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Title:

Flavescence dorée phytoplasma deregulates stomatal control of photosynthesis in *Vitis vinifera*.

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Summary

Flavescence dorée (FD) is among major grapevine diseases causing high management costs; curative methods against FD are unavailable. In FD infected plants, a decrease in photosynthesis is usually recorded, but stomatal control of leaf gas exchange during FD infection and recovery is unknown. During one year when grapevines experienced a water stress and another with no drought we measured the seasonal time course of gas exchange rates in two cultivars ('Barbera' and 'Nebbiolo') different in the response to FD infection and recovery, as assessed by symptom observation and phytoplasma detection through PCR analysis. Chlorophyll fluorescence was also evaluated at maximum symptom severity in 'Barbera', the cultivar showing the most severe stress response to FD infection, causing the highest damage in vineyards of north-western Italy. In FD infected plants, net photosynthesis and transpiration gradually decreased during the season more during the un-droughted year than upon drought. During recovery, healthy (PCR negative) plants infected two years before, but not those still infected the year before, regained the gas exchange performances measured pre-infection. The relationships between stomatal conductance and the residual leaf intercellular CO_2 concentration (c_i) discriminated healthy versus FD infected and recovered plants; at the same c_i, FD infected leaves had higher non-photochemical quenching than healthy ones. We conclude that metabolic, not stomatal, leaf gas exchange limitation in FD infected and recovered grapevines is at the basis of plant response to FD disease. In addition, we suggest that such response is limited upon water stress, by showing that water stress superimposes on FD infection in terms of stomatal and metabolic non-stomatal limitations to carbon assimilation.

Keywords

Carbon assimilation, non-photochemical quenching, stomata, grapevine.

Abbreviations

A, leaf net photosynthesis; BBCH-scale, Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie - scale, a scale used to identify the phenological development stages of a crop plant; BN, Bois noir; c_i, intercellular CO_2 concentration; E, leaf transpiration rate; FD, Flavescence dorée; F_o, F_v, F_m, minimal, variable and maximum fluorescence, respectively; F_v/F_m, intrinsic efficiency of PSII; ΦPSII, quantum yield of PSII; g_s, stomatal conductance to water vapour; mT, maximum daily temperature; mVPD, maximum vapour pressure deficit; NPQ, non-photochemical quenching; PCR, polymerase chain reaction; PSII, photosystem II; REC1, recovered-healthy (PCR negative) plants infected in the previous year; REC2, recovered-healthy (PCR negative) plants infected two years before; REC3, recovered-healthy (PCR negative) plants infected three years before; RuBP, ribulose-1,5-biophosphate.

Introduction

Phytoplasmas are wall-less non-culturable prokariotes, belonging to the class Mollicutes, a group of microorganisms phylogenetically related to Gram-positive bacteria (Marcone, 2012). They are plant-pathogenic prokaryotes restricted to the phloem sieve elements of infected hosts (Firrao *et al.,* 2007). These obligate-parasites are transmitted by phloem-sap-feeding leaf, planthoppers or psyllids to several hundred of plants around the world, including species economically important such as *Vitis vinifera* L.

Phytoplasmas associated with Flavescence dorée (FD, 16SrV-C, -D) and Bois noir (BN, 16SrXII-A) diseases are the main cause of grapevine yellows (GY) in Europe (Galetto *et al.*, 2005; Quaglino *et al.*, 2009). FD is a quarantine pest (A2 list EPPO/CABI, 2003).

Ecophysiological relations of FD phytoplasmas with host plants are still unclear. FD-infected grapevines usually show characteristic symptoms such as yellowing or reddening of the leaves, stunting, downward leaf rolling, shortening of internodes, bunch shriveling, and general decline that may result in plant death. Phytoplasma-infected vines, following the first year of symptom expression, may show a spontaneous remission of symptoms, known as "recovery" (REC) as previously reported in apple (Musetti *et al.*, 2004), apricot (Musetti *et al.*, 2005) and grapevine (Caudwell, 1990). However, mechanisms and dynamics of REC phenomenon are largely unknown and still debated (Landi and Romanazzi, 2011).

The early response of plants to pathogen attack is represented by an anomalous deposition of callose on the plates of sieve tubes, through a Ca^{2+} dependent phenomena (Knoblauch *et al.*, 2001), leading to the formation of physical barriers able to reduce or block movements of phytoplasmas in shoots. In addition, subsequent blocks in sugar and protein translocation are followed by an increase of starch in source leaves and a decrease in sink leaves and roots as previously observed in coconut palms affected by lethal yellowing (Maust *et al.*, 2003) and in maize plants affected by bushy stunt (Junqueira *et al.*, 2004). Additionally, a related decrease in chlorophyll content and in chlorophyll biosynthesis has been observed in coconut palms infected by coconut lethal yellowing (Leon *et al.*, 1996), in apple trees infected by apple proliferation (Bertamini *et al.*, 2002a) and periwinkles infected by ash yellows (Tan and Whitlow, 2001).

Currently, key ecophysiological leaf traits related to phytoplasma disease symptoms are decreased rates of gas exchange variables carbon assimilation (A) and water transpiration (E) (Tan and Whitlow, 2001; Endeshaw *et al.*, 2012). Proposed mechanisms leading to stomatal closure were related to an increase in abscisic acid (ABA) concentration (Leon *et al.*, 1996; Martinez *et al.*, 2000) and sucrose accumulation around guard cells, as reported by Lu *et al.* (1997). However, there is little information about stomatal limitation. In FD plants, it is not clear whether limitation in stomatal conductance (g_s) derives from metabolic impairment modulating apoplasm leaf CO_2 levels (c_i), as suggested by von Caemmerer and Farquhar (1981), or on the contrary, that stomatal damage reduces carbon assimilation. A previous study performed on periwinkle infected by ash yellow (Tan and Whitlow, 2001) showed that A rates were higher in healthy plants than in infected ones, with no variations in c_i. Recently, Endeshaw *et al*. (2012) observd high c_i coupled with tendentially decreasing values of A and g_s in symptomatic grapevine leaves of BN-infected grapevines.

In this work we monitored ecophysiological changes during two summer seasons of healthy, FD-infected and recovered 'Barbera' and 'Nebbiolo' grapevines located in vineyards of north-western Italy. We aimed: i) to highlight which mechanism controls leaf/atmosphere gas exchange during FD infection, ii) to monitor grapevine recovery both by ecophysiological parameters and by PCR phytoplasma detection, and iii) to investigate the response to FD of two different grapevine varieties upon either well watered or drought conditions.

Materials and methods

Vineyards

The study was carried out during four years (2008-2011) in two vineyards of north-western Italy: Monteu Roero and Cocconato, characterized by different soil and climate conditions, where 'Nebbiolo' and 'Barbera' cultivars were monitored and sampled, respectively. A meteorological station was present in each vineyard in order to record air temperature, relative humidity and rainfall during the season.

Plants were trained to vertical trellis system with *guyot* pruning; conventional agronomic and phytosanitary management were regularly applied in both vineyards.

The Monteu Roero vineyard consisted of 17 rows, for a total of about 1250 'Nebbiolo' vines, planted in 1998 on SO4 rootstock. The Cocconato vineyard consisted of 76 rows, for a total of about 8000 'Barbera' plants, planted in 1999 also on SO4 rootstock. Preliminary inspections and PCR analyses confirmed the presence of FD infection in both vineyards since 2006. Detailed mapping and labeling of infected plants started in 2008. In both vineyards, two insecticide treatments were applied at the end of June (thiamethoxam) and end of July (chlorpyriphos) against the FD vector *Scaphoideus titanus* Ball. In 2010 a severe FD outbreak occurred in the Cocconato vineyard.

Sampling and phytoplasma detection and characterization

Vineyards were surveyed three times each year: late spring (May – early June), early summer (July – early August) and late summer (September). In 2008, to establish a pool of FD-infected plants of both cultivars, samples (ten leaves per each plant) were collected from the 'Barbera' (n=16) and from the 'Nebbiolo' (n=20) plants showing grapevine yellows symptoms. In the following years, samples were taken from new 'Barbera' and 'Nebbiolo' plants showing symptoms as well as from all the plants analyzed the year before. Once a plant was identified as a FD-infected, it was re-sampled at any further sampling time every year.

Total DNA was extracted from 1.5 g of leaf veins following a phytoplasma enrichment protocol (Marzachi' *et al.*, 1999). Polymerase chain reaction (PCR) was employed for phytoplasma diagnosis with universal primers, P1/P7 (Schneider *et al.*, 1995) followed by primers R16(I)F1/R1 or R16(V)F1/R1 (Lee *et al.*, 1994). Reaction and cycling conditions were as detailed in the original papers. To discriminate between "*Candidatus* Phytoplasma asteris" and Bois Noir phytoplasma infections, two µl aliquots of fragments amplified with R16(I)F1/R1 amplicons were digested with one unit of *Mse*I (Invitrogen, Carlsbad, CA) at 37°C, according to the manufacturer's recommendations. A Piemonte strain of FD (FD-C), acquired from grape by *S. titanus* and transmitted to *Vicia faba* L., a French strain of FD (FD-D), kindly provided by Dr. E. Boudon-Padieu and graft-maintained in periwinkle in the collection of the Istituto di Virologia Vegetale, CNR, and a Sardinian strain of Stolbur from tomato (T2_92), also maintained in periwinkle (Minucci and Boccardo, 1997), were used as reference isolates and positive controls in PCR and restriction fragment length polymorphism experiments. A healthy grapevine, grown from seed and maintained in insect-proof greenhouse, was employed as healthy control in PCR experiments.

In this trial ecophysiological parameters were monitored during 2009 and 2010 productive seasons. Phytoplasma presence was assessed three times every year: late spring (May – early June), early summer (July – early August) and late summer (beginning of September). At the beginning, we started with a group of plants already monitored for phytoplasma infection in 2008, consisting in 10 FD-infected 'Nebbiolo' and 12 FD-infected 'Barbera' grapevines (Table 1) with the same number of control (healthy) plants. To follow FD and recovery dynamics, the number of observed plants increased over the years, by the identification of new FD-infected plants by diagnostic PCR (Tables 2 and 3).

Plants were divided into four groups: FD (symptomatic and PCR-positive plants), REC1 (symptomless and PCR-negative plants that were FD-infected the year before), REC2 (symptomless and PCR-negative plants for two consecutive years that were FD-infected two years before) and healthy plants. Recovered plants were negative in PCR assays at each of the three samplings of the year.

Gas exchange measurements were performed on FD (symptomatic leaves), REC1 and REC2 plants and a healthy plant in the same vineyard row was monitored as control for each FD or REC1 replicate. Ecophysiological measurements carried out in plants found FD positive in PCR diagnosis at any sampling time were considered FD plants for the whole season (Table 1).

Gas exchange and chlorophyll fluorescence

During the two years, assimilation (A), transpiration (E), stomatal conductance (g_s) and leaf internal CO_2 concentration (c_i) of three well-exposed mature leaves were measured with an LCpro+ ADC system (Analytical Development Company, Hoddesdon, UK) on 1 to 15 plants for each treatment (the exact number of plants observed in control, FD, REC1 and REC2 treatments is displayed in table 1). Measurements were taken in summer once a month during a sunny day, in central hours of the day, at ambient relative humidity (RH) and [CO_2] (about 380 ppm), under saturating light (1200-1400 μmol m^{-2} s^{-1}) using a broad-leaf chamber (6.25 cm^2 leaf area). Measurements were carried out on symptomatic leaves in FD plants for both cultivars. Only for the more symptomatic cultivar ('Barbera'), at the end of the season 2010 (11/09/2010, ripening period, BBCH scale-85) chlorophyll fluorescence using the portable gas-exchange fluorescence system GFS-3000 (Heinz Walz GmbH, Effeltrich, D) was quantified. These measurements were carried out on FD symptomatic leaves at ambient CO_2 and ambient temperature. Fluorescence levels with electron acceptors fully oxidized (F_0), and electron acceptors fully reduced (F_m) were measured on leaves after a dark-adapted period of 5 minutes, the monitor being clipped to the leaf. The maximum quantum efficiency of PSII was determined as F_v/F_m, where F_v is the difference between F_0 and F_m. Other fluorescent parameters were considered: ΦPSII calculated according to Genty *et al*. (1989), and NPQ as previously described by Schreiber *et al.* (1986).

Statistical analysis

Seasonal time courses were summarised by means and corresponding standard errors, which thus represent information about the variation and the issue of unequal replication of plants for each treatment category (healthy, FD, REC1 and REC2) and for each cultivar. One-way ANOVA was applied to data at specific time points using the SPSS (SPSS Inc., Cary, NC, USA) statistical software package. Statistical significance between the treatments was determined by an F-test result with $P < 0.05$. Pearson correlation was used to assess the strength of relationships between variables of interest. Correlated variables were displayed in figures along with regression lines plotted by means of Microsoft Excel © software.

Results

Identification of FD-infected plants and recovery trend in the two grapevine varieties

Symptomatic 'Barbera' and 'Nebbiolo' plants provided a band of the expected size following electrophoretic separation of the nested PCR amplicons (not shown). Characterization of FD phytoplasmas showed that only FD-C strain was present in Cocconato, while both FD-C and -D isolates were found in Monteu Roero vineyard.
BN phytoplasma was absent in plants chosen for ecophysiological measurements in both vineyards.
Table 2 and 3 show the number of observed plants in the vineyards and the percentage of recovered plants. In 'Barbera', annual recovery rates ranged between 51 and 62%. The percentage of plants that from FD became REC2 was around 45% and only in three cases re-infections were observed. On the contrary, 'Nebbiolo' plants showed lower percentage of recovery (REC1 around 21%), with only two plants that remained recovered for two consecutive years. For both cultivars, REC2 plants were not subjected to a re-infection by FD.

Ecophysiological characterization

Seasonal patterns of daily maximum temperature, maximum daily vapour pressure deficit (mVPD) and rainfall recorded in the two vineyards in 2009 and 2010 are shown in Figs. 1a,b and 2a,b. The driest period was recorded from the middle of July up to September with only sporadic rainfall events in both years. During the same period, the temperature was high and quite stable (26-34°C) up to the beginning of September, causing an increase in VPD. A similar trend for

VPD values were observed when they were evaluated during gas exchange measurements in central hours of the day by the E/g_s ratio (Figs. 1c,d and 2c,d), suggesting that central hours of the day are determinant for the evaporative demand.

As expected, seasonal course of E was influenced by the VPD levels, with the exception of 'Barbera' plants in late summer 2009, where transpiration significantly ($P < 0.05$, F-test) decreased even if VPD increased, this caused by stomatal response to drought (Fig. 1f,h and table 4). E, g_s and A levels showed significant differences ($P<0.05$, F-tests) among health status categories in 'Nebbiolo' during 2009 (Fig. 1e,g,i), while on the contrary 'Barbera' plants followed similar patterns in healthy, FD-infected and REC1 vines (Fig. 1f,h,j).

REC1 'Nebbiolo' plants in August showed greater reductions of all parameters in comparison with controls, but a complete recovery in the last survey date was recorded. On the contrary, the detrimental effects of FD on gas exchange in 'Nebbiolo' infected plants were observed till to the end of the season (Fig. 1e,g,i), even if not always with statistical significance.

In 2010, 'Nebbiolo' plants did not show significant ($P<0.05$, F-test) differences among sanitary categories in the first survey date, with the exception of g_s (Fig 2g). As in 2009, differences between FD and healthy 'Nebbiolo' plants were observed in the middle of the summer (August). On the contrary, during 2010 season, heavy influence of FD was found in 'Barbera', where FD-infected plants were affected detrimentally during the season (Fig. 2f,h,j). In both REC1 and REC2 plants slight differences were shown for all parameters considered, suggesting an incomplete recovery; however, REC2 plants reached performances similar to healthy ones in the last survey date (Fig. 2f,h,j).

By plotting stomatal conductance (g_s) versus leaf internal CO_2 concentration (c_i) it is possible to investigate on metabolic and stomatal limitations to photosynthesis. In healthy plants of both cultivars, g_s and c_i decreased in parallel, showing that stomatal limitations during the season limited carbon assimilation; a positive, even if not always significant, regression between g_s and c_i was recorded as assessed by the Pearson correlation, suggesting that seasonal variations of g_s controlled carbon uptake by the plants, resulting in leaf internal CO_2 changes parallel to stomatal function (Figs. 3,4a,b). On the contrary, in FD plants, high internal CO_2 concentrations were associated to a stomatal closure, especially in the last two measurement dates (Figs. 3c,d,and 4c,d), suggesting that a metabolic, non stomatal limitation to carbon assimilation occurred during FD infection. Notably, the significant positive regression recorded in healthy plants did not occur upon FD infection, leading the hypothesis that metabolic limitations on carbon metabolism caused stomatal closure by an enhancement of internal leaf CO_2 concentration, not used by an impaired carboxylation. This limitation was still evident in 2010 REC1 'Barbera' plants (significant, $P < 0.05$ F-test, negative correlation between g_s and c_i), suggesting an incomplete recovery, whereas other REC1 plants appeared to be fully recovered, even if not always significantly (Figs. 3,4e,f). In both cultivars, REC2 plants behaved similarly to healthy plants (Fig. 4g,h).

Chlorophyll fluorescence was measured to evaluate responses caused by FD on the photosynthetic apparatus. As index of thermal dissipation, in figure 5 we correlated the non-photochemical quenching (NPQ) with g_s and c_i. NPQ remained stable at g_s levels above 0.2 mol H_2O $m^{-2}s^{-1}$ and significantly increased after stomatal closure and rise of c_i (FD plants). As expected, in healthy plants we did not observe any change in thermal dissipation rate at the different g_s levels (ranging NPQ between 0.15 and 0.60 mol H_2O m^{-2} s^{-1}). Even the relationship between c_i and NPQ showed only a slight variation of NPQ for healthy plants. On the contrary, in FD-infected 'Barbera' plants we noticed a decrease in F_v/F_m (intrinsic efficiency of PSII) and ΦPSII (quantum yield of PSII) and unaltered F_0 levels (minimal fluorescence in dark-adapted leaves) (data not shown). Moreover, FD plants, when compared with healthy plants, at the same c_i had higher NPQ (Fig. 5b), showing that energy dissipation is higher when carbon cellular assimilation equilibrates CO_2 uptake upon FD infection.

Discussion

Non-stomatal control of photosynthesis during FD infection

We recorded a metabolic, not stomatal, leaf gas exchange limitation in FD-infected and REC1 (symptomless and phytoplasma PCR-negative plants that were FD-infected the year before) grapevines. In these plants a residual sub-stomatal CO_2 concentration caused stomatal closure. Through chlorophyll fluorescence measurements, we showed that as a consequence of stomata closure associated to c_i rise, during FD infection there occurred an increase in thermal dissipation *via* non-photochemical quenching (NPQ).

The cause of stomatal closure in phytoplasma-infected plants is still debated. Some authors demonstrated that in coconut lethal yellowing-infected plants it is independent from abscisic acid concentration in leaves (Leon *et al.*, 1996; Martinez *et al.*, 2000). A proposed mechanism to explain stomata closure involved sucrose accumulation in the apoplast of guard cells (Lu *et al.*, 1997). According to this hypothesis, other authors showed sucrose and starch accumulation in phytoplasma-infected periwinkle leaves (Lepka *et al.*, 1999) and in sieve tubes of grapevine (Musetti *et al.*, 2007).

As previously reported by Leon *et al.* (1996), a decrease in assimilation was improperly associated with a decrease in photosynthetic pigments and protein content. A more detailed analysis on photosynthetic apparatus described the damage of BN phytoplasma in grapevine as non-specific stress-damage similar to senescence or ageing (Bertamini *et al.*, 2002b). Studies on chlorophyll fluorescence in grapevine (Bertamini *et al.*, 2002b; Endeshaw *et al.*, 2012), apple (Bertamini *et al.*, 2002a) and periwinkle (Tan and Whitlow, 2001) phytoplasma-infected plants showed reduced F_v/F_m ratios (intrinsic efficiency of PSII) and quantum yield of PSII (ΦPSII), without increase of F_0 levels (minimal fluorescence in dark-adapted leaves). Furthermore, the repressed activity of carbonic anhydrase (Albertazzi *et al.,* 2009), carboxilation capacity of RuBisCO (Bertamini and Nedunchezhian, 2001; Tan and Whitlow, 2001) and the down-regulation of RuBisCO activase (Hren *et al.*, 2009; Margaria and Palmano, 2011) indicate decrease in regeneration rates of RuBP (ribulose-1,5-biophosphate) with down-regulation of the Calvin cycle.

Commonly, healthy grapevines maintain constant level of c_i around 280 ppm, whilst in the presence of water stress g_s decreases under 0.15 mol H_2O $m^{-2}s^{-1}$. A parallel decrease of c_i suggests that no metabolic hindrance in carbon metabolism is present, as reported by Flexas *et al*. (2002a,b). The close relation between c_i and g_s was discussed by von Caemmerer and Farquhar (1981), and on the base of this relation the role of photosynthesis in stomata control was debated (Jones, 1998; Morison and Gifford, 1983). Our results showed that 'Barbera' and 'Nebbiolo' FD infected plants experience higher c_i rates associated with low g_s levels (below 0.15 mol H_2O $m^{-2}s^{-1}$) (Figs. 3 and 4c,d,e,f). Elevated c_i has been reported by Endeshaw *et al*. (2012) in BN infected grapevine. Probably, the disease slows down the Calvin cycle, causing a hindrance to metabolism that results in a lack of environmental CO_2 demand with a consequent rise of c_i.

It is notable that we observed c_i values even higher than environmental CO_2 concentration, this is probably due to an increase either in respiration or photorespiration. NPQ could be associated to a high photorespiration (Horton *et al.*, 1996) and its enhancement could indicate the need to dissipate excess of light energy in FD infected plants.

Effects of vintage-climate and cultivar on FD infection and recovery

The severe reaction of FD-infected 'Barbera' plants was associated with a significant detrimental effect on E, g_s and A rates, mainly observed in 2010, in absence of water stress. Our results are similar to those reported for symptomatic Chardonnay leaves of Bois Noir-infected plants (Endeshaw *et al.*, 2012). However, in 2009, the FD-infection effect in 'Barbera' was reduced or masked, probably due to a water stress period that had impaired ecophysiological performances of FD, REC1 and above all healthy plants. In healthy controls, as well as in FD plants, drought reduced transpiration and photosynthesis, showing that water stress superimposed on FD infection in terms of stomatal and metabolic non-stomatal limitations to carbon assimilation

(Lovisolo *et al.*, 2010). It is tempting to speculate that upon water stress, a likely reduction of vessel development and/or of their hydraulic conductivity (Lovisolo and Schubert, 1998; Lovisolo *et al.*, 2002), a common grapevine adaptation, hindered FD spread in plants. A reduced interchange of water from xylem and apoplasm to phloem could not counterbalance high concentrations of solute in the phloem, required by the sieve elements to maintain turgor and to continue to function when the plant is under severe water stress (Turgeon, 2010), hindering in turn FD phytoplasma development.

This work showed that the different symptom severity was correlated with ecophysiological parameters. 'Barbera' is known to be more susceptible to FD than 'Nebbiolo'. Moreover, a higher FD phytoplasma titre has been estimated in 'Barbera' compared to 'Nebbiolo' plants (Marzachì *et al.* in preparation). The two cultivars showed a different attitude to recovery from FD. We found that the cultivar with the most severe stress response to FD infection is also the cultivar with the highest recovery attitude. Our recovery rates were similar to those reported by Bellomo *et al*. (2007). We showed that among the 24 plants monitored in 2008 and 2009, 11 'Barbera' plants reached the *status* of REC2, whereas only two 'Nebbiolo' grapevines did so. The achievement of REC2 condition led to a stable remission of symptoms, although this was recorded on a low number of plants. Several REC1 plants of both cultivars showed FD-symptoms the next year, probably due to an incomplete clearance of the phytoplasma from the phloem, even though re-infection could not be excluded.

Musetti *et al*. (2007) suggested that recovery phenomena in grapevine occurs when a long-term accumulation of H_2O_2 in phloem sieve tubes takes place. It would be interesting to compare H_2O_2 accumulation in the phloem of 'Barbera' and 'Nebbiolo', to further explain the different recovery attitudes.

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Tables

Table 1. Population of plants of 'Barbera' and 'Nebbiolo' used for ecophysiological measurements in the two vineyards, and its evolution during the experimental period. Plants are divided in healthy, Flavescence dorée infected FD, recovery REC1, and REC2, according to their belonging to different categories, as detailed in Materials and Methods (* three plants were added in 2010).

Cultivar		2008	2009	2010
'Barbera'	healthy	12	12	12+3*
	FD	12	7	5+3*
	REC1		5	2
	REC2			5
	Tot	24	24	30
'Nebbiolo'	healthy	10	10	10
	FD	10	9	7
	REC1		1	2
	REC2			1
	Tot	20	20	20

Table 2. Schematic representation of recovery during the four experimental years in Cocconato 'Barbera' vineyard. In the first row are the numbers of starting FD infected plants every year. In the boxes along the diagonal (same grey shade), the percent of evolution from FD to REC plants is reported (in brackets it is shown the number of plants that became REC1, REC2 and REC3). Plants that are not recovered fall within the number of FD infected plants of the following year.

'Barbera'	2008	2009	2010	2011
FD	n=13	n=11	n=70	n=72
REC1		61.5 % (n=8)	54.5% (n=6)	51.4% (n=36)
REC2			75 % (n=6)	83.3% (n=5)
REC3				100% (n=6)

559 **Table 3.** Schematic representation of recovery percentage during the four experimental years in
560 Monteu Roero 'Nebbiolo' vineyard. In the first row are the numbers of starting FD infected
561 plants. In the boxes along the diagonal (same grey shade), the percent of evolution from FD to
562 REC plants is reported (in brackets it is shown the number of plants that became REC1, REC2
563 and REC3). Plants that are not recovered fall within the number of FD infected plants of the
564 following year.
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'Nebbiolo'	2008	2009	2010	2011
FD	n=13	n=11	n=13	n=17
REC1		23.1% (n=3)	9% (n=1)	30.7% (n=4)
REC2			33.3% (n=1)	100% (n=1)
REC3				100% (n=1)

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568 **Table 4.** Pearson correlation (positive, +, or negative, -), R^2 values and their corresponding P-
569 values, between E and VPD for each of the treatment groups in both cultivars and years.

E/VPD	2009						2010					
	'Nebbiolo'			'Barbera'			'Nebbiolo'			'Barbera'		
	R^2	P-value	correlation	R^2	P-value	correlation	R^2	P-value	correlation	R^2	P-value	correlation
Healthy	0.81	<0.05	+	0.56	<0.05	-	0.81	<0.05	+	0.60	<0.05	+
FD	0.78	<0.05	+	0.56	<0.05	-	0.78	<0.05	+	0.51	<0.05	+
REC1	0.83	<0.05	+	0.80	<0.05	-	0.66	<0.05	+	0.77	<0.05	+
REC2	/	/	/	/	/	/	0.69	<0.05	+	0.34	n.s.	

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Figure legends

Figure 1

Seasonal time course (2009) of maximum temperature (mT, solid line), midday vapour pressure deficit (mVPD, dotted line), rainfall (solid bars) (**a**,**b**), and comparison between calculated VDP from gas exchange data (E/g_s) (solid line) and environmental mVPD (dotted line) (**c**,**d**). Seasonal time course (2009) of transpiration E (**e**,**f**), stomatal conductance g_s (**g**,**h**) and carbon assimilation A (**i**,**j**), for the three analyzed plant categories: healthy (solid line, diamonds), FD infected on symptomatic leaves (dotted line, triangles) and recovery REC1 (dashed line, squares). Graphs in the first column represent data derived from 'Nebbiolo' (**a**,**c**,**e**,**g**,**i**) while the second column from 'Barbera' (**b**,**d**,**f**,**h**,**j**). Data are the means ± standard error. The numbers of plants which constitute the means are specified in table 1. * Asterisk indicates a significant difference between treatments, $P < 0.05$, F-test.

Figure 2

Parameters referred to 2010 as in figure 1. In addition, in frames e-j, REC2 seasonal time course is displayed (long-dashed line, circles).

Figure 3

Relationship observed in 2009 season between g_s and c_i, in healthy (**a**,**b**), FD infected on symptomatic leaves (**c**,**d**) and REC1 (**e**,**f**) plants. All plots are divided for experimental day. Graphs **a**,**c**,**e** derived from 'Nebbiolo', while graphs **b**, **d**, **f** derived from 'Barbera'. Number of plants observed in all categories is displayed in table 1. At the bottom of the figure the legend representing symbols and dates, respectively, is displayed. (*) Asterisk marks significance of the Pearson correlation between stomatal conductance (g_s) and leaf internal CO_2 concentration (c_i) at $P<0.05$, F-test; n.s. not significant.

Figure 4

Relationship observed in 2010 as in figure 3. In addition, in frames **g** and **h**, REC2 relationship is displayed.

Figure 5

Relationship between non-photochemical quenching (NPQ) and (**a**) stomatal conductance (g_s)or (**b**) internal carbon (c_i) obtained with coupled measurement of gas exchange and chlorophyll fluorescence. Comparison between 'Barbera' healthy and FD infected (FD) plants in the last date of measurement (11/09/2010). (*) Asterisk marks significance of the Pearson correlation between non-photochemical quenching (NPQ) and both stomatal conductance (g_s) and leaf internal CO_2 concentration (c_i) at $P<0.05$, F-test; n.s. not significant.

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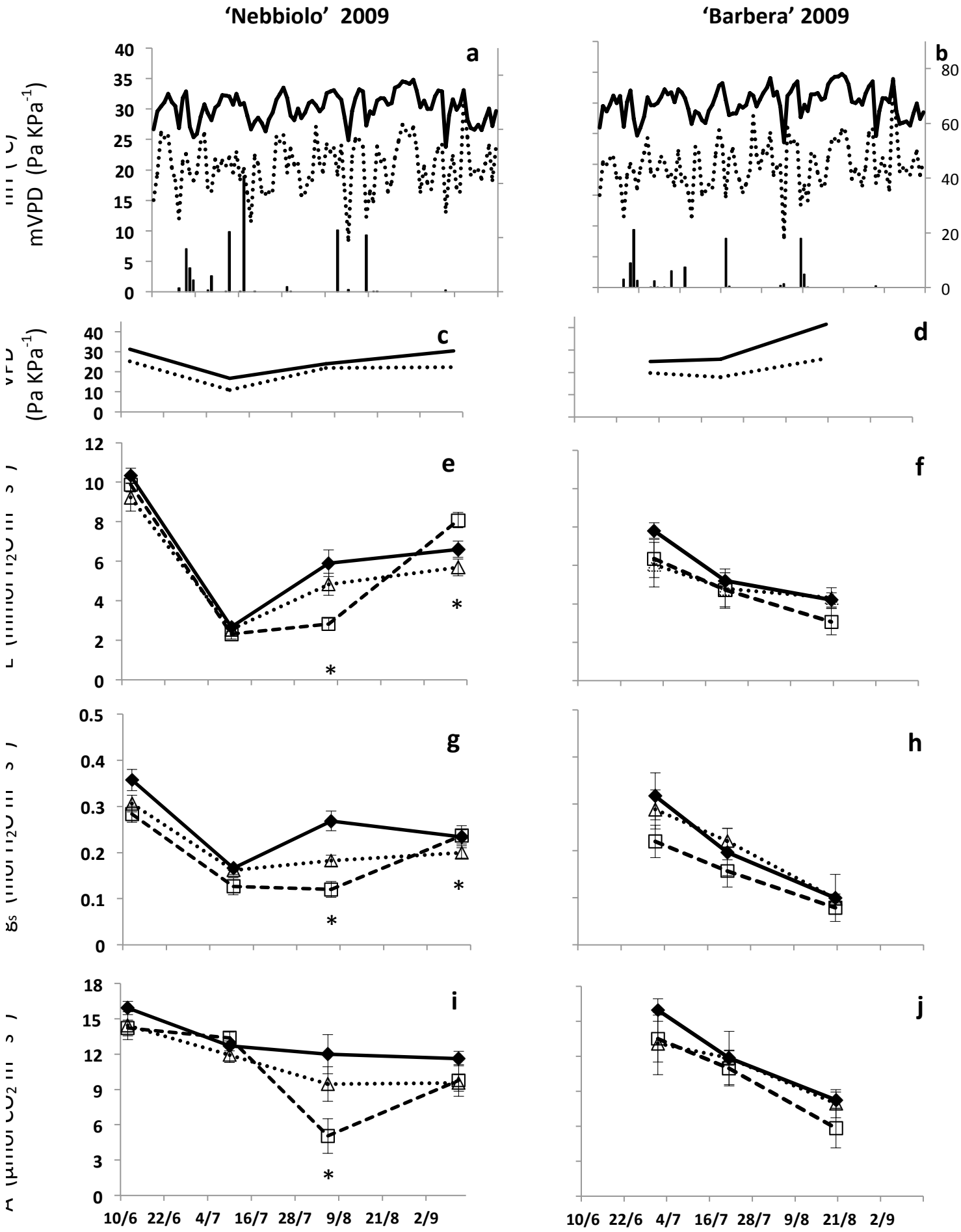
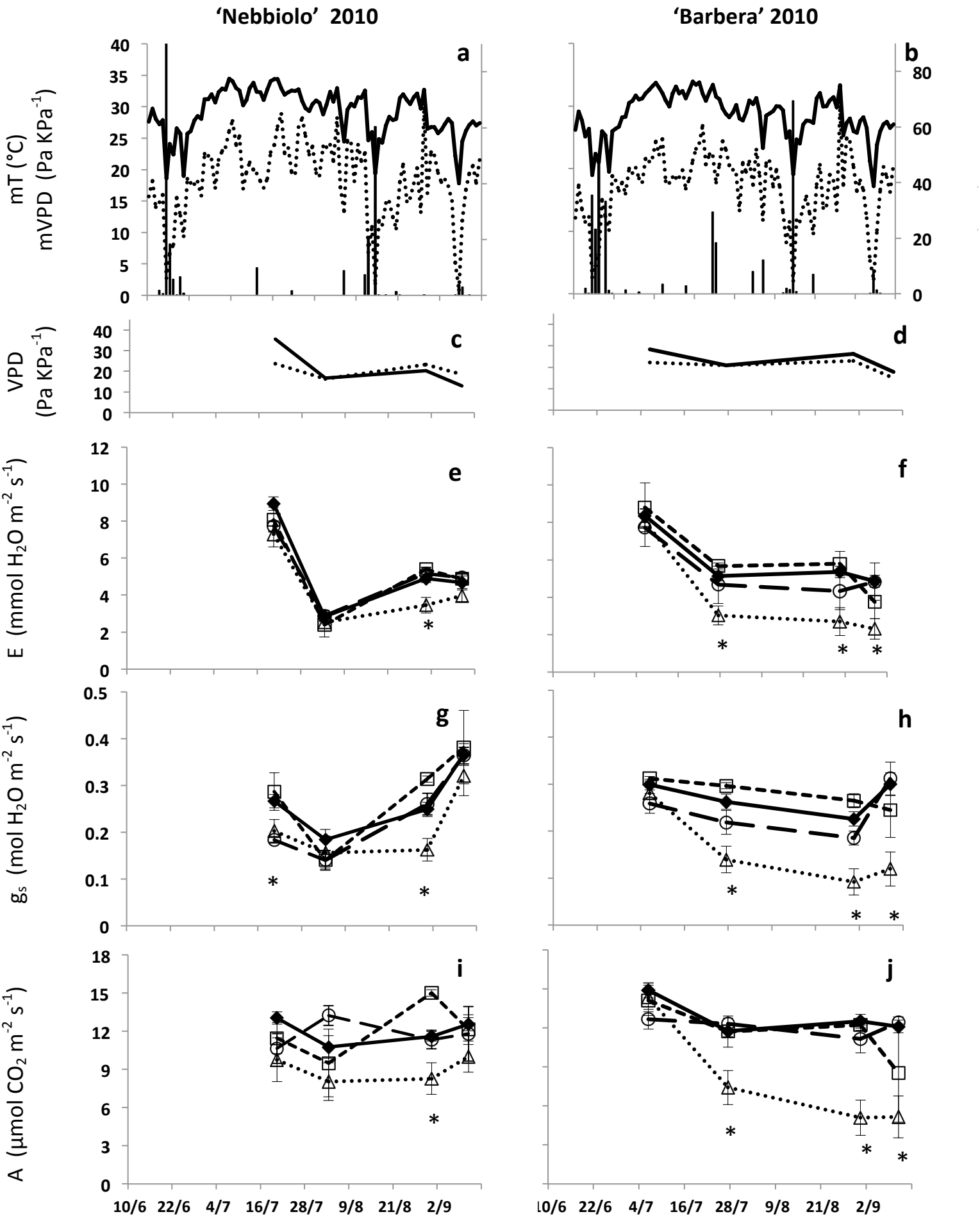
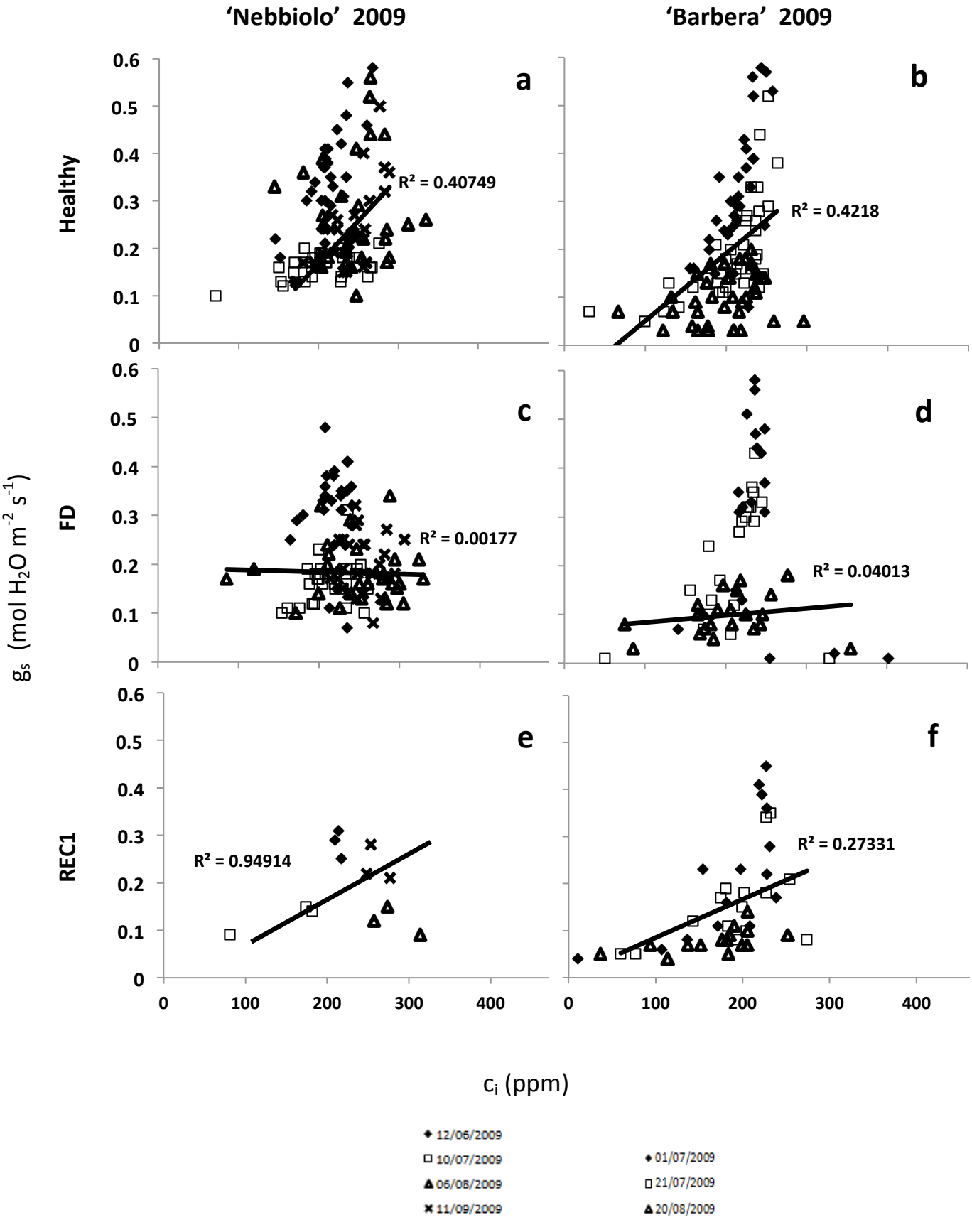


615 Figure 1


'Nebbiolo' 2010
'Barbera' 2010
a
b
c
d
e
f
g
h
i
j
mT (°C)
mVPD (Pa KPa-1)
VPD (Pa KPa-1)
E (mmol H2O m-2 s-1)
gs (mol H2O m-2 s-1)
A (µmol CO2 m-2 s-1)
10/6 22/6 4/7 16/7 28/7 9/8 21/8 2/9

617 Figure 2



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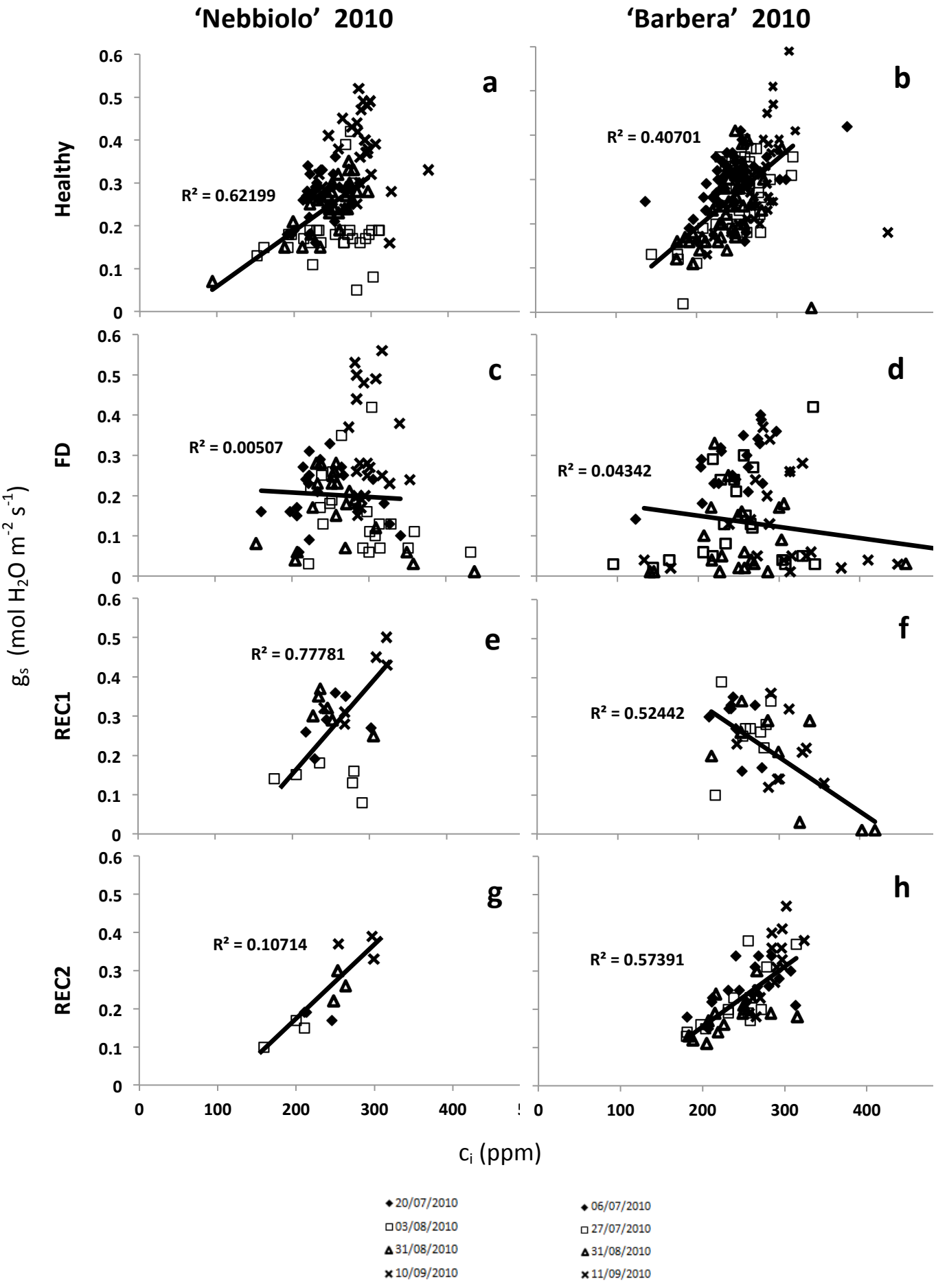
620 Figure 3



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622 Figure 4

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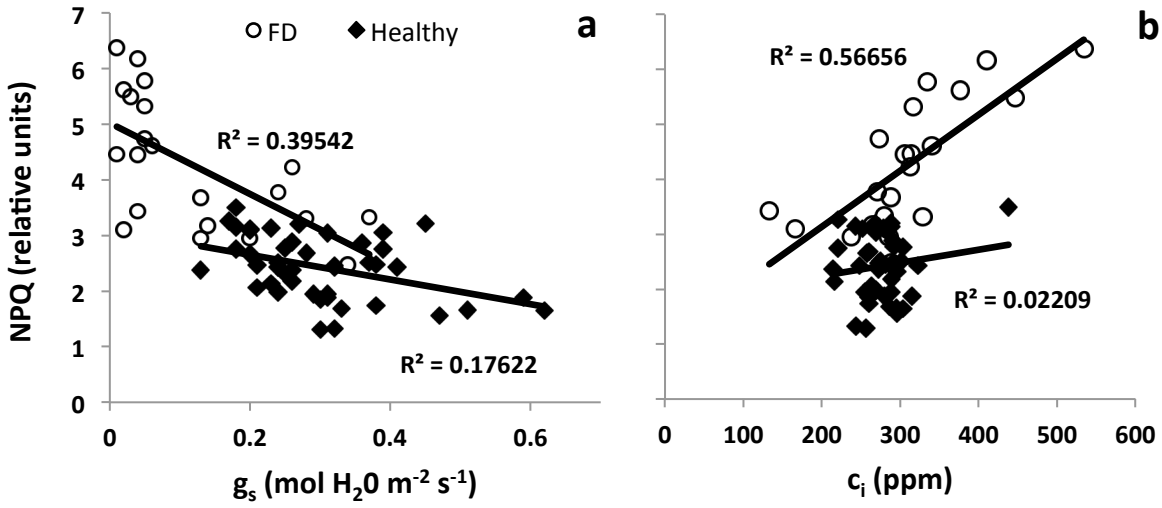

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628 Figure 5

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