assimilation performance and even in the long term is carbon isotope discrimination ($\Delta$, a measure of the $^{13}C/^{12}C$ ratio in plant tissues compared to the air). It is long used as an indirect assessment of leaf-level WUE since it was found to relate with the ratio of intercellular to ambient $CO_2$ concentrations ($c_i/c_a$); intrinsic water use efficiency ($WUE_i$, the ratio of $CO_2$ assimilation rate to stomatal conductance) is also associated with $c_i/c_a$ and thus, the relationship (negative) between $\Delta$ and $WUE_i$ is apparent (Farquhar and Richards, 1984). Interestingly, $\Delta$ has been proved a reliable, indirect and long-term indicator of WUE at biomass level (the ratio of biomass produced to the water consumed to produce it) for many $C_3$ species (Turner, 1996). In wheat, flag leaf at anthesis and mature grains were the most suitable organs for $\Delta$ determinations showing strong correlations with yield (Merah et al., 2002). It was found that autotrophic vegetative tissues (e.g. leaves, stems) are more depleted in $^{13}C$ (higher $\Delta$) compared to wheat grains indicating post-photosynthetic fractionation that further modifies the isotopic signatures of plant organs (Badeck et al., 2005). The difference in $\Delta$s between the vegetative parts (leaves, stems) and grains can be a measure of the contribution of biomass translocation from the vegetative parts to filling grains (Merah et al., 2018).

Along with carbon isotopes, nitrogen isotope composition ($\delta^{15}N$) in plant tissues is used as a tool to get insights of the processes N undergone in soil and plant having implications on N uptake and metabolism (Serret et al., 2008; Yousfi et al., 2009).

Employing a suite of long-term assessments mainly, exercised on canopy (CTD), flag leaf (SPAD, SLA, $\Delta_{FL}$, $\delta^{15}N_{FL}$, and leaf N) at the end of anthesis and grain ($\Delta_G$, and $\delta^{15}N_G$) at harvest maturity, we aimed to study the effects of pyraclostrobin, applied at two growth stages, on wheat physiology in regard with diseases suppression. Works employing long-term assessments to study the putative “plant health” effects of strobilurins on durum wheat under Mediterranean conditions are lacking.

## 2. Materials and methods

### 2.1. Site and experiment set up

A field experiment was conducted for two growth seasons [2010–11 (hereafter 2011) and 2011–12 (hereafter 2012)] at the Cereal Institute (40°32′2 N, 23°00′1 E, 20 m), ELGO-“Demeter”, Thermi, Greece. Two cultivars, Agapi and Elpida, registered by the Cereal Institute, were hand-seeded at a rate of 400 seeds $m^{-2}$; breeder's seeds were used for both cultivars. Seeding took place on 20 November 2010 and 23 November 2011. Before seeding, 80 kg N $ha^{-1}$, 40 kg $P_2O_5$ $ha^{-1}$, and 48 kg S $ha^{-1}$ were applied and incorporated into the soil. Ammonium nitrate (40 kg N $ha^{-1}$) was supplemented as top-dressing when two nodes were detectable (BBCH 32) according to the universal scale (Lancashire et al., 1991). The preceding crop was fallow in 2011 and common vetch (*Vicia sativa* L.) in 2012. Weeds were suppressed by spraying BROMOTRIL 40 EC (Bromoxynil 40%) between mid March (2011) and 20 March (2012). No irrigation was supplied.

The soil was a Typic Xerorthent loam and some of its characteristics are given in Table 1. The monthly rainfall and mean temperature during the growth seasons (November to May) are presented in Fig. 1.

The experiment was arranged in a split-plot design with five foliar treatments in the main plots and cultivars in the subplots. Each treatment was quadruplicated and blocks were separated by 2.5 m buffer zone. The subplots were consisted of 10 rows at 25 cm separation and