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Interactions between bois noir and the esca disease complex in a Chardonnay vineyard in Italy

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Summary. Grapevine yellows bois noir (BN) and the grapevine trunk disease esca complex (EC) cause serious yield losses in European vineyards and are often widespread in the same vineyard. In a Chardonnay vineyard in north-eastern Italy, evolution of the two diseases from 2007 to 2020 was compared and their possible interaction was investigated. Evolution of symptomatic grapevines over the 16 years was very different between the two diseases, with a substantial linear increase for BN and an exponential increase for EC. The BN increase from one year to another was associated with the abundance of *Hyalesthes obsoletus*, the BN-phytoplasma vector, whereas the exponential increase in EC was likely due to the amount of inoculum and the increased size of pruning cuts over time. The courses of the two diseases were also very different, with a much greater occurrence of dead grapevines from EC than from BN. Some grapevines showed symptoms of both diseases, but the probability was less that a grapevine symptomatic for BN or EC showed symptoms of the other disease. Examinations of the spatial distribution of the two diseases showed dissociation between them. Data indicated that mechanisms of induced defense were involved in the lower probability that a grapevine affected by one showed symptoms of the other.

Keywords. Grapevine yellows, phytoplasmas, grapevine trunk diseases, symptom evolution, induced defense.

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INTRODUCTION

Grapevine age positively influences yields, rooting depths, plant resilience and wine quality (Riffle *et al.*, 2022), so factors that influence grapevine longevity can compromise the economic sustainability of vineyards. In European vineyards, yield losses, often associated with early grapevine death, are caused by grapevine yellows (GYs) [i.e., flavescence dorèe (FD) and bois noir (BN)] (Garau *et al.*, 2007; Belli *et al.*, 2010; Pavan *et al.*, 2012; Romanazzi *et al.*, 2013), and trunk diseases [esca complex (EC), in particular)] (Larignon and Dubos, 1997; Mugnai *et al.*, 1999; Bertsch *et al.*, 2013; Bruez *et al.*, 2013; De la Fuente *et al.*, 2016).

GYs are associated with phytoplasmas that colonize phloem vessels (Santi *et al*., 2013; Angelini *et al*., 2018), and are vectored by auchenorrhyncha phloem-feeding insects (Alma *et al*., 2019). Bois noir (BN), unlike FD, is widespread in all Euro-Mediterranean grape-growing regions (Angelini *et al*., 2018). BN is associated with '*Candidatus* Phytoplasma (*Ca*. P.) solani' (subgroup 16SrXII-A) (Quaglino *et al*., 2013; Angelini *et al*., 2018), and the most important vector of this pathogen is the planthopper *Hyalesthes obsoletus* Signoret (*Homoptera, Cixiidae*) (Maixner, 1994; Alma *et al*., 2019; Kosovac *et al*., 2019).

Different susceptibilities and sensitivities of grapevine cultivars to BN have been reported (Bellomo *et al*., 2007; Garau *et al*., 2007; Panassiti *et al*., 2015; Zahavi *et al*., 2013). After symptom appearance, the course of the disease in BN-symptomatic grapevines during subsequent vegetative seasons can lead to recovery or death of the infected grapevines (Osler *et al*., 1993, 2002; Garau *et al*., 2007; Pavan *et al*., 2012; Zahavi *et al*., 2013). In general, recovery from GYs involves phytoplasma-induced grapevine response mechanisms (Musetti *et al*., 2007; Albertazzi *et al*., 2009; Romanazzi *et al*., 2009; Landi and Romanazzi, 2011; Margaria and Palmano, 2011; Santi *et al*., 2013; Paolacci *et al*., 2017; Bertazzon *et al*., 2019; Pacifico *et al*., 2019; Mátai *et al*., 2020; Pagliarani *et al*., 2020; Nutricati *et al*., 2023). Furthermore, fungal and bacterial endophytes have been suggested to affect grapevine recovery from GYs (Martini *et al*., 2009; Bulgari *et al*., 2016).

Grapevine trunk diseases (GTDs) are caused by fungi that penetrate host plants through wood wounds and invade the vascular systems (Bertsch *et al*., 2013; Bruez *et al*., 2013; Claverie *et al*., 2020). Esca complex (EC) is the most widespread GTD, and is characterised by inner necrosis in grapevine wood tissues and external plant symptoms ("tiger-striped" leaves or "black measles" on the berries) (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Graniti *et al*., 2000; Úrbez-Torres, 2011; Mondello *et al*., 2018; Fischer and Peighami-Asnaei, 2019; Nerva *et al*., 2019; Bruez *et al*., 2020). When grapevines show typical leaf symptoms, the EC is defined as grapevine leaf stripe disease (GLSD), whereas when grapevines dry out and die, the EC shows apoplectic symptoms (Surico *et al*., 2006; Surico, 2009). Grapevines can produce defensive compounds in response to wood invasion by esca-associated fungi (Ramírez-Suero *et al*., 2014; Stempien *et al*., 2018; Goufo *et al*., 2019). Activity of non-pathogenic fungi (e.g., *Epicoccum* spp., *Pythium oligandrum*, *Trichoderma* spp.) or bacteria [e.g., *Bacillus pumilus* (S32), *Paenibacillus* sp. (S19)] in preventing EC fungal infections has been reported, and different mechanisms were considered, including production of inhibitory compounds, competition for nutrients and space, triggering of grapevine resistance, and interference with pathogenicity genes of esca-associated fungi (Haidar *et al*., 2016; Del Frari *et al*., 2019; Bigot *et al*., 2020; Yacoub *et al*., 2020; Romeo- Oliván *et al*., 2022). Susceptibility to EC can be influenced by *V. vinifera* cultivar, rootstock genotype, and cultivar × rootstock combination (Fischer and Peighami-Ashnaei, 2019). Chardonnay was classified as intermediate in EC susceptibility (Borgo *et al*., 2016).

No studies have been reported on comparative evolution of GYs and EC within the same vineyard, or the possible interaction between the GYs and EC. For this purpose, grapevines in a large Chardonnay vineyard in north-eastern Italy were annually monitored from 2007 to 2020. This allowed comparison of the evolution of the two diseases at vineyard level, and determination of whether the two diseases can coexist in individual host plants. This also would give knowledge of the degree of interaction (none, synergistic, or antagonistic) between the two in manifestation of host symptoms.

MATERIALS AND METHODS

Vineyard studied

The study was conducted from 2007 to 2020, in a vineyard in north-eastern Italy (Friuli Venezia Giulia region, Gorizia district, Cormons locality; 45°56'34"N, 13°26'45"E, 44 m a.s.l.). The vineyard was planted in 2000, with approx. 3.5 ha of Chardonnay R8 clone grafted onto SO4. The vineyard had 21 rows of length 370 m (westernmost row) to 408 m (easternmost row). The grapevines were trained to the double-arched Guyot system, and were at spacing of 3.5 m between rows and 1.0 m within rows.

In the vineyard, the only GY detected up to 2022 was BN. All the 128 GY symptomatic samples randomly collected and analysed by qPCR/HRM from 2010 to 2021 tested positive for '*Ca*. P. solani' and negative for FD phytoplasma (Mori *et al*., 2020; Pavan *et al*., 2024; Martini *et al*., unpublished data). No symptoms of virus or soil-borne diseases were observed in the vineyard.

During the years of the study, FD had not been reported in the vineyards of the wine-growing area where the vineyard was located, but mandatory insecticide treatments against *Scaphoideus titanus* were applied each year, at the beginning of July. Since the active ingredients used were not basipetal systemic insecticides (organophosphate in the early years, thiamethoxam more recently), the insecticides would not have affected *H. obsoletus* nymphs living in the roots of herbaceous plants. Even against *H. obsoletus* adults, the efficacy of insecticides is not high, as

the insects emerge gradually and can colonize vineyards, even from outside. Only mechanical weeding was used in the vineyard. After winter pruning, no fungicide treatments were applied to protect the pruning cuts.

Sampling and data analyses

Mapping of the vineyard

In 2007, the vineyard rows and plants were mapped, by referring each grapevine to a row (numbered 1 to 21, starting from the west), a supporting pole along the row (numbered from the north end of each row), and a position (designated a, b, c, or d) between neighbouring poles. From 2007 to 2020, each grapevine was checked each year in early September, by the same observers every year, to determine which plants showed GY or EC symptoms. Replacement of dead grapevines occurred in only a few rows in 2019, but newly planted grapevines were not considered in this study.

Grapevines with BN symptoms were identified based on their characteristic GY symptoms, including leaf rolling, sectorial leaf blade discolourations, poorly lignified and falling shoots, and shriveled berries. Grapevines with the GLSD form of EC were identified based on tiger-striped leaves or black measles on the berries. Grapevines with the apoplexy form of EC were identified based on necrosis and shoots without leaves. Since these symptoms were visible both in grapevines that had or had not previously manifested GLSD symptoms, death of these plants was attributed to EC, even when the GLSD form of EC had not previously manifested. The grapevines that died from BN could not be confused with those that died from EC, as BN-affected plants had green shoots in the previous year, which only in the following year were necrotic due to frost damage, as they were not lignified.

Symptom evolution in BN and EC symptomatic grapevines

Annual records were made to check whether grapevines were affected by BN, and if the symptoms were noted for the first time. In subsequent sampling years, these grapevines were assigned to three categories: "still symptomatic", "asymptomatic", or "dead". Grapevines that became asymptomatic were recorded as "recovered" only after three consecutive years without symptoms, and taking into account that pollarding can mask GY symptoms for some years (Pavan *et al.*, 1997; Mutton *et al.*, 2002). Grapevines that did not have lignified shoots at the time of sampling, or that did not sprout in the following year, were considered dead. The annual sampling of grapevines affected by BN allowed recording of the evolution of symptoms for individual grapevines, from the year of the first symptom appearance (year = 0) to year n (maximum n = 13 for grapevines that showed symptoms in the first sampling year).

For each grapevine exhibiting EC symptoms, the following details were noted: (i) the year in which symptoms first appeared, and whether they were in the form of GLSD or apoplexy, and (ii) the year in which apoplexy occurred for grapevines that had previously exhibited GLSD symptoms. To determine when the grapevines affected by EC showed apoplexy (0, or more years), only grapevines during the period 2015 to 2020 were considered, which enabled certainty for exclusion of grapevines that could have presented symptoms of GLSD in the years preceding the start of mapping.

Interactions between BN- and EC-affected grapevines

For comparison of symptom evolution of BN and EC with time, and disease interactions, the grapevines were grouped into those displaying symptoms of only BN or EC, or of both diseases. For both diseases, the following parameters were calculated: (i) Proportions (%) of dead grapevines to total live grapevines in 2007; (ii) Proportions (%) of dead grapevines to total symptomatic grapevines; (iii) Accumulated numbers of dead grapevines; (iv) Accumulated numbers of total symptomatic grapevines (symptomatic live plus dead grapevines). Grapevines with symptoms of both diseases over the sampling years were also divided into three groups: (1) grapevines that showed EC symptoms for the first time in years following their first year of exhibiting BN symptoms; (2) grapevines showing EC symptoms for the first time one or more years after recovering from BN; or (3) grapevines that showed BN symptoms for the first time in the years following their first year of exhibiting EC symptoms.

Fisher's exact test was used to compare two proportions in each dataset, while Rayan's test was used to compare three proportions. Dynamics of BN or EC dead grapevines over the years were determined using polynomial regressions of best fit to the experimental data for the two diseases.

Comparisons of spatial distribution of BN- and EC-symptomatic grapevines

SADIE red-blue analysis (Perry *et al.*, 1999) was used to determine spatial distributions of cumula-

tive BN- and EC-symptomatic grapevines within the vineyard. The sampling units were groups of 16 grapevines (four inter-poles), and the total number of grapevines with symptoms of either or both diseases in each year was determined for each 16 plant unit. This analysis identified areas with high-density counts (disease patches) or low or zero counts (gaps), and calculated indices of clustering (vi; vj) for each unit, which measured local contribution to either patch or gap. The clustering significance ($\alpha = 0.05$) for each variable was determined by comparing the mean values of vi and vj with their corresponding values under the null hypothesis (Perry *et al.*, 1999).

A two-dimensional map depicting the spatial distribution of local clustering indices (vi; vj) for each variable was generated using linear kriging with SURFER (Golden Software 191 Inc.). The red-blue analysis datasets were then used to assess similarity between spatial patterns of BN-symptomatic grapevines and EC-symptomatic grapevines. An algorithm was used to derive overall indices of spatial association (X_k), and their statistical significance (P_x) was determined through a randomisation test (Perry and Dixon, 2002). This test determined if the spatial patterns of two variables were associated ($P_x < 0.025$), unassociated ($0.025 \leq P_x \leq 0.975$), or dissociated ($P_x > 0.975$), with association indicating the coincidence of a patch cluster for one variable with a patch cluster for the other, or the coincidence of two gaps, and dissociation indicating that a patch for one variable coincides with a gap for the other (Perry, 1998).

RESULTS

Evolution of grapevines affected by BN

In the first sampling year (2007), 199 grapevines had symptoms of BN, and cumulative numbers of grapevines with these symptoms rose to 831 in the last sampling year (2020). The status of most of the grapevines affected by BN changed to "recovered" or "dead" in the years following the first onset of symptoms (Figure 1). A substantial proportion of the grapevines (11%) showed symptoms for at least 13 years, highlighting that the infection can last for many years. Probability of recovery of a symptomatic grapevine was greater in the first few years than later, as more than half of recovered plants were recorded within the following 3 years. Likelihood of a symptomatic grapevine dying was also greater in the first few years than later, as more than two-thirds of symptomatic plants died within the first 5 years after first symptom observation.

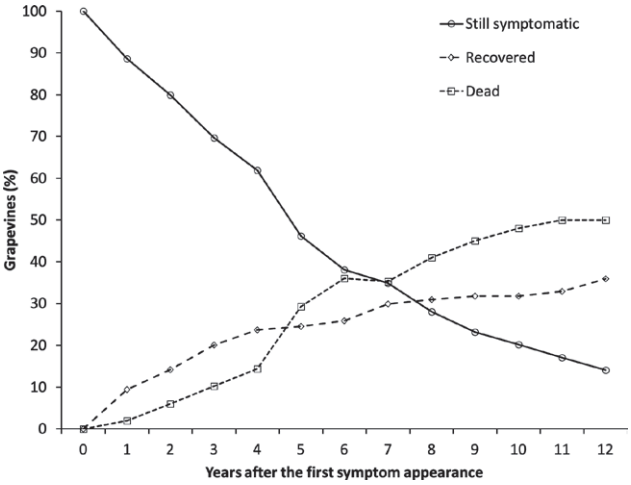

Figure 1. Proportions of grapevines that from 1 to 13 years after first appearance of BN symptoms (year 0) either remained symptomatic, recovered, or died.

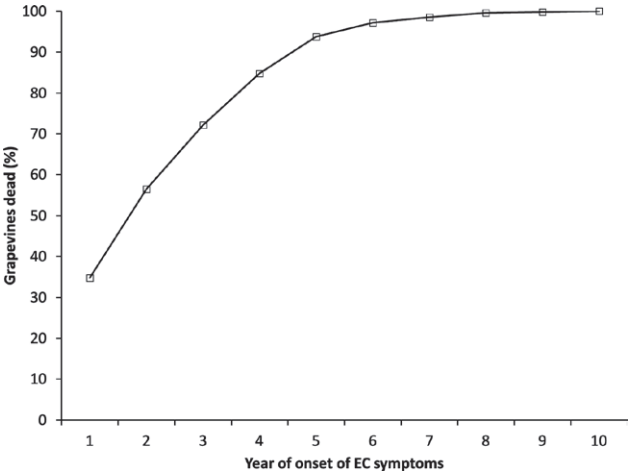

Figure 2. Proportions of dead grapevines during the first 10 years following the occurrence of EC symptoms. Grapevines that were dead at year = 1 exhibited the apoplexy form of EC without first showing the GLSD form.

Evolution of grapevines affected by EC

From 2015 to 2020, 1126 grapevines died for apoplexy. Apoplexy was observed in a third of the grapevines without them having previously shown GLSD symptoms (Figure 2). Over 95% of the grapevines with GLSD symptoms exhibited apoplexy within 5 years. Three grapevines died 9 years after first showing GLSD symptoms, and two grapevines did not develop apoplexy more than 9 years (10 and 12, respectively) after the first appearance of GLSD symptoms in 2008, which increased initially very slowly.

Table 1. Mean percentages of grapevines dead from 2008 to 2020 after showing symptoms of BN or EC, or both diseases, calculated on the total number of live vines in 2007 and on the number of symptomatic vines. Means in each column accompanied by the same letter are not different ($P > 0.05$).

Symptoms	Dead grapevines (%)	
	Calculated on total live in 2007	Calculated on symptomatic
BN exclusively	2.96 b	27.55 a
EC exclusively	24.45 c	86.26 c
Both BN and EC	0.74 a	69.51 b

Comparisons of BN and EC evolution

From 2008 to 2020, 2236 grapevines died (29.2% of the live grapevines in 2007), 1952 of which had EC symptoms, 227 had BN symptoms, and 57 had symptoms of both diseases. The proportion of dead grapevines from EC was eight times greater ($P \leq 0.05$) than from BN (Table 1). Approx. 1% of the grapevines died after showing symptoms of both diseases. Grapevine deaths from EC started 1 year after the BN deaths, but accumulated death numbers subsequently increased more rapidly, as the ratios of EC deaths to BN deaths rose from 2.0 in 2009 to 8.6 in 2020 (Figure 3 A). The trend towards increases in dead grapevines over time was sinusoidal for BN ($Y = -0.2486X^3 + 1502X^2 - 3E + 06X + 2E+09$; $R^2 = 0.9947$), and more than proportional for EC ($Y = 13.41X^2 - 53854X + 5E + 07$; $R^2 = 0.9968$).

Considering the total number of symptomatic grapevines (i.e. "symptomatic alive" plus "dead"), the differences between BN and EC were less marked (Figure 3 B). From 2007 to 2020, 2262 grapevines exhibited symptoms of EC, 831 of BN, and 82 of both diseases. The number of accumulated symptomatic grapevines was greater for BN up to 2013 and greater for EC in the subsequent years.

The EC / BN ratio of the total number of affected grapevines (2.74) was three times smaller than that of the dead grapevines (8.60), due to the different mortality rates between the EC and BN. The percentage of "dead" grapevines relative to the total of "symptomatic" grapevines was greater ($P \leq 0.05$) for EC than for BN, with EC being three times greater than BN (Table 1). There was also a smaller proportion of grapevines that died with symptoms of both diseases than grapevines dying only with EC symptoms ($P \leq 0.05$).

Interactions between BN and EC

The percentage of grapevines displaying EC symptoms was less among grapevines that had exhibited BN

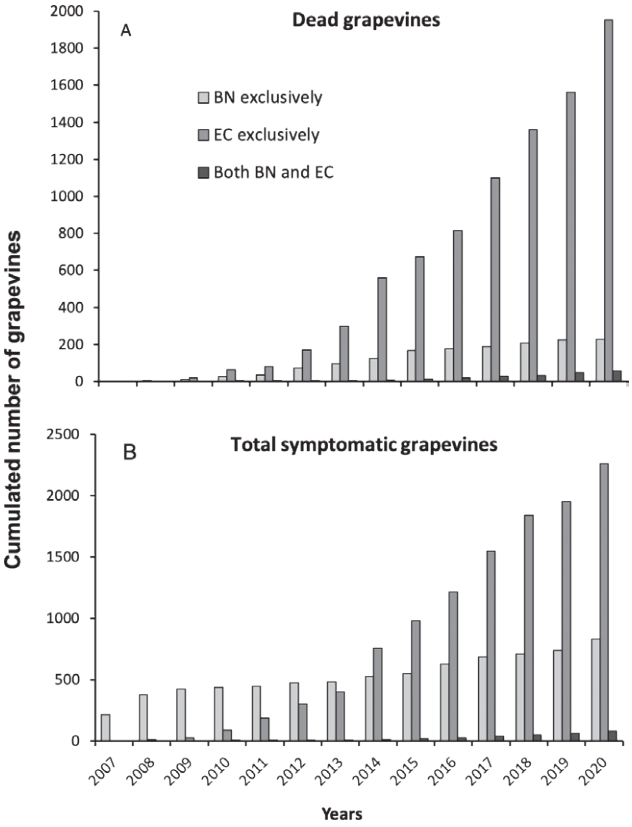

Figure 3. Accumulated numbers of dead and total symptomatic grapevines ("symptomatic alive" and "dead") due to EC, BN or both diseases during the years 2007 to 2020.

symptoms in previous years than among those that had never shown BN symptoms (Figure 4 A). Within the BN-symptomatic grapevines, the recovered grapevines showed greater proportions with EC symptoms than the non-recovered grapevines (Figure 4 B), but were still less likely to show EC symptoms than grapevines that never showed BN symptoms (Figure 4, A and B).

The percentage of grapevines succumbing to EC was less in those that had previously exhibited symptoms of BN than in those that had never shown BN symptoms (Figure 4 C). The proportion of dead grapevines among symptomatic ones (86.3%) was greater ($P = 0.017$; Fisher's exact test) for those without previous BN symptoms than for those with prior BN symptoms (76.0%). Within the BN-symptomatic grapevines, the recovered grapevines died from EC at a greater proportion than the non-recovered grapevines (Figure 4 D), but they still died at lower percentage ($P \leq 0.05$) than grapevines that had never exhibited BN symptoms (Figure 4, C and D).

Among grapevines that displayed GLSD symptoms, only seven plants (2.3%) later had symptoms of BN, and this contrasted with grapevines that had never exhibited

GLSD symptoms, which had a greater rate (13.8%) of BN symptoms ($P < 0.00001$; Fisher's exact test).

Spatial distribution of BN- and EC-symptomatic grapevines

The distribution of BN-symptomatic grapevines was aggregated. The most important patches were found in the first 100 m from the northern edge of the vineyard (vi = 6.365, $P_{vi} < 0.001$), while gaps occurred in the remaining vineyard area and predominantly in the south-western part of the vineyard (vj = - 5.691; $P_{vj} < 0.001$) (Figure 5 A).

The distribution of EC-symptomatic grapevines was aggregated. The most significant patches occurred along the eastern edge of the vineyard, between the central and southern parts (vi = 5.08; $P_{vi} < 0.001$), while gaps occurred along the western vineyard edge in the first 100 m from the northern edge (vj = -5.312, $P_{vj} < 0.001$) (Figure 5 B).

The distributions of BN- and EC-symptomatic grapevines were dissociated, with the areas of greatest dissociation coinciding with the major patches of BN- and EC-symptomatic grapevines (X_k = - 0.25; $P_x > 0.999$) (Figure 8 C).

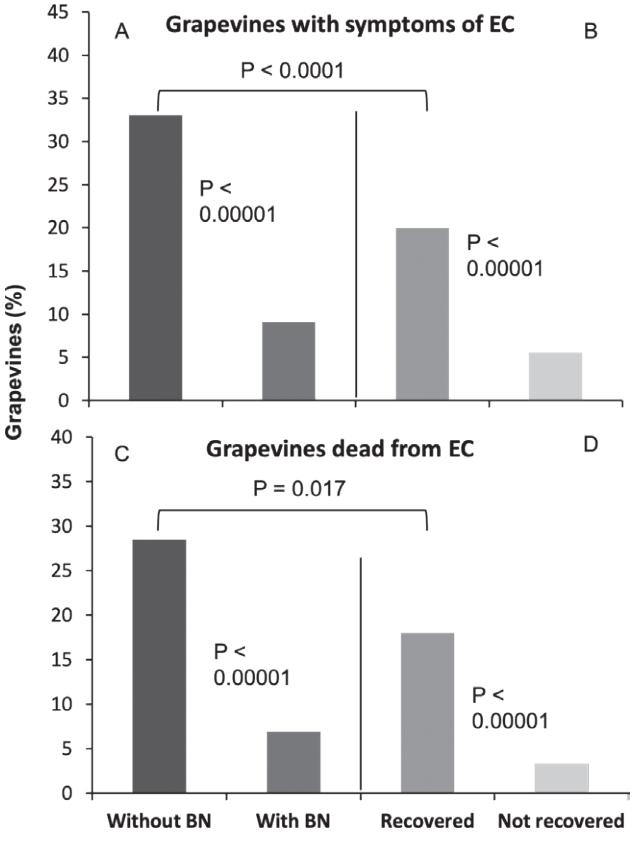

Figure 4. Mean proportions (%) of grapevine plants with symptoms of EC, and among these, percentages of dead vines that never showed BN symptoms (without BN; 4 A), or had previously shown symptoms of BN (with BN) (4 C). Among the BN symptomatic vines, the percentages of those "Recovered" or "Not recovered" (i.e., still symptomatic; 4 B) at the occurrence of EC symptoms, or dead from EC (4 D), are indicated.

DISCUSSION

This 14-year-long descriptive epidemiology study was carried out in a large vineyard, and aimed to investigate potential synergistic or antagonistic interactions between two important grapevine diseases. The selected vineyard was in a wine-growing area where the exclusive presence of BN among GY had been reported until the last study year (2020), and also during the monitoring survey conducted in 2021 and 2022 seasons. This made it possible to obtain descriptive results, as discussed below.

Evolution of grapevines affected by BN

In the vineyard studied, 50% of the grapevines died, and 10% still showed BN symptoms 13 years after first detection of these symptoms. This evolution was less favourable than that observed for the same cultivar (Chardonnay) in a multi-year study (1987–2000) conducted in ten vineyards in another wine-growing area of north-eastern Italy (Friuli Venezia Giulia region, Pordenone district: Pavan *et al.*, 2012). In that study: (i) only 10% of the affected grapevines had died after 9 years (45% in the present study), and only 1.1% of grapevines showed symptoms after 13 years (11% after 13 years in the present study). These differences could be due to differing virulence of the phytoplasma strains (Langer and Maixner, 2004; Pierro *et al.*, 2022), or to differing host susceptibility. Differences in grapevine susceptibility could have been due to differences in clone-rootstock combinations and/or grapevine training systems. In the study of Pavan *et al.* (2012), many clone-rootstock combinations were assessed, including some polyclonal vineyards, and the grapevine training system was Sylvoz. In the present study, the vineyard was monoclonal, and the training system was double-arched Guyot. In the Pavan *et al.* (2012) study, there were three to five canes originating from points of each permanent horizontal trunk, the canes were distant from each other, and the symptoms only rarely seriously affected all the canes. The affected plants also almost always had some lignified shoots. In the present study, each grapevine had two canes originating from the trunk heads, and

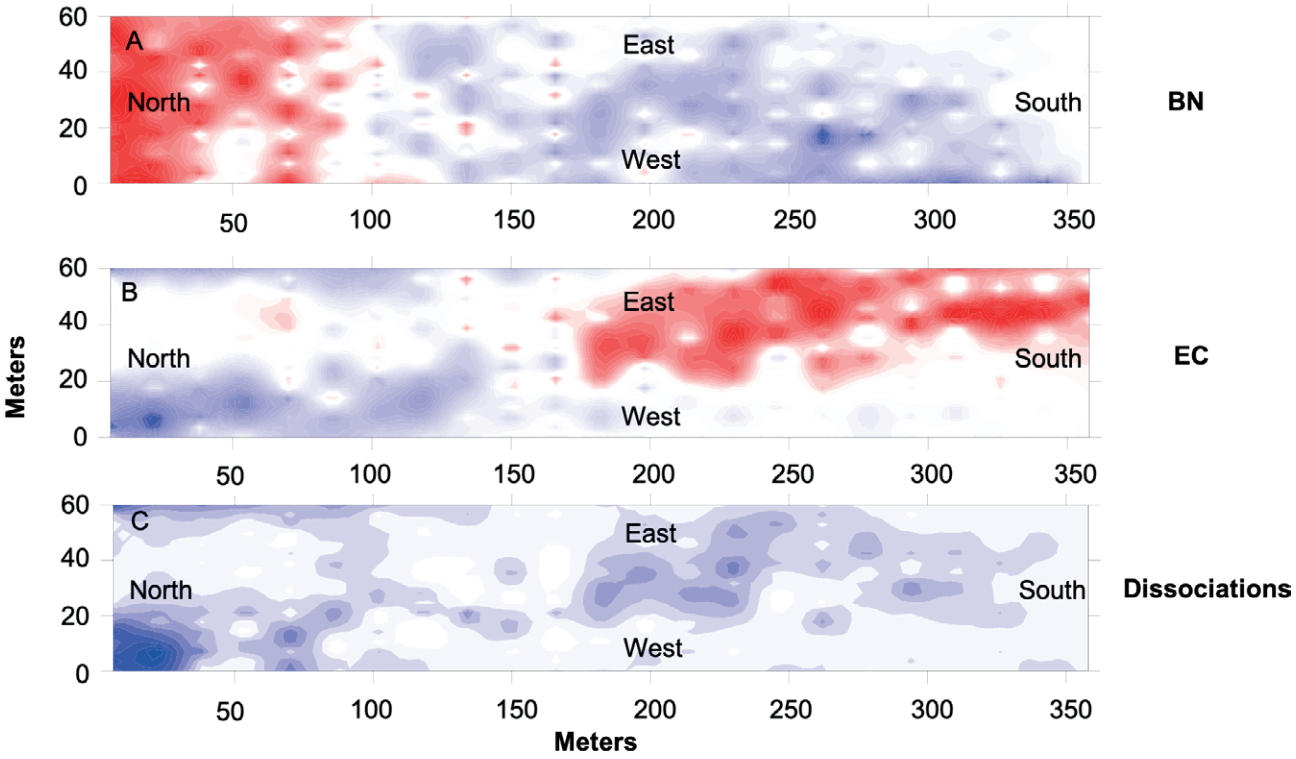

Figure 5. Spatial distributions of BN and EC symptomatic grapevines (cumulative numbers over sampling years from 2007 to 2020). Red areas indicate aggregation (patches: vi ≥ 1.5), and blue areas indicate dispersion (gaps: vj ≤ -1.5). The map of Dissociations has blue areas indicating areas with statistically significant dissociations between the two diseases.

the symptoms often affected both canes. As there were no lignified shoots, these were probably killed by winter frosts, so the grapevines did not sprout in the following springs.

Comparisons of BN and EC evolution

The appearance of BN before EC is explained by the different epidemiology of the two diseases. For BN, the likelihood that a healthy grapevine may become infected does not depend on the vineyard age, but depends on the number of infectious vectors that can inoculate grapevines with BN phytoplasmas, regardless of grapevine age. In contrast, the likelihood that grapevines are affected by trunk diseases increases with vineyard age, because the overall numbers of pruning cuts increase with time, favouring fungal infections (Mugnai *et al.*, 1999; Amponsah *et al.*, 2011; Kovacs *et al.*, 2017). With increasing vineyard age and EC progression, pathogen inoculum related to EC also increases with time, increasing infection probability. Although EC appeared after BN in the studied vineyard, the number of grapevines affected by EC at the end of the survey years was three times greater than for BN. This occurred because the increase in BN over study period was substantially linear, with some fluctuations. In contrast, the increase in EC was more than proportional. This difference between the two disease syndromes could be due to BN inoculum not originating from the grapevines, which are the final hosts of the phytoplasma (Maixner, 2010; Alma *et al.*, 2019). For EC, the infected grapevines are the inoculum sources. For BN, primary inoculum arrives from other host plants via *H. obsoletus*, so the pathogen inoculum is likely to be relatively stable, which may explain the low variability in increase with time. In contrast, EC inoculum derives from infected grapevines whose numbers increase with time. This explains the greater than proportional increase in EC-affected grapevines. However, some annual variation in rate of increase also occurs for BN, because the number of infectious vectors can vary relative to the number of host plants that are sources of BN phytoplasma, and relative to the population density of the vector *H. obsoletus* (Mori *et al.*, 2008; 2012; 2020; Panassiti *et al.*, 2015).

In the present study, EC was more harmful to the grapevines than BN, as indicated by the greater number of symptomatic grapevines and the greater incidence of dead grapevines among those infected.

Interactions between BN and EC

Grapevines previously affected by BN were less likely to show symptoms of EC and die from EC than grapevines without BN. However, the probability that grapevines exhibited symptoms of EC was greater for BN-recovered grapevines than for those still symptomatic, although the likelihood of EC symptoms was always less than for grapevines that had never shown BN symptoms. These two data sets could suggest that in BN-infected grapevines, there are specific defense mechanisms induced by infections that prevent EC infections or symptom expression, and that these mechanisms are less present in BN-recovered grapevines (therefore no longer symptomatic) than in those that have not recovered (therefore still symptomatic) but have active defense mechanisms.

It is known that induced defense in host plants confers resistance, not only towards the pathogen that activated the resistance mechanisms, but also against other pathogens (Sticher *et al*., 1997; Durrant and Dong, 2004; Vlot *et al*., 2021; Cooper and Ton, 2022). Activation of defense mechanisms is well documented for GYs. Recent studies have shown that concentrations of defense compounds (phenolic substances), including stilbenoids, flavonols and flavanols, are increased in the presence of BN or FD infections (Rusjan *et al*., 2015; Pagliarani *et al*., 2020; Casarin *et al*., 2023). Other studies have reported that after infection of grapevines with '*Ca*. P. solani', most jasmonic acid (JA) and salicylic acid (SA) biosynthetic genes are up-regulated, as compared to uninfected plants (Paolacci *et al*., 2017; Rotter *et al*., 2018; Dermastia, 2019). In stably recovered plants, increased levels of endogenous H_2O_2 and increased concentrations of specific stilbenoids, including viniferin, have been demonstrated (Musetti *et al*., 2007; Gambino *et al*., 2013; Pagliarani *et al*., 2020). Activation of defense genes is also linked to JA-dependent signalling, and suppression of SA-dependent signalling is important for establishment and maintenance of host recovery (Paolacci *et al*., 2017; Pagliarani *et al*., 2020). The role of mechanisms of induced resistance in recovered grapevines has been indirectly confirmed by the efficacy of resistance inducers in improving the recovery of BN-affected grapevines (Romanazzi *et al*., 2009).

EC infections also significantly reduce the likelihood of BN infections. In this case, it would be the EC infections that induce traits in grapevines that act as resistance factors against phytoplasmas. Several reports have indicated involvement of upregulations of the phenylalanine ammonia-lyase (PAL) gene, which encodes the first enzyme of the phenylpropanoid pathway, along with the chalcone synthase (CHS) and stilbene synthase (STS) genes, coding for enzymes of the flavonoid and stilbenoid pathways (Kenfaoui *et al*., 2022; Garcia *et al*., 2022). Recent data also suggested a JA-dependent signalling mechanism activated after invasion of wood by EC-associated fungi, that induces accumulation of secondary metabolites such as phytoalexins and pathogenesis-related proteins (PRs) (Goufo *et al*., 2020). EC-symptomatic plants are also unattractive for the vector *H. obsoletus*, as happened in grapevines treated with a chemical elicitor emitting volatiles that repelled adult planthoppers (Minuz *et al*., 2020).

Grapevines previously symptomatic for BN were less likely to show EC symptoms, and non-BN symptomatic plants were more likely than BN symptomatic plants to develop EC symptoms, probably influenced the spatial distribution of grapevines with symptoms of each disease. This might explain why where there are patches of one disease there are gaps of the other, and *vice versa*. The present study has therefore highlighted that spatial distribution of a disease within a crop can be influenced by the presence of a separate pathogen.

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