Molecular and spatial analyses reveal new insights on Bois noir epidemiology in Franciacorta vineyards

Fabio Quaglino [1], Alessandro Passera[1], Monica Faccincani[2], Abdelhameed Moussa[1], Alberto Pozzebon[3], Francesco Sanna[3], Paola Casati[3], Piero Attilio Bianco[3], Nicola Mori[4]

[1]Department of Agricultural and Environmental Sciences – Production, Landscape, Agroenergy, University of Milan, via Celoria 2, 20133, Milan, Italy

[2]The Consortium for the protection of Franciacorta, via G. Verdi 53, 25030, Erbusco, BS, Italy

[3]Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padua, Agripolis, viale dell'Università 16, Legnaro, Padua, Italy

[4]Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy

Correspondence: Fabio Quaglino, Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DiSAA), University of Milan, via Celoria 2, 20133 Milano, Italy. Tel: +39-02-50316789; e-mail: fabio.quaglino@unimi.it

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SUMMARY

Bois noir (BN) grapevine disease is associated with '*Candidatus* Phytoplasma solani' (CaPsol), a pathogen with a complex ecology including multiple insect vectors and plant hosts. A key point to improve the effectiveness of BN control strategies consists in determining the epidemiological role of ground-cover weeds. The present study employed a multidisciplinary approach, based on the application of spatial (Spatial Analysis by Distance IndicEs) and molecular (*stamp* gene typing) analyses, to identify weeds with a potential role in BN epidemiology in Northern Italy. Generated data showed that, in addition to *Convolvulus arvensis*, one of the main CaPsol inoculum source, *Chenopodium album*, *Polygonum aviculare*, and *Trifolium repens* were found associated with BN epidemiology. CaPsol molecular typing highlighted that the strains prevalent in symptomatic grapevines were characterized by *stamp* sequence variants St19, St11 (nettle-related), and St5 (bindweed-related). The latter was prevalent also in *Hyalesthes obsoletus* and weeds, suggesting their main association with bindweed-related epidemiology. On the other hand, nettle-related CaPsol strains were occasionally found in *H. obsoletus* and weeds. Considering that *H. obsoletus*-mediated transmission of CaPsol occurs mainly with young instars, further investigations will confirm if, in addition to bindweed and nettle, weeds associated with BN epidemiology in Franciacorta can represent larval developmental hosts, and, consequently, act as CaPsol reservoirs for transmission to grapevine. Moreover, other studies are needed to clarify the relationship between such weeds and CaPsol alternative vectors.

Keywords: '*Candidatus* Phytoplasma solani'; grapevine; spatial analysis; *stamp*; weeds; *Hyalesthes obsoletus*

1 INTRODUCTION

Bois noir (BN), the most widespread disease of the grapevine yellows complex, is associated with '*Candidatus* Phytoplasma solani' (CaPsol) (Quaglino et al., 2013). It has been present in Europe from a long time (Belli et al., 2010), while it was only recently reported in South America and Asia (Gajardo et al., 2009; Duduk et al., 2010; Jamshidi et al., 2019). In Europe, CaPsol is transmitted to grapevine dead-end host mainly by *Hyalesthes obsoletus* Sign., a polyphagous cixiid living preferentially on non-crop plants (*Convolvulus arvensis* L., *Urtica dioica* L., *Vitex agnus-castus* L., and *Crepis foetida* L.) that represent the phytoplasma inoculum source (Langer & Maixner, 2004; Kosovac et al., 2016, 2019; Moussa et al., 2019). In the last years, studies of molecular epidemiology based on CaPsol typing by sequence analysis of variable (*secY*) and hypervariable genes (*stamp*, *vmp1*) highlighted the presence of previously unknown plant hosts and vectors involved in CaPsol spreading in vineyards. For example, other polyphagous insects, feeding on non-crop and crop plants, were identified as alternative CaPsol vectors to grapevine in northern Italy and Serbia (Cvrkovic et al., 2014; Quaglino et al., 2019a). Moreover, additional CaPsol transmission routes in vineyards, involving putative insect vectors *Anaceratagallia ribauti* (Oss.) (Riedle-Bauer et al., 2008; Aryan et al., 2014; Šafářová et al., 2018), *Macrosteles quadripunctulatus* (Kir.) (Batlle et al., 2008), and *Reptalus artemisiae* (Becker) (Chuche et al., 2016; Pierro et al., 2020), formerly know as *Reptalus quinquecostatus* (Duf.) (Emeljanov, 2020), have been recently proposed. The complexity of this epidemiological scenario is increased by two factors: (i) the broad range of cultivated and wild plants, reported as CaPsol hosts, that could act as inoculum source for its vector-related transmission to grapevine (Cvrkovic et al., 2014; Oliveri et al., 2015; Quaglino et al., 2019b); (ii) the considerable genetic diversity among CaPsol strains, determined through comparison of multiple gene sequences, possibly reflecting differences in their biological features (Landi et al., 2015; Murolo & Romanazzi, 2015; Quaglino et al., 2016; Pierro et al., 2018a, 2018b). Due to the CaPsol multifaceted ecology, including multiple insect vectors and plant hosts, it is extremely difficult to develop efficient control strategies for BN.

As no effective control measures directly targeting phytoplasmas are available, preventive measures are applied including the sanitary status check, hot water treatment of propagation material, and control of vectors before their emergence from the ground (Bertaccini et al., 2014; Bianco et al., 2019). Concerning the main CaPsol vector *H. obsoletus*, insecticide treatments on grapevine canopy are completely ineffective due to its life cycle including subterranean lifestyle at nymph stage and a spotted distribution of the adults on the preferred weeds (Maixner et al., 1994; Mori et al., 2008). Consequently, some control strategies focused instead on suppressing *C. arvensis* and *U. dioica*, the vector's main plant hosts in Europe, applying repeated treatments of mechanical or chemical weeding (Langer et al., 2003; Mori et al., 2012; 2014). Considering the polyphagia of all the CaPsol insect vectors, a central point to allow the improvement of the knowledge of CaPsol diffusion routes, and consequently the effectiveness of BN control strategies, consists in establishing the epidemiological role of ground-cover weeds. Based on previous studies focused on other phytoplasma-associated diseases (Navratil et al., 2009; Bonnot et al., 2010; Rappussi et al., 2012), a multidisciplinary approach, based on the synergic application of spatial (Spatial Analysis by Distance Indices, SADIE) and molecular (RFLP-based *tuf* type determination) analyses, revealed the association of *Chenopodium album* L. and *Malva sylvestris* L. with, and the separation of *Trifolium repens* L. to BN spreading in Veneto region (north-eastern Italy) vineyards (Mori et al., 2015b).

The present study aimed to apply spatial analyses, coupled with *stamp* gene based CaPsol strain typing, to investigate the weeds associated with BN epidemiology in vineyards located in Franciacorta (northern Italy).

2 MATERIALS AND METHODS

2.1 Investigated vineyards

The investigation on BN epidemiology was conducted in the years 2013-2015 in Franciacorta, a grape-growing area of the Lombardy Region (Northern Italy) famous worldwide to produce

104 sparkling wine, in two Chardonnay vineyards, located in Erbusco (45°35'55.2"N, 9°57'38.9"E) and
105 Borgonato di Corte Franca (45°37'29.2"N 10°00'30.4"E). The vineyard in Erbusco (4614 vines) is
106 composed by 38 rows North-South oriented, bordered with other vineyards, and arable meadows
107 and with the A4 Milan-Venice highway in the southern side. Grape vines were trained using the
108 Guyot system in 13 rows (distance between rows 2.3m; plant distance along the row 0.9 m), and the
109 Sylvoz system in 25 rows (distance between rows 2.8m; plant distance along the row 2.5 m). The
110 vineyard in Corte Franca (12419 vines) is composed by 112 rows East-West oriented, bordered with
111 other vineyards and with the Provincial Road North XI in the west side. Grapevines were trained
112 using the spurred cord system (distance between rows 1.25m; plant distance along the row 0.8 m).
113
114 **2.2 Sampling and spatial distribution of symptomatic grapevines, weeds, and *H. obsoletus***
115 In 2013 and 2014, grapevine plants, weeds, and *H. obsoletus* adults were monitored, mapped,
116 and sampled in both vineyards following the experimental scheme reported by Mori et al. (2015). In
117 September of each year, when the BN symptoms are evident on diseased plants, the grapevines
118 were classified as symptomatic or asymptomatic during a monitoring activity conducted by two
119 people inspecting both sides of the plants. BN incidence was calculated as percentage of
120 symptomatic plants on the total number of plants in the vineyards. Moreover, in 2014 and 2015, the
121 presence of new symptomatic grapevines (plants showing grapevine yellows symptoms for the first
122 time in the studied year) was calculated in comparison with the asymptomatic grapevines in the
123 previous years, and their incidence expressed as percentage of overall symptomatic plants in the
124 season. The grapevine spatial distribution maps were referred to 256 block units in Erbusco [96 in
125 the Guyot system (26±2 plants per block; width 4.6m ± 1m, length 11.7m ± 0,9m); 160 in the
126 Sylvoz system (16±2 plants per block; width 5.6m, length 11.25m ± 1.25 m)], and 308 block units
127 in Corte Franca (40±2 plants per block; width 5m, length 10m ± 0.8m). Each block was geo-tagged
128 with GPS spatial coordinates. In both vineyards, in each block unit used for insect grid (see below),

129 leaf petioles were collected from each of two symptomatic grapevine plants and stored at -30°C
130 until total nucleic extraction for CaPsol identification and typing analyses was performed.

131 As spontaneous grasses are not known as CaPsol host plants, investigation was conducted on
132 broadleaf species. In July of each year, the weed species observed in correspondence to the space
133 occupied by each grapevine and its intra- and inter-row surroundings (here called "spatial cluster"),
134 were recorded and geo-tagged. The weed spatial distribution maps were referred in both vineyards
135 to the block units described above for grapevine. Weed spatial incidence on the total area of each
136 vineyard was calculated as the ratio between occupied and total clusters (4164 in Erbusco; 12419 in
137 Corte Franca). After monitoring and mapping, in each block used for the insect grid (see below),
138 five to ten leaves were collected from at least one plant per observed weed species and stored at -
139 30°C until total nucleic extraction for CaPsol identification and typing analyses was performed. As
140 weeds were symptomless, they were sampled randomly within each block.

141 *H. obsoletus* distribution inside the vineyards was referred to a regular grid constituted by 32
142 block units in Erbusco [12 in the Guyot system (195±16 plants per block; width 16.1m ± 1.15m,
143 length 27m ± 0,9m); 20 in the Sylvoz system (149±11 plants per block; width 16.8m ± 1.4m, length
144 30m ± 1.25m)] and 40 block units in Corte Franca (384 plants per block; width 20m, length 19.2m
145 ± 1.6m). Each block was geo-tagged with GPS spatial coordinates. In both vineyards, the presence
146 of *H. obsoletus* was monitored every week from June to September by using yellow sticky traps (21
147 cm X 40 cm, SuperColor Giallo®, Serbios) placed in the center of each block unit. Incidence of *H.*
148 *obsoletus* was expressed as total number of specimens captured during the season. All the *H.*
149 *obsoletus* specimens captured were stored in ethanol 90% at 4°C until total nucleic extraction for
150 CaPsol identification and typing analyses.

151 To compare the spatial distribution of grapevines, weeds and *H. obsoletus*, the plant maps and
152 insect grid were overlapped.

153

154 **2.3 CaPsol identification and typing**

155 Considering both vineyards, in 2013-2014 total nucleic acids were extracted from leaf
156 samples collected from 288 symptomatic grapevines and 2233 weeds using the CTAB-based
157 protocol described by Angelini et al. (2001), and 282 *H. obsoletus* specimens using the CTAB-
158 based protocol described by Marzachì et al. (1998).

159 Extracted total nucleic acids were utilized as templates in nested PCR reactions conducted for
160 CaPsol specific identification through the amplification of the *stamp* gene. Direct PCRs were
161 performed using the primer pair StampF/StampR0, followed by nested PCRs with the primer pair
162 StampF1/StampR1. Primer sequences and reaction conditions were as previously described (Fabre
163 et al., 2011). Total nucleic acids from periwinkle plants infected by phytoplasma strain STOL ('*Ca*.
164 P. solani'), AY1 ('*Ca*. P. asteris'), and EY1 ('*Ca*. P. ulmi') were used as reference controls. The
165 reaction mixture devoid of nucleic acids was used as negative control. PCR products were verified
166 by electrophoresis on 1% agarose gel in TBE buffer and visualized under a UV transilluminator.

167 An overall total of 523 StampF1/StampR1 amplicons (233 from grapevines, 223 from weeds,
168 and 67 from *H. obsoletus*) were sequenced in both strands by a commercial sequencing service
169 (Eurofins Genomics, Germany). Nucleotide sequences were assembled by the Contig Assembling
170 Program and trimmed to the *stamp* gene start and stop codons in the software BioEdit version 7.2.6
171 (Hall, 1999). *Stamp* gene nucleotide sequences, obtained in this study from CaPsol strains identified
172 in grapevines, weeds, and *H. obsoletus* specimens, were aligned using the ClustalW Multiple
173 Alignment program in the software BioEdit and analyzed by Sequence Identity Matrix to calculate
174 their genetic diversity. Finally, *stamp* sequence variants, identified in this study, were aligned with
175 representative sequences of previously defined *stamp* sequence variants (Pierro et al., 2018a,
176 2018b); a nucleotide sequence identity of 100% was necessary for the sequence variant attribution.

177 Nucleotide sequences of CaPsol representative strains of *stamp* sequence variants, identified
178 in this and in previous studies (Pierro et al., 2018a, 2018b), were aligned and used for generating

179 unrooted phylogenetic trees by Neighbor-Joining method performed using the Jukes-Cantor model
180 and bootstrap replicated 1000 times in the MEGAX software (Kumar et al., 2018).
181
182 **2.4 Spatial Analysis by Distance Indices (SADIE)**
183 As reported in Mori et al. (2015b), SADIE methodology was applied on vineyard monitoring
184 and molecular data to detect spatial patterns of symptomatic grapevines, weeds, *H. obsoletus*
185 captures, and CaPsol-infected *H. obsoletus* specimens within the season. Briefly, the spatial
186 clustering of each variable into patches and gaps (red-blue analysis) was determined by calculating,
187 for each sampling point (the block unit), the indexes of clustering (v_i; v_j) that measure the local
188 contribution to patch or to gap, respectively. Clustering significance ($\alpha = 0.05$) was provided by
189 comparing the v_i and v_j mean value with their corresponding values under the null hypothesis (Perry
190 et al., 1999). For each variable, a two-dimensional map showing the spatial distribution of local
191 aggregation indexes (v_i; v_j) was generated using linear kriging with SURFER (Golden Software
192 Inc., CO). Datasets from red-blue analysis were used to evaluate the similarity among the spatial
193 patterns of symptomatic grapevines, weeds, and *H. obsoletus*. A specific algorithm was used to
194 derive an overall index of spatial association (X), which significance (Px) was established through a
195 randomization test (Perry & Dixon, 2002). This test determines whether the spatial patterns of two
196 variables are associated ($Px < 0.025$), unassociated ($0.025 \leq Px \leq 0.975$) or dissociated ($Px >$
197 0.975). As grapevine plants normally show BN symptoms from at least one year after the
198 phytoplasma infection, spatial patterns of new symptomatic grapevines (plants showing BN
199 symptoms for the first time) were compared with the spatial patterns of weeds and insects detected
200 in the previous year.
201
202 **2.5 Criteria determining the epidemiological role of weeds**
203 The determination of which weeds could have an epidemiological role was carried out as
204 reported in Mori et al. (2015b) with some modifications (CaPsol typing based on *stamp* gene

instead of *tuf* gene). The criteria used are: (i) if the weed harbors the same CaPsol strains characterized by *stamp* gene sequence variants found also in symptomatic grapevines, (ii) statistically significant association with overall and/or new symptomatic grapevines, and (iii) statistically significant association with *H. obsoletus*. Based on these criteria the weeds identified in the examined vineyards were ranked in four epidemiological groups, as follows. Group 1 (weeds associated with BN epidemiology): weeds harboring the same CaPsol strains as grapevine, and associated with symptomatic grapevines and/or *H. obsoletus* captures in both years in at least one of the studied vineyards; Group 2 (weeds possibly associated with BN epidemiology): weeds harboring the same CaPsol strains as grapevine, and associated with symptomatic grapevines and/or *H. obsoletus* captures in either 2013 or 2014 in at least one of the studied vineyards; Group 3 (weeds uncertainly associated with BN epidemiology): weeds harboring the same CaPsol strains as grapevine, not associated with symptomatic grapevines and *H. obsoletus* captures, or weeds non-harboring the same CaPsol strains as grapevine but associated with symptomatic grapevines and/or *H. obsoletus* captures in 2013 and/or 2014 in at least one of the studied vineyards; Group 4 (weeds not associated with BN epidemiology): weeds not harboring the same CaPsol strains as grapevine, and not associated with symptomatic grapevines and *H. obsoletus* captures.

3 RESULTS

3.1 Incidence of symptomatic grapevines, weeds and *H. obsoletus*

Incidence of symptomatic grapevines was stable in Erbusco [11.8% (545 out of 4614 vines) in 2013; 11.3% (520 vines) in 2014; 11.1% (510 vines) in 2015], while decreased in Corte Franca [4% (494 out of 12419 vines) in 2013; 1.9% (230 vines) in 2014; 0.4% (48 vines) in 2015]. However, among the overall symptomatic grapevines, a higher incidence of grapevines showing symptoms for the first time was registered in Corte Franca (54.7% in 2014; 45.8% in 2015) than in Erbusco (32.9% in 2014; 20.8% in 2015). In 2013-2014, leaf samples were collected from 128 symptomatic grapevines in Erbusco and 160 in Corte Franca (Table 1).

231 In 2013-2014, 33 (18 annual and 15 perennial) and 26 (15 annual and 11 perennial) weed
232 species were identified and monitored in Erbusco and Corte Franca vineyards, respectively. All
233 weeds present in Corte Franca (except *Achillea millefolium* L.) were found also in Erbusco, while
234 eight weed species (*Bellis perennis* L., *Capsella bursa-pastoris* (L.) Medik, *Geranium dissectum* L.,
235 *Matricaria chamomilla* L., *Oxalis* sp., *Papaver rhoeas* L., *Prunella vulgaris* L., and *Silene vulgaris*
236 (Moench) Garcke) were present only in Erbusco. In detail, 24 and 22 weed species were present in
237 both years in Erbusco and Corte Franca vineyards, respectively. On the other hand, nine weeds were
238 observed only in 2013 in Erbusco, and four weeds only in 2014 in Corte Franca. Incidence of weeds
239 common to both vineyards was higher in Erbusco in 2013 and 2014. In detail, six weeds in Erbusco
240 (*C. arvensis*, *Plantago major* L., *Rumex acetosa* L., *Trifolium pratense* L., *T. repens*, *Veronica*
241 *persica* Poir.) and one weed (*Taraxacum officinale* (L.) Weber) in Corte Franca had an incidence
242 >10% in both considered years (Table 2). Leaf samples were collected from 781 weeds (belonging
243 to 33 species) in 2013, and 419 weeds (24 species) in 2014 in Erbusco vineyard, and from 584
244 weeds (22 species) in 2013, and 449 weeds (26 species) in 2014 in Corte Franca vineyard (Table 1,
245 2).

246 Based on sticky trap captures, *H. obsoletus* was found in most of the vineyard blocks during
247 the investigated period. In both vineyards, *H. obsoletus* specimens were captured from the end of
248 June to the begin of September. Its flight curve showed the main peak in July 23 in Erbusco, and in
249 August 06 in Corte Franca. The number of *H. obsoletus* captured specimens was higher in 2013
250 (120 and 133 in Erbusco and Corte Franca, respectively) than in 2014 (eight and 21 in Erbusco and
251 Corte Franca, respectively) (Table 1).

252

253 **3.2 Molecular identification and infection rate of CaPsol**

254 Nested PCR-based amplification of *stamp* gene detected CaPsol in: (i) 89% (57 out of 64 in
255 2013) and 75% (48 out of 64 in 2014) of symptomatic grapevines in Erbusco vineyard, and 84% (67
256 out of 80 in 2013) and 76% (61 out of 80 in 2014) of symptomatic grapevines in Corte Franca

vineyard; (ii) 14% (113 out of 781 in 2013) and 22% (94 out of 419 in 2014) of weeds sampled in Erbusco, and 11% (63 out of 584 in 2013) and 5% (21 out of 449) of weeds sampled in Corte Franca; (iii) 43% (51 out of 120 in 2013) and 37% (three out of eight) of *H. obsoletus* specimens captured in Erbusco, and 5% (seven out of 133 in 2013) and 21% (six out of 21 in 2014) of *H. obsoletus* specimens captured in Corte Franca (Table 1).

Concerning the weeds, in Erbusco vineyard CaPsol was identified in 29 out of 33 species in 2013, and in 21 out of 24 species in 2014; in fact, *C. album, P. rhoeas, Potentilla reptans* L., and *Senecio vulgaris* L. were uninfected in 2013, while *Lactuca serriola* L.*, Oxalis* sp., and *Silene vulgaris* in 2014. Fifteen weeds showed an infection rate ≥ 10% in both years; *C. arvensis* (61%), *Medicago lupolina* L. (45%), and *C. bursa-pastoris* (28%) had the highest infection rate in 2013, while *M. lupolina* (50%), *C. arvensis* (38%), and *Erigeron annuus* (L.) Pers. (36%) in 2014 (Table 2). In Corte Franca vineyard, CaPsol was identified in 19 out of 22 species in 2013, and in 8 out of 26 species in 2014; in fact, *Artemisia vulgaris* L.*, Cirsium arvense* (L.) Scop, and *R. acetosa* were uninfected in 2013, while *Amaranthus retroflexus* L., *C. album*, *C. arvensis*, *L. serriola*, *Polygonum aviculare* L., *Portulaca oleracea* L., *Sonchus oleraceus* L., and *T. repens* were CaPsol-infected in 2014. Two weeds (*C. album* and *P. oleracea*) showed an infection rate ≥ 10% in both years (Table 2).

PCR products of *stamp* gene amplified from 313 samples (105 grapevines, 154 weeds, and 54 insects) in Erbusco vineyard, and 210 samples (128 grapevines, 69 weeds, and 13 insects) in Corte Franca vineyards were sequenced for CaPsol strain typing.

3.3 Spatial Analysis by Distance Indices

In Erbusco vineyard, significant clustering into patch/gap was found in the distributions of overall symptomatic grapevines and 20 weed species observed in 2013, while *P. rhoeas* and *T. repens* distributions were significantly clustered only into patch and gap, respectively (Figure 1, Table S1). In 2014, significant clustering into patch/gap was detected in the distributions of overall

283 symptomatic grapevines and 19 weed species, while *B. perennis*, *P. aviculare* and *Solanum nigrum*
284 L. distributions were significantly clustered only into gap, and *Plantago lanceolata* L. only into
285 patch (Figure 1, Table S1).

286 In Corte Franca vineyard, significant clustering into patch/gap was found in the distributions
287 of eight weed species observed in 2013, while *S. nigrum* and *T. officinale* distributions were
288 significantly clustered only into patch, and *M. sylvestris* only into gap (Figure 1, Table S2). In 2014,
289 significant clustering into patch/gap was detected in the distributions of eight weeds, while *S.*
290 *oleraceus* and *T. officinale* distributions were significantly clustered only into gap, and *S. nigrum*
291 only into patch (Figure 1, Table S2).

292 In Erbusco vineyard in 2013, spatial association analyses showed that the distribution of
293 overall symptomatic grapevines was significantly associated with *C. bursa-pastoris*, and
294 significantly dissociated from *E. annuus*, *M. sylvestris*, *S. oleraceus*, *T. officinale*, and *V. persica*.
295 Distribution of new symptomatic grapevines observed in 2014 was associated with *C. arvensis*, *L.*
296 *serriola*, *T. officinale*, and *T. repens* (Figure 2), and dissociated from *T. pratense*. No associations
297 were found between distributions of captured and CaPsol-infected insect specimens and weeds
298 (Table 3). In 2014, distribution of overall symptomatic grapevines was associated with *L. serriola*,
299 and dissociated from *C. bursa-pastoris*, *E. annuus*, *M. sylvestris*, *P. major*, *S. nigrum*, *T. repens*,
300 and *V. persica*. Distribution of new symptomatic grapevines observed in 2015 was associated with
301 *C. arvensis* and *T. repens* (Table 3).

302 In Corte Franca vineyard in 2013, distribution of overall symptomatic grapevines was
303 significantly associated with *S. oleraceus*. Distribution of new symptomatic grapevines observed in
304 2014 was found significantly associated with captured *H. obsoletus* specimens, *C. album*, *C.*
305 *arvense*, *M. sylvestris*, *P. aviculare*, *T. repens*, and *V. persica* (Figure 3). Distribution of captured *H.*
306 *obsoletus* was significantly associated with *P. reptans* and *V. persica* and dissociated from *C.*
307 *arvensis* and *T. officinale*. Distribution of CaPsol-infected *H. obsoletus* was significantly associated
308 with *Erigeron canadensis* (L.) Cronquist, *P. reptans*, and *V. persica*, and dissociated from *Crepis*

309 sp., *L. serriola*, *S. oleraceus*, and *T. officinale* (Table 4). In 2014, distribution of new symptomatic
310 grapevines observed in 2015 was found significantly associated with *C. album*, *C. arvense*, *M.*
311 *sylvestris*, *P. aviculare*, *T. repens*, and *V. persica,* and dissociated from *A. vulgaris* and *L. serriola*.
312 Distribution of captured *H. obsoletus* was significantly associated with *P. reptans* and *V. persica*
313 and dissociated from *T. officinale*. Distribution of CaPsol-infected *H. obsoletus* was significantly
314 associated with *V. persica* and dissociated from *T. officinale* (Table 4).
315
316 **3.4 CaPsol strain typing**
317 Based on *stamp* gene nucleotide sequence identity, CaPsol strains identified in the examined
318 vineyards were attributed to 22 *stamp* gene sequence variants, from St_Fc1 to St_Fc22 (Table 5).
319 The six most prevalent variants, St_Fc2 (321 out of 523 CaPsol strains), St_Fc1 (93), St_Fc5 (35),
320 St_Fc4 (22), St_Fc8 (13), and St_Fc10 (10), were found in 94% of CaPsol strains in both vineyards
321 in the years 2013 and 2014. Only the variant St_Fc2 was present in grapevines, weeds, and *H.*
322 *obsoletus* in both vineyards during the seasons considered, albeit showing different abundance
323 between hosts, vineyards, and seasons. Among other prevalent variants, St_Fc1, St_Fc4, St_Fc5,
324 and St_Fc8 were identified with diverse abundance in grapevines, weeds, and *H. obsoletus* in
325 Erbusco and/or Corte Franca in 2013 and/or 2014. Variant St_Fc10 was found only in weeds and *H.*
326 *obsoletus*. Considering the remnant variants, St_Fc6 was found in grapevines, weeds, and *H.*
327 *obsoletus* only in Corte Franca in 2013; St_Fc7 in grapevine in Corte Franca and weeds in Erbusco
328 only in 2013; St_Fc3 and St_Fc9 were found only in grapevines; the others (from St_Fc11 to
329 St_Fc22) were sporadically found only in weeds in Erbusco and/or Corte Franca vineyard in 2013
330 or 2014 (Table 5, S3, S4). Moreover, variants St_Fc1 and St_Fc2 had a similar prevalence in
331 symptomatic grapevines in both vineyards in both seasons, but St_Fc2 was largely prevalent in
332 weeds and *H. obsoletus* specimens, while St_Fc1 was poorly present in weeds and *H. obsoletus* in
333 both vineyards in both seasons. A comparable distribution was evidenced also for the variant
334 St_Fc5, abundant similarly to St_Fc1 and St_Fc2 in grapevine in Erbusco, but never identified in

335 the other hosts (Table 5, S3, S4). *Stamp* sequence variant distribution in weeds highlighted that in
336 Erbusco 22 species out of 24 in 2013 (except *G. dissectum* and *Trifolium pretense*), and 20 species
337 out of 20 in 2014 harbored CaPsol strains characterized by *stamp* sequence variants identified also
338 in grapevine. In detail, St_Fc2 was found largely prevalent in all CaPsol-infected weed species;
339 St_Fc1 in *M. sylvestris* and *S. nigrum*, St_Fc7 in *A. retroflexus*, *C. arvensis* and *R. acetosa*; St_Fc8
340 in *C. arvensis*. In Corte Franca vineyard, 16 weed species out of 16 in 2013, and eight out of eight
341 in 2014 harbored CaPsol strains characterized by *stamp* sequence variants identified also in
342 grapevine. In detail, St_Fc2 was found in all CaPsol-infected weeds except *A. retroflexus* and
343 *Senecio vulgaris*; St_Fc1 in *A. retroflexus*, *E. canadensis*, and *P. oleracea*; St_Fc4 in *C. album*, *S.*
344 *oleraceus*, and *T. repens*; St_Fc5 in *T. repens*; St_Fc6 in *Senecio vulgaris*.
345 Comparison with *stamp* gene dataset previously published (Pierro *et al*., 2020) showed that 11
346 sequence variants identified in CaPsol strain populations in Franciacorta are identical with
347 previously published *stamp* variants as follows: St_Fc1 to St19; St_Fc2 to St5; St_Fc3 to St9;
348 St_Fc4 to St10; St_Fc5 to St11; St_Fc6 to St30; St_Fc7 to St38; St_Fc8 to St8; St_Fc10 to St18;
349 St_Fc15 to St36; St_Fc18 to St32. The remaining 11 *stamp* variants were found for the first time in
350 the present study and named as follows: St_Fc9 (St60), St_Fc11 (St61), St_Fc12 (St62), St_Fc13
351 (St63), St_Fc14 (St64), St_Fc16 (St65), St_Fc17 (St66), St_Fc19 (St67), St_Fc20 (St68), St_Fc21
352 (St69), St_Fc22 (St70). A representative sequence for each variant was deposited at NCBI GenBank
353 at the accession numbers MT777487 to MT777508 (Table 5).
354 Phylogenetic analysis showed that CaPsol strains typed by *stamp* sequence variants St_Fc1,
355 St_Fc3, St_Fc5, St_Fc8, St_Fc10, and St_Fc22, representing 55% of grapevine-harbored strains,
356 19% of *H. obsoletus*-harbored strains, and 7% of weeds-harbored strains, grouped within nettle-
357 related sub-clusters a1 and a2; on the other hand, CaPsol strains typed by the remaining 15 variants,
358 representing 45% of grapevine-harbored strains, 81% of *H. obsoletus*-harbored strains, and 93% of
359 weeds-harbored strains, grouped within bindweed-related clusters b-I, b-II, and b-III (Figure 4).
360

3.5 Weed attribution to epidemiological groups

Based on infection by CaPsol strain carrying *stamp* gene sequence variant found also in symptomatic grapevines, and spatial distribution significantly association with overall and/or new symptomatic grapevines and/or *H. obsoletus*, weeds were ranked in four epidemiological groups as follows: (group 1) *C. album*, *C. arvensis*, *P. aviculare*, and *T. repens*; (group 2) *C. bursa-pastoris*, *E. canadensis*, *L. serriola*, *M. sylvestris*, *S. oleraceus*, *T. officinale*, and *V. persica*; (group 3) *A. millefolium*, *A. retroflexus*, *A. vulgaris*, *B. perennis*, *C. arvense*, *Crepis* sp., *E. annuus*, *G. dissectum*, *M. lupolina*, *P. lanceolata*, *P. major*, *P. oleracea*, *P. reptans*, *P. vulgaris*, *R. acetosa*, *Senecio vulgaris*, *S. nigrum*, and *T. pratense*; (group 4) *Lampsana communis* L., *M. chamomilla, Oxalis* sp., *P. rhoeas*, and *Silene vulgaris* (Table 6).

4 DISCUSSION

CaPsol transmission to grapevine by *Hyalesthes obsoletus* is influenced by the presence of weeds on which it feeds, both its preferred hosts (nettle, bindweed, chaste tree, stinking hawksbeard) (Langer & Maixner, 2004; Kosovac et al., 2016, 2019; Moussa et al., 2019) and other plants that could be associated with CaPsol ecology. Thus, several studies have been carried out to clarify the involvement of such plants in BN epidemiology (Marchi et al., 2015; Oliveri et al., 2015). For example, a multidisciplinary approach combining field surveys, spatial analyses, and molecular typing of '*Ca*. P. solani' (CaPsol), the etiological agent of the disease, was applied in the years 2010-12 in north-eastern Italian vineyards highlighting that in addition to *Urtica dioica* (nettle) and *C. arvensis* (bindweed) also *C. album* and *M. sylvestris* could play a role in BN diffusion (Mori et al., 2015b). Distinct season and geographic areas, even if close to one another, offer different environmental and ecological features that can shape insect vector populations, weed presence and abundance, and the strain composition of phytoplasma populations (Cai et al., 2008; Wu et al., 2012; Pierro et al., 2018a; Quaglino et al., 2019b). In the last years, the utilization of variable (*secY*) and hyper-variable genes (*stamp*, *vmp1*) allowed more in-depth characterization of

CaPsol strain populations and identification of BN epidemiological routes involving previously undescribed plant hosts and insect vectors (Cvrkovic et al., 2014; Kosovac et al., 2016, 2019; Quaglino et al., 2019b; Jakovljević et al., 2020; Pierro et al., 2020). Based on these evidence and considerations, we decided to apply the experimental approach described by Mori et al. (2015) with the following differences: (i) another location, Franciacorta area in northern Italy; (ii) the period analyzed (2013-15); (iii) the molecular marker employed (the more variable *stamp* gene instead of *tufB* gene).

Field surveys revealed that the disease incidence in the two vineyards (Erbusco and Corte Franca) had different trends: in Erbusco the overall BN incidence remained stable (11.8% in 2013; 11.1% in 2015), while it plummeted in Corte Franca (4% in 2013; 0.4% in 2015). These variations seem to indicate that BN disease was not spreading within the vineyards, nevertheless many grapevines showing symptoms for the first time was observed each year. Considering that most symptomatic grapevines were not replaced throughout the years, it follows that many vines previously diseased did not show any symptoms in the following seasons. Such dynamics confirmed the existence of balanced equilibrium between driving forces acting on BN impact (Rotter et al., 2018; Murolo et al., 2020). In particular, vector-mediated CaPsol transmission to grapevines (new infections) and symptom remission were equally strong drivers in Erbusco, while the latter was prevalent in Corte Franca.

The combination of spatial analyses and CaPsol molecular typing generated data suitable for the attribution of the weeds observed in the examined vineyards to BN epidemiological groups, ranked based on their relevance from 1 (max) to 4 (min). Among the 34 weed species observed in the vineyards in 2013-14, four belong to group 1, seven to group 2, 18 to group 3, and five to group 4.

The weeds associated with BN epidemiology in Franciacorta vineyards (group 1) were *C. album*, *C. arvensis*, *P. aviculare*, and *T. repens*. The robustness of the utilized methodology was proved by the ranking of *C. arvensis*, a weed part of the established epidemiological system *C.*

413 *arvensis* / *H. obsoletus* / grapevine (Langer and Maixner, 2004), in group 1. Even if a spatial
414 relation of *H. obsoletus* and weeds can be caused by their similarities of habitat requirements, in
415 previous study *H. obsoletus* was found able to survive both on *C. album*, *P. aviculare*, and *C.*
416 *arvensis* for three days, a time sufficient for the acquisition of CaPsol from such weeds (Mori et al.,
417 2015a). In a previous study, *T. repens* was reported as uninfected and not associated with
418 symptomatic grapevines and/or insect vectors in north-eastern Italy (Mori et al., 2015b).
419 Intriguingly, here it was found as the only weed harboring CaPsol strains found also in symptomatic
420 grapevines and spatially associated with new symptomatic grapevines in both vineyards in 2013 and
421 2014. Additional research should be performed to investigate its association with *H. obsoletus* and
422 other vectors reported by Quaglino et al. (2019b). Furthermore, two annual weeds (*C. album* and *P.*
423 *aviculare*) were attributed to group 1. *C. album* belonged to group 1 also in vineyards in Veneto
424 region in the years 2010-11. Hypotheses on the contribution of annual plants to BN epidemiology
425 were formulated in Mori et al. (2015), including seed-mediated CaPsol vertical transmission
426 throughout seasons (Olivier et al., 2010; Calari et al., 2011) and role as acquisition source of
427 alternative vectors present in the vineyard as adults for a longer period compared to *H. obsoletus*
428 (June – September). This last hypothesis is strengthened by alternative vectors recently discovered
429 in Franciacorta (Quaglino et al., 2019b) which have a longer adult stage or overwinter as nymphs or
430 adults (*Dicranotropis hamata*, *Philaenus spumarius*, *Euscelis incisus*, *Euscelidius variegatus*),
431 allowing the overwintering of CaPsol as well (Nickel et al., 2002; Holzinger et al., 2003;
432 Biedermann & Niedringhaus, 2009).

433 Group 2 included seven weeds that gave opposite results in the two seasons, meaning that
434 their association with BN epidemiology should be clarified. Moreover, group 2 composition was
435 different from that determined in Veneto: in Franciacorta *M. sylvestris* (group 1 in Veneto), *E.*
436 *canadensis*, *S. oleraceus*, *T. officinale*, and *V. persica* (group 3 in Veneto), and *L. serriola* (group 4
437 in Veneto) (Mori et al., 2015b) were included. Special attention should be given to *V. persica*, the
438 only weed spatially associated with new symptomatic grapevines and *H. obsoletus*.

439 Group 3 included 18 weeds which majority harbors grapevine-infecting CaPsol strains but are
440 not spatially associated with symptomatic grapevines and *H. obsoletus*. Therefore, it is reasonable
441 to suggest that such weeds can play a role as CaPsol inoculum source in alternative BN
442 transmission routes to grapevine. This is the case of *Crepis* sp. and *P. reptans*, two plants found in
443 the gut of CaPsol alternative insect vectors to grapevine recently identified in Franciacorta: *Crepis*
444 sp. in *Dictyophara europaea*, *P. reptans* in *Philaenus spumarius* and *Euscelis incisus* (Quaglino et
445 al., 2019b).
446 Group 4 included four weeds not observed in the Veneto survey (*L. communis*, *Oxalis* sp., *P.*
447 *rhoeas*, *Silene vulgaris*), and *M. chamomilla* (group 3 in Veneto). Due to the absence of grapevine-
448 infecting CaPsol strains and spatial association with symptomatic grapevines and *H. obsoletus*, such
449 weeds are not associated with BN epidemiology in examined vineyards in Franciacorta.
450 Molecular and spatial analyses, conducted in this study, provided a useful basis for further
451 research on BN epidemiology. Considering that *H. obsoletus*-mediated transmission of CaPsol
452 occurs mainly with young instars (Darimont & Maixner, 2001), to verify the real epidemiological
453 role of the weeds, associated with symptomatic grapevines and/or *H. obsoletus* and identified as
454 CaPsol hosts in Franciacorta, it would be required to prove their role in *H. obsoletus* larval
455 development and CaPsol inoculum source by transmission experiments. Given that alternative
456 CaPsol insect vectors, recently reported in Franciacorta (Quaglino et al., 2019b), have an adult stage
457 longer than *H. obsoletus* and can overwinter as nymphs or adults (Nickel et al., 2002; Holzinger et
458 al., 2003; Biedermann & Niedringhaus, 2009), studies are needed to investigate their phenology and
459 ecology, with a special focus on the role of weeds here attributed to group 1 in larval development
460 and CaPsol inoculum source for such insects.
461 Molecular typing of *stamp* gene nucleotide sequences highlighted that: (i) CaPsol strains
462 prevalent in symptomatic grapevine were characterized by *stamp* sequence variants St5 (37.8% of
463 CaPsol-infected vines), St19 (34.3%), and St11 (24.2%); (ii) CaPsol strains carrying the variant St5
464 (CaPsol-St5) was largely prevalent in *H. obsoletus* (68.7% of the CaPsol-infected specimens) and

465 weeds (83.9% of the CaPsol-infected weeds); (iii) CaPsol-St19 and -St11 were poorly identified in
466 *H. obsoletus* (11.9% and 1.5%, respectively) and weeds (2.2% and 0.4%, respectively). No weeds
467 of group 1 were found infected by CaPsol-St19, while only one plant of *T. repens* was found
468 infected by CaPsol-St11 in Corte Franca vineyard. Several studies demonstrated that CaPsol strains
469 associated with nettle and bindweed ecologies grouped in separate phylogenetic clusters determined
470 by *stamp* gene nucleotide sequence analysis, mirroring the distinction obtained on the basis of the
471 *tufB* gene (Aryan et al., 2014; Atanasova et al., 2015; Plavec et al., 2015). Here, phylogenetic
472 analyses showed that CaPsol-St5 grouped within the subcluster b-II, associated with the bindweed-
473 related epidemiological system. On the other hand, CaPsol-St11 and -St19 grouped within the
474 subclusters a1 and a2, respectively, associated with the nettle-related epidemiological system.
475 CaPsol-St5, -St11 and -St19 were largely reported in Europe in association with BN (Fabre et al.,
476 2011; Aryan et al., 2014; Cvrkovic et al., 2014; Kostadinovska et al., 2014; Atanasova et al., 2015;
477 Murolo & Romanazzi, 2015; Kosovac et al., 2016). Interestingly, CaPsol-St5 were reported as
478 prevalent in BN-diseased vineyards in Franciacorta, and it was experimentally proved that *H.*
479 *obsoletus* and eight alternative polyphagous insect vectors can transmit such strains to grapevine.
480 CaPsol-St11 and -St19 were also largely found in symptomatic grapevines, but no insect vectors
481 were found able to transmit them to grapevine (Quaglino et al., 2019b). Evidence from the present
482 study suggests that bindweed- and nettle-related epidemiological systems are equally involved in
483 CaPsol transmission routes to grapevine in the examined vineyards. However, infected *H. obsoletus*
484 and weeds of group 1 harbored prevalently CaPsol strains associated with the bindweed-related
485 ecology, suggesting their main association with this BN epidemiological system. Nettle-related
486 CaPsol-St11 and -St19 were found in low number of *H. obsoletus* specimens, and only CaPsol-St19
487 in weeds attributed to groups 2 and 3 (*A. retroflexus*, *E. canadensis*, *M. sylvestris*, *P. oleracea*, *S.*
488 *nigrum*). Occurrence of nettle-related CaPsol strains in hosts aside from nettle, grapevine, and *H.*
489 *obsoletus* is newly reported in this study, suggesting the existence of overlapping transmission
490 routes of nettle-related CaPsol strains not only to grapevine, but between weeds. Further

investigation is necessary to clarify if such weeds could be inoculums source of nettle-related CaPsol strains to grapevine.

Bindweed-related CaPsol-St10, so far identified only with other hosts and recently reported as widespread in BN-diseased vines in Tuscany vineyards in association with a newly proposed BN epidemiological cycle (Pierro et al., 2018a, 2018b, 2020), was identified in symptomatic grapevines with increasing infection rate throughout the years (1.5% in 2013; 11.5% in 2014) in Corte Franca vineyard. Here, this CaPsol strains were identified also in *H. obsoletus* (infection rate: 14.3% in 2013; 33.3% in 2014) and in three weed species (*C. album* and *T. repens*, group 1; *S. oleraceus*, group 2). Considering the diffusion of CaPsol-St10 in Tuscany, its presence in Franciacorta should be monitored.

Most new *stamp* sequence variants grouped with the bindweed-related CaPsol strains and were identified exclusively in weeds attributed to epidemiological groups 2 and 3. It could be hypothesized that such strains are potentially involved in other CaPsol-associated diseases.

In conclusion, the new insights acquired in this study (i) confirmed the role of *C. arvensis* in BN epidemiology in different agroecosystems in northern Italy; (ii) showed that *H. obsoletus* and weeds, along with recently reported alternative CaPsol vectors to grapevine (Quaglino et al., 2019b), are mainly associated with bindweed-related CaPsol transmission routes to grapevine in Franciacorta; (iii) reinforced the evidence that *stamp* gene-based molecular markers are the most suitable for epidemiological studies on CaPsol-associated diseases; (iv) remarked that BN epidemiology is extremely complicated, including different actors that change in relation to environmental and ecological features of distinct geographic areas and seasons; (v) underlined the need of further investigations, focused mainly on weeds associated with BN epidemiology (*C. album*, *P. aviculare*, and *T. repens*), to check if they can represent developmental hosts and CaPsol inoculum sources for *H. obsoletus* and/or alternative insect vectors.

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Table S1. Average indexes of clustering into patch (mean v_i) and into gap (mean v_j) with associated probability (P) from randomisation test in Erbusco. Numbers in bold indicate significant results at randomization test ($\alpha = 0.05$).

Folder	2013				2014			
	Mean v_i	P (mean v_i)	Mean v_j	P (mean v_j)	Mean v_i	P (mean v_i)	Mean v_j	P (mean v_j)
Overall symptomatic grapevines	**1.471**	**0.0251**	**-1.465**	**0.0277**	**1.462**	**0.0226**	**-1.696**	**0.0031**
2014-new symptomatic grapevines	0.945	0.5708	-0.986	0.4569				
2015-new symptomatic grapevines					0.765	0.4732	-1.582	0.3511
Hyalesthes obsoletus captured	0.769	0.9590	-0.745	0.9821	0.921	0.8340	-0.847	0.8121
Hyalesthes obsoletus CaPsol-infected	1.092	0.2308	-1.037	0.3656	1.255	0.4450	-0.987	0.5271
Amaranthus retrofexus L.	1.296	0.0759	**-1.456**	**0.0292**	**1.677**	**0.0031**	**-1.542**	**0.0128**
Artemisia vulgaris L.	1.111	0.2497	-0.984	0.4692	0.898	0.2867	-1.045	0.3431
Bellis perennis L.	**1.433**	**0.0292**	**-1.477**	**0.0159**	1.231	0.1185	**-1.591**	**0.0062**
Capsella bursa pastoris (L.) Medik	1.197	0.1467	-1.123	0.2154	1.004	0.4215	-0.864	0.7236
Chenopodium album L.	**1.659**	**0.0021**	**-1.515**	**0.0128**	**1.956**	**<0.0001**	**-1.943**	**<0.0001**
Cirsium arvense (L.) Scop	1.094	0.2518	-1.266	0.0887	1.270	0.0764	-1.310	0.0626
Convolvulus arvensis L.	**1.413**	**0.0292**	**-1.584**	**0.0103**	**1.368**	**0.0492**	**-1.623**	**0.0062**
Crepis sp.	**1.781**	**0.0005**	**-1.422**	**0.0272**	**1.687**	**0.0046**	**-1.550**	**0.0154**
Erigeron annuus (L.) Pers.	**1.573**	**0.0087**	**-1.474**	**0.0190**	**1.565**	**0.0123**	**-1.575**	**0.0113**
Erigeron canadensis (L.) Cronquist	**1.807**	**0.0005**	**-1.552**	**0.0103**	**1.838**	**<0.0001**	**-1.577**	**0.0062**
Geranium dissectum L.	**1.677**	**0.0015**	**-1.734**	**0.0005**	**1.366**	**0.0338**	**-1.263**	**0.0687**
Lactuca serriola L.	**1.834**	**<0.0001**	**-1.845**	**<0.0001**	**1.981**	**0.0005**	**-2.112**	**<0.0001**
Lampsana communis L.	1.010	0.4046	-1.083	0.2559	**1.924**	**0.0005**	**-1.845**	**0.0005**
Malva sylvestris L.	**1.681**	**0.0010**	**-1.737**	**0.0010**	**1.649**	**0.0041**	**-1.845**	**<0.0001**
Matricaria chamomilla L.	1.090	0.2431	-1.014	0.3851				
Medicago lupolina L.	**1.415**	**0.0236**	**-1.555**	**0.0072**	**1.395**	**0.0364**	**-1.611**	**0.0067**
Oxalis sp.	**1.518**	**0.0123**	**-1.477**	**0.0179**	1.300	0.0733	-1.089	0.2697
Papaver rhoeas L.	**1.620**	**0.0051**	-1.282	0.0733				
Plantago lanceolata L.	**1.420**	**0.0251**	**-1.665**	**0.0046**	**1.696**	**0.0015**	**-1.781**	**0.0015**
Plantago major L.	**1.585**	**0.0072**	**-1.510**	**0.0108**	**1.598**	**0.0046**	-1.315	0.0662
Polygonum aviculare L.	**1.479**	**0.0144**	**-1.605**	**0.0046**	1.335	0.0544	**-1.376**	**0.0462**
Portulaca oleracea L.	**1.547**	**0.0113**	**-1.724**	**0.0015**	**1.725**	**0.0010**	**-1.731**	**0.0026**
Potentilla reptans L.	0.907	0.6703	-0.890	0.7123	0.913	0.6738	-1.133	0.2292
Prunella vulgaris L.	1.169	0.1687	-1.105	0.2241	1.306	0.0692	-1.063	0.2969
Rumex acetosa L.	1.279	0.0764	-1.214	0.1226	1.197	0.1215	-1.150	0.1779
Senecio vulgaris L.	1.233	0.1051	-1.268	0.0703	1.347	0.0533	-1.322	0.0605
Silene vulgaris (Moench) Garcke	**1.587**	**0.0041**	**-1.397**	**0.0292**	**1.615**	**0.0046**	**-1.376**	**0.0451**
Solanum nigrum L.	1.260	0.0959	-1.223	0.1354	1.155	0.0769	**-1.334**	**0.0436**
Sonchus oleraceus L.	**1.649**	**0.0041**	**-1.619**	**0.0062**	**1.715**	**0.0031**	**-1.968**	**<0.0001**
Taraxacum officinale (L.) Weber	**1.573**	**0.0108**	**-1.687**	**0.0036**	**1.532**	**0.0133**	**-1.711**	**0.0021**
Trifolium pratense L.	**1.437**	**0.0236**	**-1.727**	**0.0026**	**1.420**	**0.0287**	**-1.676**	**0.0036**
Trifolium repens L.	1.119	0.2210	**-1.355**	**0.0405**	**1.556**	**0.0113**	**-1.749**	**0.0010**
Veronica persica Poir.	**1.677**	**0.0041**	**-1.877**	**<0.0001**	**1.559**	**0.0087**	**-1.726**	**0.0015**

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713

714 **Table S2.** Average indexes of clustering into patch (mean v_i) and into gap (mean v_j) with associated
715 probability (P) from randomisation test in Corte Franca. Numbers in bold indicate significant results
716 at randomization test ($\alpha = 0.05$).

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Folder	2013				2014			
	Mean v_i	**P (mean v_i)**	**Mean v_j**	**P (mean v_j)**	**Mean v_i**	**P (mean v_i)**	**Mean v_j**	**P (mean v_j)**
Overall symptomatic grapevines	1.021	0.3641	-1.002	0.4179	1.069	0.261	-1.019	0.3549
2014-new symptomatic grapevines	1.096	0.2323	-1.057	0.2991				
2015-new symptomatic grapevines					1.562	0.1821	-1.373	0.4619
Hyalesthes obsoletus captured	1.249	0.0744	-1.183	0.1262	0.944	0.4670	-1.116	0.5693
Hyalesthes obsoletus CaPsol-infected	1.116	0.2005	-1.197	0.1205	1.012	0.3780	-1.344	0.2471
Achillea millefolium L.	**1.463**	**0.0133**	**-1.569**	**0.0072**	1.112	0.2128	-1.147	0.1826
Amaranthus retroflexus L.					1.226	0.0882	-1.259	0.0779
Artemisia vulgaris L.	0.815	0.9221	-0.811	0.8856	0.877	0.7682	-0.932	0.5990
Chenopodium album L.	1.135	0.1754	-1.121	0.1979	0.893	0.7231	-0.933	0.6036
Cirsium arvense (L.) Scop	**1.375**	**0.0241**	**-1.493**	**0.0118**	0.892	0.6928	-0.908	0.6641
Convolvulus arvensis L.	1.140	0.1774	-1.225	0.1128	**1.556**	**0.0072**	**-1.468**	**0.0169**
Crepis sp.	**1.356**	**0.0385**	**-1.301**	**0.0528**	**1.319**	**0.0395**	**-1.356**	**0.0477**
Erigeron annuus (L.) Pers.					0.958	0.5385	-0.954	0.5492
Erigeron canadensis (L.) Cronquist	0.395	0.7103	-1.086	0.2621	1.042	0.3082	-1.237	0.0913
Lactuca serriola L.	1.063	0.2862	-1.132	0.1785	0.939	0.5815	-0.865	0.7790
Lampsana communis L.					1.008	0.3774	-0.989	0.4441
Malva sylvestris L.	1.220	0.0995	**-1.399**	**0.0262**	1.037	0.3405	-1.272	0.0785
Medicago lupolina L.	0.857	0.8436	-0.829	0.8610	**1.295**	**0.0426**	**-1.379**	**0.0226**
Plantago lanceolata L.	**1.347**	**0.0359**	**-1.324**	**0.0472**	1.182	0.1267	-1.254	0.0826
Plantago major L.	1.275	0.0754	-1.239	0.0851	1.076	0.2769	-1.031	0.3615
Polygonum aviculare L.	**1.667**	**0.0036**	**-1.627**	**0.0021**	1.205	0.1108	-1.032	0.3487
Portulaca oleracea L.	0.981	0.4400	-1.030	0.3374	**1.406**	**0.0179**	**-1.502**	**0.0113**
Potentilla reptans L.	**2.124**	**<0.0001**	**-1.756**	**<0.0001**	**2.083**	**<0.0001**	**-1.853**	**<0.0001**
Rumex acetosa L.	0.940	0.5805	-0.910	0.6554	0.878	0.7774	-0.843	0.8277
Senecio vulgaris L.	0.910	0.6713	-0.965	0.5067	**1.316**	**0.0472**	**-1.452**	**0.0205**
Solanum nigrum L.	**1.314**	**0.0456**	-1.176	0.1338	**1.315**	**0.0405**	-1.179	0.1144
Sonchus oleraceus L.	1.048	0.3256	-0.910	0.6872	1.299	0.0523	**-1.341**	**0.0441**
Taraxacum officinale (L.) Weber	**1.452**	**0.0144**	-1.318	0.0518	1.309	0.0533	**-1.336**	**0.0390**
Trifolium pratense L.					0.990	0.4333	-0.923	0.6149
Trifolium repens L.	**1.876**	**<0.0001**	**-1.837**	**0.0005**	**1.733**	**0.0010**	**-1.662**	**0.0041**
Veronica persica Poir.	**1.367**	**0.0344**	**-1.269**	**0.0790**	**1.758**	**0.0010**	**-1.664**	**0.0026**

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729 **Table S3.** Distribution of *stamp* sequence variants in weeds in 2013. Sequence variants with * are
730 those identified in grapevines. E: Erbusco; C: Corte Franca.

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Species	Vineyard	St_Fc1 (St19)*	St_Fc2 (St5)*	St_Fc6 (St30)*	St_Fc7 (St38)*	St_Fc8 (St8)*	St_Fc10 (St18)	St_Fc11	St_Fc12	St_Fc13	St_Fc14	St_Fc15 (St36)	St_Fc16	St_Fc17	St_Fc18 (St32)	St_Fc19	St_Fc20	Total
Achillea millefolium L.	C		1															1
Amaranthus retroflexus L.	E		2		1													3
Bellis perennis L.	E		2															2
Capsella bursa pastoris (L.) Medik	E		4								1							5
Chenopodium album L.	C		2															2
Cirsium arvense (L.) Scop	E		1															1
Convolvulus arvensis L.	E		14		1	1	2						1					19
	C		4				1								1			6
Crepis sp.	E		4															4
	C		3															3
Erigeron annuus (L.) Pers.	E		2															2
Erigeron canadensis (L.) Cronquist	E		4															4
	C	1	2															3
Geranium dissectum L.	E						1											1
Lactuca serriola L.	E		3					1										4
	C		2															2
Malva sylvestris L.	E	1	2															3
	C		1															1
Medicago lupolina L.	E		1						1									2
	C		1															1
Plantago lanceolata L.	E		1							1								2
	C		4															4
Plantago major L.	E		4															4
	C		3															3
Polygonum aviculare L.	E		2				1					1						4
	C		2															2
Portulaca oleracea L.	E		3															3
	C		3															3
Prunella vulgaris L.	E		1															1
Rumex acetosa L.	E				1					1								2
Senecio vulgaris L.	C			1														1
Solanum nigrum L.	E		2								1							3
	C		4															4
Sonchus oleraceus L.	E		3											1				4
	C		3															3
Taraxacum officinale (L.) Weber	E		1															1
Trifolium pratense L.	E						1											1
Trifolium repens L.	E		2				1											3
	C		3															3
Veronica persica Poir.	E		1															1
	C		4													1	1	6
	Total	2	101	1	3	1	7	1	1	2	2	1	1	1	1	1	1	127

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Table S4. Distribution of *stamp* sequence variants in weeds in 2014. Sequence variants with * are those identified in grapevines. E: Erbusco; C: Corte Franca.

Species	Vineyard	St_Fc1 (St19)*	St_Fc2 (St5)*	St_Fc4 (St10)*	St_Fc5 (St11)*	St_Fc21	St_Fc22	Total
Amaranthus retroflexus L.	**E**		1				1	2
	C	1						1
Artemisia vulgaris L.	**E**		1					1
Bellis perennis L.	**E**		3					3
Capsella bursa pastoris (L.) Medik	**E**		1					1
Chenopodium album L.	**E**		1					1
	C		4	1				5
Cirsium arvense (L.) Scop	**E**		3					3
Convolvulus arvensis L.	**E**		11					11
	C		5					5
Erigeron annuus (L.) Pers.	**E**		4					4
Geranium dissectum L.	**E**		4			1		5
Lactuca serriola L.	**C**		2					2
Malva sylvestris L.	**E**		3					3
Medicago lupolina L.	**E**		2					2
Plantago major L.	**E**		5					5
Polygonum aviculare L.	**E**		1					1
	C		2					2
Portulaca oleracea L.	**C**	1	1					2
Prunella vulgaris L.	**E**		1					1
Rumex acetosa L.	**E**		6					6
Solanum nigrum L.	**E**	1	10					11
Sonchus oleraceus L.	**E**		2					2
	C			1				1
Trifolium pratense L.	**E**		2					2
Trifolium repens L.	**E**		5					5
	C			2	1			3
Veronica persica Poir.	**E**		6					6
Total		3	86	4	1	1	1	96

TABLES

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TABLE 1. Grapevine, *H. obsoletus*, and weed samples collected, determined as infected and sequenced

Vineyard	Host	Year	Samples		Infection %	Sequenced
			Collected	Infected		
Erbusco	*V. vinifera*	2013	64	57	89	57
		2014	64	48	90	48
	H. obsoletus	2013	120	51	42	51
		2014	8	3	37	3
	Weeds	2013	781	113	14	79
		2014	419	94	22	75
Cortefranca	*V. vinifera*	2013	80	67	84	67
		2014	80	61	76	61
	H. obsoletus	2013	133	7	5	7
		2014	21	6	21	6
	Weeds	2013	584	63	11	48
		2014	449	21	5	21

750 **TABLE 2**. Incidence and CaPsol-infection rate in weeds observed in Erbusco and Corte Franca vineyard

Species	Cycle	Erbusco 2013 - 2014					Corte Franca 2013 - 2014				
		No. of cluster	Incidence	No. of collected samples	No. of CaPsol-infected samples	Infection %	No. of cluster	Incidence	No. of collected samples	No. of CaPsol-infected samples	Infection %
Achillea millefolium L.	P						25 - 46	0.2 - 0.3	15 - 3	1 - 0	7 - 0
Amaranthus retroflexus L.	A	429 - 405	8.1 - 7.6	22 - 17	5 - 3	23 - 18	0 - 67	0 - 0.4	0 - 5	0 - 1	0 - 20
Artemisia vulgaris L.	P	241 - 288	4.6 - 5.4	23 - 19	1 - 4	4 - 21	25 - 34	0.2 - 0.2	8 - 2	0 - 0	0 - 0
Bellis perennis L.	P	390 - 367	7.3 - 6.9	27 - 15	2 - 3	7 - 20					
Capsella bursa-pastoris (L.)	P	417 - 49	7.9 - 0.9	25 - 1	7 -1	28 - 100					
Chenopodium album L.	A	171 - 386	3.2 - 7.3	18 - 15	0 - 1	0 - 7	181 - 972	1.2 - 6.3	27 - 36	6 - 5	22 - 14
Cirsium arvense (L.) Scop	P	265 - 458	5.0 - 8.6	18 - 14	1 - 3	6 - 21	23 - 7	0.2 - 0.1	4 - 1	0 - 0	0 - 0
Convolvulus arvensis L.	P	1833 - 1908	34.5 - 41.4	33 - 12	20 - 12	61 - 38	621 - 414	4 - 2.7	39 - 33	3 - 5	8 - 15
Crepis sp.	A	1079 - 0	20.3 - 0	31 - 0	5 - 0	16 - 0	408 - 332	2.7 - 2.2	35 - 15	6 - 0	17 - 0
Erigeron annuus (L.) Pers.	A/P	115 - 347	2.2 - 6.5	15 - 14	3 - 5	20 - 36	0 - 8	0 - 0.1	0 - 1	0 - 0	0 - 0
Erigeron canadensis (L.)	A	969 - 442	18.2 - 8.2	33 - 13	7 - 5	21 - 8	40 - 66	0.3 - 0.4	20 - 5	3 - 0	15 - 0
Geranium dissectum L.	A	741 - 525	13.9 - 9.9	31 - 30	4 - 7	13 - 23					
Lactuca serriola L.	A/P	473 - 39	8.9 - 0.7	28 - 4	5 - 0	18 - 0	576 - 744	3.8 - 4.8	40 - 39	4 - 2	10 - 5
Lampsana communis L.	A	156 - 0	2.9 - 0	13 - 0	1 - 0	8 - 0	0 - 25	0 - 0.2	0 - 2	0 - 0	0 - 0
Malva sylvestris L.	A/P	603 - 392	11.4 - 7.4	30 - 20	2 - 4	7 - 20	50 - 65	0.3 - 0.4	14 - 3	1 - 0	7 - 0
Matricaria chamomilla L.	A	42 - 0	0.8 - 0	12 - 0	1 - 0	8 - 0					
Medicago lupolina L.	A/P	144 - 152	2.7 - 2.9	11 - 6	5 - 3	45 - 50	28 - 13	0.2 - 0.1	11 - 1	1 - 0	9 - 0
Oxalis sp.	P	53 - 34	1 - 0.6	6 - 2	1 - 0	17 - 0					
Papaver rhoeas L.	A	45 - 0	0.8 - 0	8 - 0	0 - 0	0 - 0					
Plantago lanceolata L.	P	2091 - 0	39.3 - 0	32 - 0	2 - 0	6 - 0	283 - 296	1.8 - 1.9	24 - 6	4 - 0	17 - 0
Plantago major L.	P	867 - 2007	16.3 - 37.8	31 - 31	5 - 5	16 - 16	719 - 1242	4.7 - 8.1	40 - 41	7 - 0	18 - 0
Polygonum aviculare L.	A	627 - 35	11.8 - 0.7	31 - 3	5 - 1	16 - 33	1390 - 1009	9.1 - 6.6	39 - 39	2 -2	5 - 5
Portulaca oleracea L.	A	861 - 0	16.2 - 0	28 - 0	2 - 0	7 - 0	227 - 142	1.5 - 0.9	36 -11	4 - 2	11 - 18
Potentilla reptans L.	P	92 - 0	1.7 - 0	8 - 0	0 - 0	0 - 0	15 - 30	0.1 - 0.2	8 - 3	1 - 0	13 - 0
Prunella vulgaris L.	P	18 - 110	0.3 - 2.1	9 - 6	1 -1	11 -17					
Rumex acetosa L.	P	1720 - 1984	32.4 - 37.3	31 - 32	3 - 6	10 - 19	13 - 18	0.1 - 0.1	6 - 2	0 - 0	0 - 0
Senecio vulgaris L.	A	261 - 0	4.9 - 0	27 - 0	0 - 0	0 - 0	483 - 342	3.1 - 2.2	36 - 13	1 - 0	3 - 0
Silene vulgaris (Moench) Garcke	P	85 - 92	1.6 - 1.7	16 - 12	1 - 0	6 - 0					
Solanum nigrum L.	A	515 - 1302	9.7 - 24.5	30 - 32	6 - 11	20 - 34	390 - 625	2.5 - 4.1	38 - 34	4 - 0	11 - 0
Sonchus oleraceus L.	A/P	1535 - 206	28.9 - 3.9	29 - 11	5 - 3	17 - 27	517 - 719	3.4 - 4.7	28 - 38	2 - 1	7 - 3
Taraxacum officinale (L.) Weber	P	3066 - 0	57.7 - 0	34 - 0	1 - 0	3 - 0	1852 - 1976	12.1 - 12.9	39 - 41	1 - 0	3 - 0
Trifolium pratense L.	P	637 - 1093	12 - 20.6	28 - 26	4 - 4	14 - 15	0 - 19	0 - 0.1	0 - 1	0 - 0	0 - 0
Trifolium repens L.	P	2753 - 3482	51.8 - 65.5	31 - 32	3 - 7	10 - 22	632 - 744	4.1 - 4.8	39 - 36	4 - 3	10 - 8
Veronica persica Poir.	A	2460 - 3505	46.3 - 66	32 - 32	5 - 9	16 - 28	322 - 629	2.1 - 4.1	38 - 38	8 - 0	21 - 0
			Total	781 - 419	113 - 94	14 - 22		**Total**	584 - 449	63 - 21	11 - 5

751 **TABLE 3**. Probability associated to spatial association index in Erbusco. Numbers in bold indicate
752 associations (P < 0.025), while numbers underlined indicate dissociations (P > 0.975)
753

Folder	2013				2014			
	Overall sympt. vines	***Hyalesthes obsoletus* captured**	***Hyalesthes obsoletus* CaPsol-infected**	**2014-new sympt. vines**	**Overall sympt. vines**	***Hyalesthes obsoletus* captured**	***Hyalasthes. obsoletus* CaPsol-infected**	**2015-new sympt. vines**
Overall sympt. vines		0.2458	0.8412	0.7234		0.1300	0.9016	0.3748
2014-new sympt. vines	0.7234	0.6545	0.5792					
2015-new sympt. vines					0.3748	0.1732	0.5673	
H. obsoletus captured	0.2458		**0.0137**	0.6545	0.1300		**0.0216**	0.1732
H. oobsoletus CaPsol-infected	0.8412	**0.0137**		0.5792	0.9016	**0.0216**		0.5673
Amaranthus retrofexus L.	0.4469	0.6180	0.3442	0.7641	0.1730	0.1543	0.0782	0.6342
Artemisia vulgaris L.	0.7263	0.4848	0.4283	0.2632	0.9610	0.7564	0.1699	0.1356
Bellis perennis L.	0.9657	0.6195	0.2626	0.0943	0.9631	0.3452	0.8732	0.1723
Capsella bursa pastoris (L.) Medik	**0.0048**	0.3614	0.9684	0.7145	0.9739	0.8534	0.5583	0.6591
Chenopodium album L.	0.0585	0.5812	0.5546	0.9548	0.6326	0.6573	0.3264	0.8963
Cirsium arvense (L.) Scop	0.4663	0.0775	0.5120	0.1876	0.6268	0.0745	0.9712	0.3561
Convolvulus arvensis L.	0.9561	0.6138	0.3534	**0.0005**	0.9118	0.2369	0.0457	**0.0125**
Crepis sp.	0.8923	0.0787	0.3968	0.0475				
Erigeron annuus (L.) Pers.	0.9752	0.6044	0.2405	0.0382	0.9821	0.6901	0.1893	0.7845
Erigeron canadensis (L.) Cronquist	0.9182	0.4047	0.4312	0.0383	0.8546	0.7592	0.7563	0.1105
Geranium dissectum L.	0.9681	0.7948	0.2877	0.1308	0.9079	0.0367	0.9561	0.3815
Lactuca serriola L.	0.9362	0.4920	0.4444	**0.0201**	**0.0084**	0.8799	0.0345	0.6773
Lampsana communis L.	0.6548	0.2397	0.4809	0.1035				
Malva sylvestris L.	0.9877	0.3462	0.0424	0.0441	0.9988	0.7569	0.8230	0.0672
Matricaria chamomilla L.	0.0336	0.0300	0.3483	0.3614				
Medicago lupolina L.	0.9658	0.6115	0.2813	0.0319	0.9060	0.1287	0.5634	0.0723
Oxalis sp.	0.3898	0.3291	0.5444	0.3851	0.8508	0.2135	0.8674	0.6453
Papaver rhoeas L.	0.5856	0.3596	0.8785	0.2018				
Plantago lanceolata L.	0.8830	0.6325	0.5373	0.2160				
Plantago major L.	0.9808	0.7481	0.2524	0.3719	0.9966	0.7791	0.3672	0.4372
Polygonum aviculare L.	0.6942	0.8316	0.4189	0.3662	0.3674	0.3102	0.5749	0.2471
Portulaca oleracea L.	0.0866	0.5109	0.6125	0.7579				
Potentilla reptans L.	0.4346	0.2369	0.5174	0.0912				
Prunella vulgaris L.	0.6326	0.9233	0.9534	0.3888	0.9555	0.8910	0.3645	0.5732
Rumex acetosa L.	0.9438	0.7237	0.3655	0.0931	0.8931	0.4991	0.4563	0.5792
Senecio vulgaris L.	0.1530	0.0769	0.3432	0.5726				
Silene vulgaris (Moench) Garcke	0.3040	0.8167	0.6554	0.7282	0.4953	0.6325	0.8571	0.1673
Solanum nigrum L.	0.2736	0.8802	0.7572	0.9544	0.9788	0.6578	0.0346	0.8674
Sonchus oleraceus L.	0.9931	0.5927	0.2664	0.0462	0.7007	0.3461	0.9634	0.0678
Taraxacum officinale (L.) Weber	0.9839	0.7515	0.3425	**0.0106**				
Trifolium pratense L.	0.0274	0.4904	0.3533	0.9780	0.0431	0.8871	0.0673	0.7764
Trifolium repens L.	0.9166	0.5141	0.4719	**0.0028**	0.9781	0.2456	0.9681	**0.0054**
Veronica persica Poir.	0.9941	0.5157	0.0366	0.0742	0.9781	0.0841	0.1152	0.1921

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757 **TABLE 4**. Probability associated to spatial association index in Corte Franca. Numbers in bold
758 indicate associations (P < 0.025), while numbers underlined indicate dissociations (P > 0.975)
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Folder	**2013**				**2014**			
	Overall sympt. vines	***Hyalesthes obsoletus* captured**	***Hyalesthes obsoletus* CaPsol-infected**	**2014-new sympt. vines**	**Overall sympt. vines**	***Hyalesthes obsoletus* captured**	***Hyalasthes. obsoletus* CaPsol-infected**	**2015-new sympt. vines**
Overall sympt. vines		0.1838	0.6089	**0.0011**		0.0463	0.2387	0.0351
2014-new sympt. vines	**0.0011**	**0.0160**	0.0417					
2015-new sympt. vines					0.0283	0.5721	0.6735	
H. obsoletus captured	0.1838		**0.0001**	**0.0160**	0.0463		**0.0231**	0.0871
H. obsoletus CaPsol-infected	0.6089	**0.0001**		0.0417	0.2387	**0.0231**		0.0965
Achillea millefolium L.	0.6326	0.9691	0.8685	0.4641	0.0954	0.9534	0.9744	0.5641
Amaranthus retroflexus L.					0.4038	0.5784	0.9732	0.2371
Artemisia vulgaris L.	0.3644	0.9372	0.6745	0.2711	0.1680	0.6784	0.4531	0.9831
Chenopodium album L.	0.2396	0.3974	0.3347	**0.0086**	0.6215	0.4463	0.3875	**0.0203**
Cirsium arvense (L.) Scop	0.3291	0.1737	0.1071	**0.0022**	0.4217	0.2431	0.7649	**0.0215**
Convolvulus arvensis L.	0.2034	0.9805	0.9729	0.8351	0.6727	0.6748	0.8643	0.4985
Crepis sp.	0.0987	0.4792	0.9968	0.6395	0.0953	0.3845	0.7851	0.8452
Erigeron annuus (L.) Pers.					0.0863	0.1388	0.0989	0.1097
Erigeron canadensis (L.) Cronquist	0.5197	0.2205	**0.0021**	0.0372	0.0740	0.0274	0.0302	0.5612
Lactuca serriola L.	0.8162	0.9647	0.9753	0.9594	0.3338	0.7896	0.8997	0.9987
Lampsana communis L.					0.0986	0.2018	0.3876	0.7591
Malva sylvestris L.	0.1437	0.8479	0.8003	**0.0247**	0.0799	0.0577	0.0734	**0.0207**
Medicago lupolina L.	0.4906	0.8944	0.8898	0.3162	0.1484	0.6754	0.5498	0.2564
Plantago lanceolata L.	0.2438	0.1218	0.9612	0.3408	0.1388	0.7643	0.1907	0.7651
Plantago major L.	0.3815	0.5534	0.4794	0.3344	0.0644	0.7654	0.5873	0.6843
Polygonum aviculare L.	0.1575	0.4193	0.1626	**0.0012**	**0.0232**	0.3121	0.0278	**0.0138**
Portulaca oleracea L.	0.5514	0.9239	0.4955	0.3698	0.1164	0.4563	0.8567	0.1785
Potentilla reptans L.	0.1919	**0.0064**	**0.0009**	0.0959	0.5280	**0.0235**	0.0354	0.0312
Rumex acetosa L.	0.8659	0.6638	0.3069	0.4106	0.6633	0.5673	0.7864	0.5632
Senecio vulgaris L.	0.0906	0.8496	0.9588	0.4914	0.3855	0.1982	0.8711	0.9332
Solanum nigrum L.	0.0694	0.9152	0.8795	0.0654	0.0493	0.7651	0.8892	0.0258
Sonchus oleraceus L.	**0.0241**	0.8315	0.9776	0.6945	0.7045	0.7895	0.6423	0.9531
Taraxacum officinale (L.) Weber	0.1276	0.9992	0.9995	0.9140	0.5784	0.9921	0.9932	0.7966
Trifolium pratense L.					0.0673	0.4572	0.3379	0.1905
Trifolium repens L.	0.3527	0.8306	0.9235	**0.0241**	0.0367	0.2167	0.7692	**0.0192**
Veronica persica Poir.	0.0756	**0.0069**	**0.0001**	**0.0093**	0.1022	**0.0145**	**0.0231**	**0.0063**

760 **TABLE 5.** Distribution of *stamp* sequence variants among CaPsol strains identified in Erbusco and Corte Franca vineyards in 2013-14

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***Stamp* sequence variant**	**Accession number**	**Number of strains**												**Total (%)**
		Erbusco 2013			**Erbusco 2014**			**Corte Franca 2013**			**Corte Franca 2014**			
		grapevine	***H. obsoletus***	**weeds**	**grapevine**	***H. obsoletus***	**weeds**	**grapevine**	***H. obsoletus***	**weeds**	**grapevine**	***H. obsoletus***	**weeds**	
St_Fc1 (St19)	MT777487	14	7	1	16	1	1	27	0	1	23	0	2	93 (17.8)
St_Fc2 (St5)	MT777488	15	37	59	22	2	72	26	4	42	25	3	14	321 (61.4)
St_Fc3 (St9)	MT777489	3	0	0	0	0	0	2	0	0	0	0	0	5 (1)
St_Fc4 (St10)	MT777490	0	4	0	3	0	0	1	1	0	7	2	4	22 (4.2)
St_Fc5 (St11)	MT777491	15	0	0	5	0	0	7	0	0	6	1	1	35 (6.7)
St_Fc6 (St30)	MT777492	0	0	0	0	0	0	2	1	1	0	0	0	4 (0.8)
St_Fc7 (St38)	MT777493	0	0	3	0	0	0	2	0	0	0	0	0	5 (1)
St_Fc8 (St8)	MT777494	9	0	1	2	0	0	0	1	0	0	0	0	13 (2.5)
St_Fc9 (St60)	MT777495	1	0	0	0	0	0	0	0	0	0	0	0	1 (0.2)
St_Fc10 (St18)	MT777496	0	3	6	0	0	0	0	0	1	0	0	0	10 (1.9)
St_Fc11 (St61)	MT777497	0	0	1	0	0	0	0	0	0	0	0	0	1 (0.2)
St_Fc12 (St62)	MT777498	0	0	1	0	0	0	0	0	0	0	0	0	1 (0.2)
St_Fc13 (St63)	MT777499	0	0	2	0	0	0	0	0	0	0	0	0	2 (0.38)
St_Fc14 (St64)	MT777500	0	0	2	0	0	0	0	0	0	0	0	0	2 (0.38)
St_Fc15 (St36)	MT777501	0	0	1	0	0	0	0	0	0	0	0	0	1 (0.2)
St_Fc16 (St65)	MT777502	0	0	1	0	0	0	0	0	0	0	0	0	1 (0.2)
St_Fc17 (St66)	MT777503	0	0	1	0	0	0	0	0	0	0	0	0	1 (0.2)
St_Fc18 (St32)	MT777504	0	0	0	0	0	0	0	0	1	0	0	0	1 (0.2)
St_Fc19 (St67)	MT777505	0	0	0	0	0	0	0	0	1	0	0	0	1 (0.2)
St_Fc20 (St68)	MT777506	0	0	0	0	0	0	0	0	1	0	0	0	1 (0.2)
St_Fc21 (St69)	MT777507	0	0	0	0	0	1	0	0	0	0	0	0	1 (0.2)
St_Fc22 (St70)	MT777508	0	0	0	0	0	1	0	0	0	0	0	0	1 (0.2)

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TABLE 6. Weed attribution to BN epidemiological groups

Species	Criteria for epidemiological group attribution[a]				Group
	Erbusco		Corte Franca		
	2013	2014	2013	2014	
Achillea millefolium L.			y-n-n	n-n-n	3
Amaranthus retroflexus L.	y-n-n	y-n-n		y-n-n	3
Artemisia vulgaris L.	n-n-n	y-n-n	n-n-n	n-n-n	3
Bellis perennis L.	y-n-n	y-n-n			3
Capsella bursa-pastoris (L.) Medik	y-y-n	y-n-n			2
Chenopodium album L.	n-n-n	y-n-n	y-y-n	y-y-n	1
Cirsium arvense (L.) Scop	y-n-n	y-n-n	n-y-n	n-y-n	3
Convolvulus arvensis L.	y-y-n	y-y-n	y-n-n	y-n-n	1
Crepis sp.	y-n-n		y-n-n	n-n-n	3
Erigeron annuus (L.) Pers.	y-n-n	y-n-n		n-n-n	3
Erigeron canadensis (L.) Cronquist	y-n-n	n-n-n	y-n-y	n-n-n	2
Geranium dissectum L.	n-n-n	y-n-n			3
Lactuca serriola L.	y-y-n	n-y-n	y-n-n	y-n-n	2
Lapsana communis L.	n-n-n			n-n-n	4
Malva sylvestris L.	y-n-n	y-n-n	y-y-n	n-y-n	2
Matricaria chamomilla L.	n-n-n				4
Medicago lupolina L.	y-n-n	y-n-n	y-n-n	n-n-n	3
Oxalis sp.	n-n-n	n-n-n			4
Papaver rhoeas L.	n-n-n				4
Plantago lanceolata L.	y-n-n		y-n-n	n-n-n	3
Plantago major L.	y-n-n	y-n-n	y-n-n	n-n-n	3
Polygonum aviculare L.	y-n-n	y-n-n	y-y-n	y-y-n	1
Portulaca oleracea L.	y-n-n		y-n-n	y-n-n	3
Potentilla reptans L.	n-n-n		y-n-n	y-n-n	3
Prunella vulgaris L.	y-n-n	y-n-n			3
Rumex acetosa L.	y-n-n	y-n-n	n-n-n	n-n-n	3
Senecio vulgaris L.	n-n-n		y-n-n	n-n-n	3
Silene vulgaris (Moench) Garcke	n-n-n	n-n-n			4
Solanum nigrum L.	y-n-n	y-n-n	y-n-n	n-n-n	3
Sonchus oleraceus L.	y-n-n	y-n-n	y-y-n	y-n-n	2
Taraxacum officinale (L.) Weber	y-y-n		n-n-n	n-n-n	2
Trifolium pratense L.	n-n-n	y-n-n		n-n-n	3
Trifolium repens L.	y-y-n	y-y-n	y-y-n	y-y-n	1
Veronica persica Poir.	y-n-n	y-n-n	y-y-y	n-y-y	2

[a] y (yes) or n (no) indicate if the following criteria are met: (i) if the weed harbors the same CaPsol strains characterized by *stamp* gene sequence variants found also in symptomatic grapevines, (ii) statistically significant association with overall and/or new symptomatic grapevines, and (iii) statistically significant association with *H. obsoletus*

779 **FIGURE LEGENDS**

780 Quaglino *et al*. [New insights on Bois noir epidemiology]

781

782 **Figure 1.** Graphical representation of clustering into patch and into gap of symptomatic grapevines, *H.*
783 *obsoletus* and weeds in Erbusco and Corte Franca vineyards in 2013 and 2014. Blue squares represent
784 statistically significant results, according to randomization test ($\alpha = 0.05$); green squares represent non-
785 significant results, according to randomization test ($\alpha = 0.05$); white squares indicate not applicable data (for
786 example, weeds not observed). Average indexes of clustering into patch (mean v_i) and into gap (mean v_j)
787 with associated probability (P) from randomization test are available in Tables S1 and S2.

788 **Figure 2.** Map of counts and clustering indexes of overall symptomatic grapevines in 2013 (a), new
789 symptomatic grapevines in 2014 (b), *Convolvulus arvensis* in 2013 (c), and *Trifolium repens* in 2013 (d) in
790 Erbusco vineyard. The maps show an example of statistically significant association of two weeds (*C.*
791 *arvensis* and *T. repens*) of epidemiological group 1 with grapevines. Dots represent number of plants or
792 insects observed in each plot. Red areas represent patches with interpolated cluster index $v_i > 1.5$. Blue areas
793 are gaps with interpolated cluster index $v_j < -1.5$. Values on axis indicate coordinates in meters.

794 **Figure 3.** Map of counts and clustering indexes of overall symptomatic grapevines in 2013 (a), new
795 symptomatic grapevines in 2014 (b), *Chenopodium album* in 2013 (c), *Polygonum aviculare* in 2013 (d), and
796 *Trifolium repens* in 2013 (e) in Corte Franca vineyard. The maps show an example of statistically significant
797 association of three weeds (*C. album*, *P. aviculare*, and *T. repens*) of epidemiological group 1 with
798 grapevines. Dots represent number of plants or insects observed in each plot. Red areas represent patches
799 with interpolated cluster index $v_i > 1.5$. Blue areas are gaps with interpolated cluster index $v_j < -1.5$. Values
800 on axis indicate coordinates in meters.

801 **Figure 4.** Unrooted phylogenetic tree inferred from *stamp* gene nucleotide sequences of CaPsol strains
802 representative of *stamp* sequence variants previously described and identified in this study (Table 5);
803 minimum evolution analysis was performed using the neighbor-joining method and bootstrap replicated
804 1000 times. Names of strains are reported on the image. GenBank accession number of each sequence is
805 given in parenthesis; gene sequences obtained in the present study are indicated in bold. Clusters are shown
806 as delimitated by parentheses.

807 **Figure 1.**

808

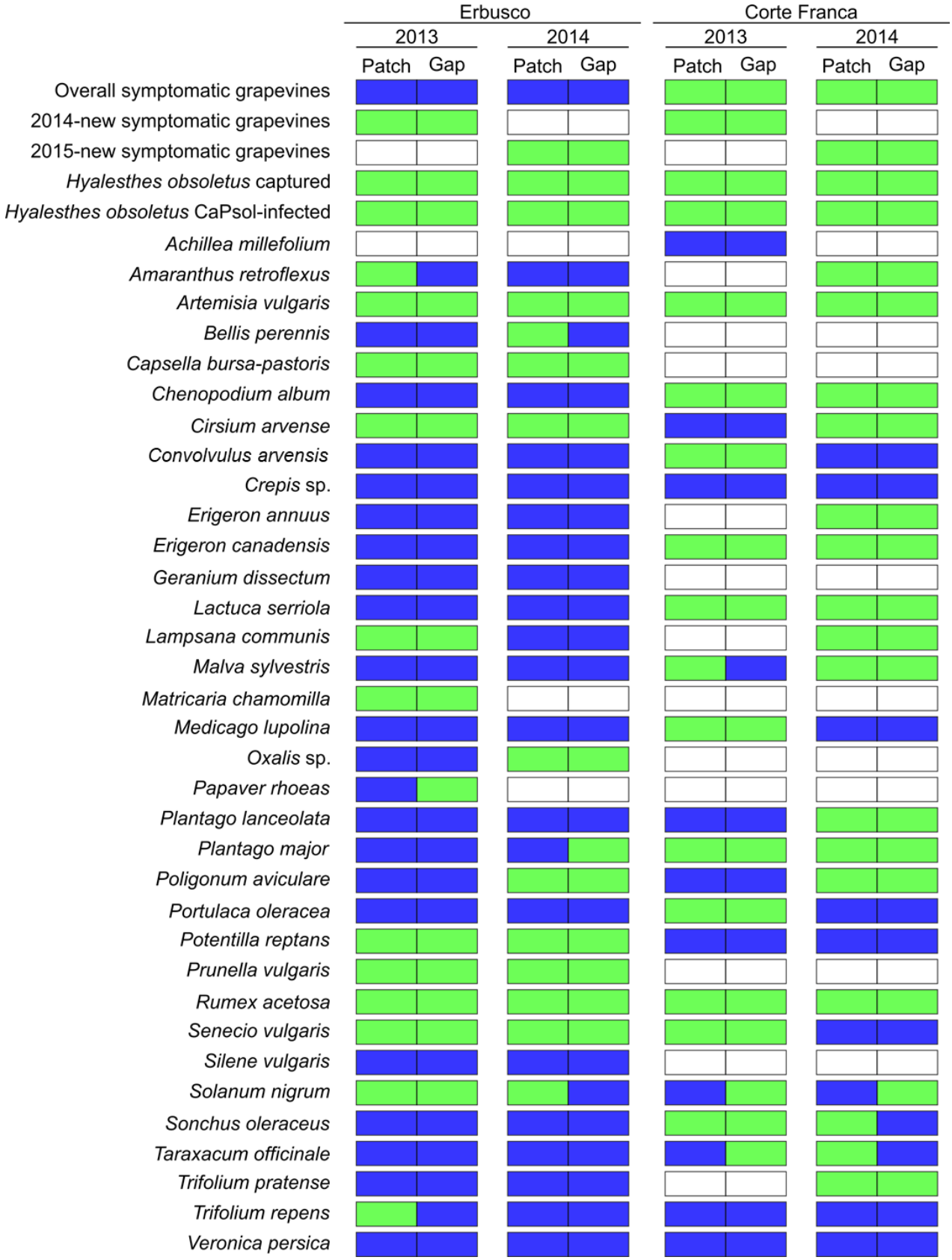

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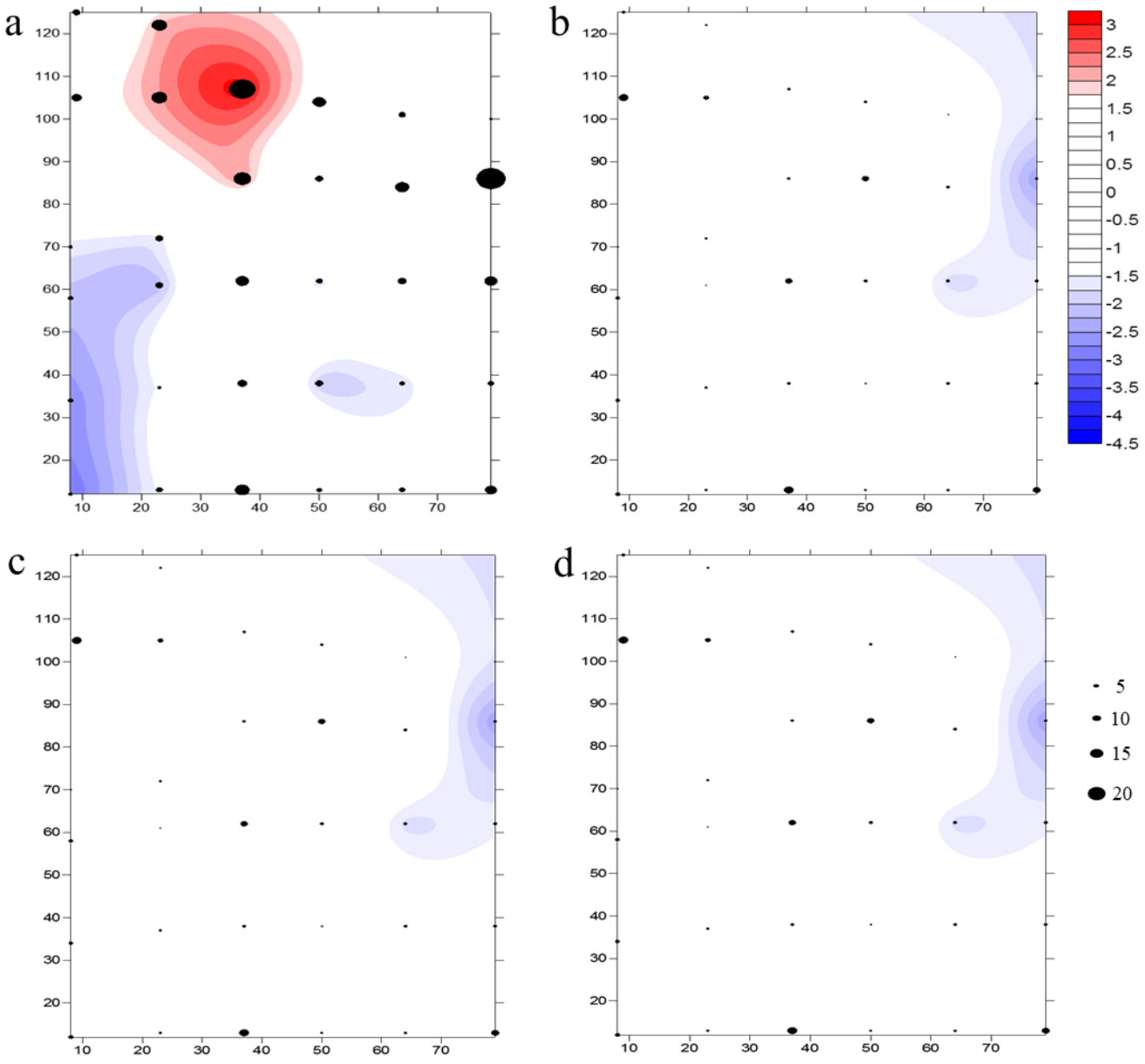
Figure 2.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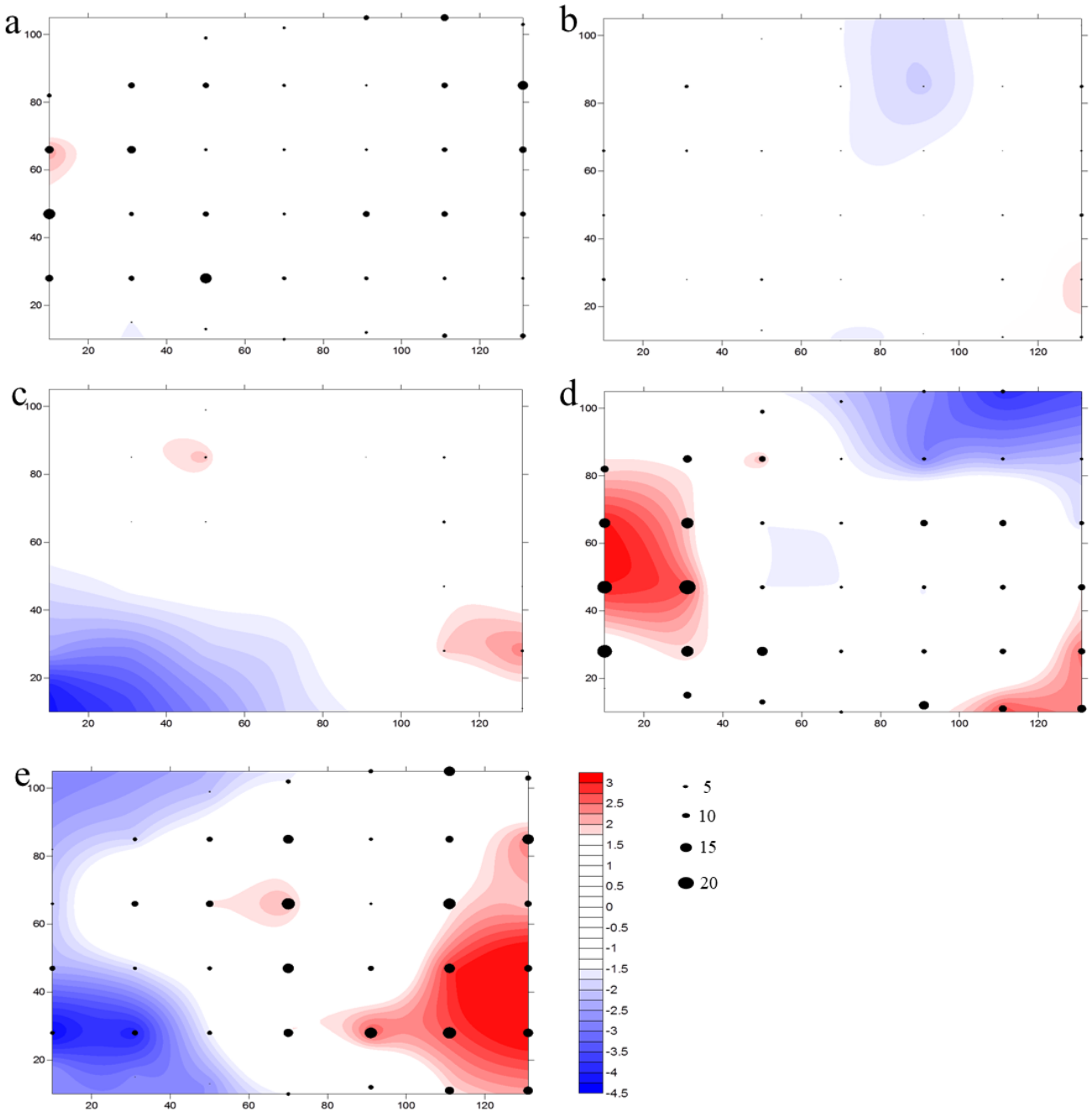
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
5
10
15
20

822

Figure 3.



823

824

825

826

827

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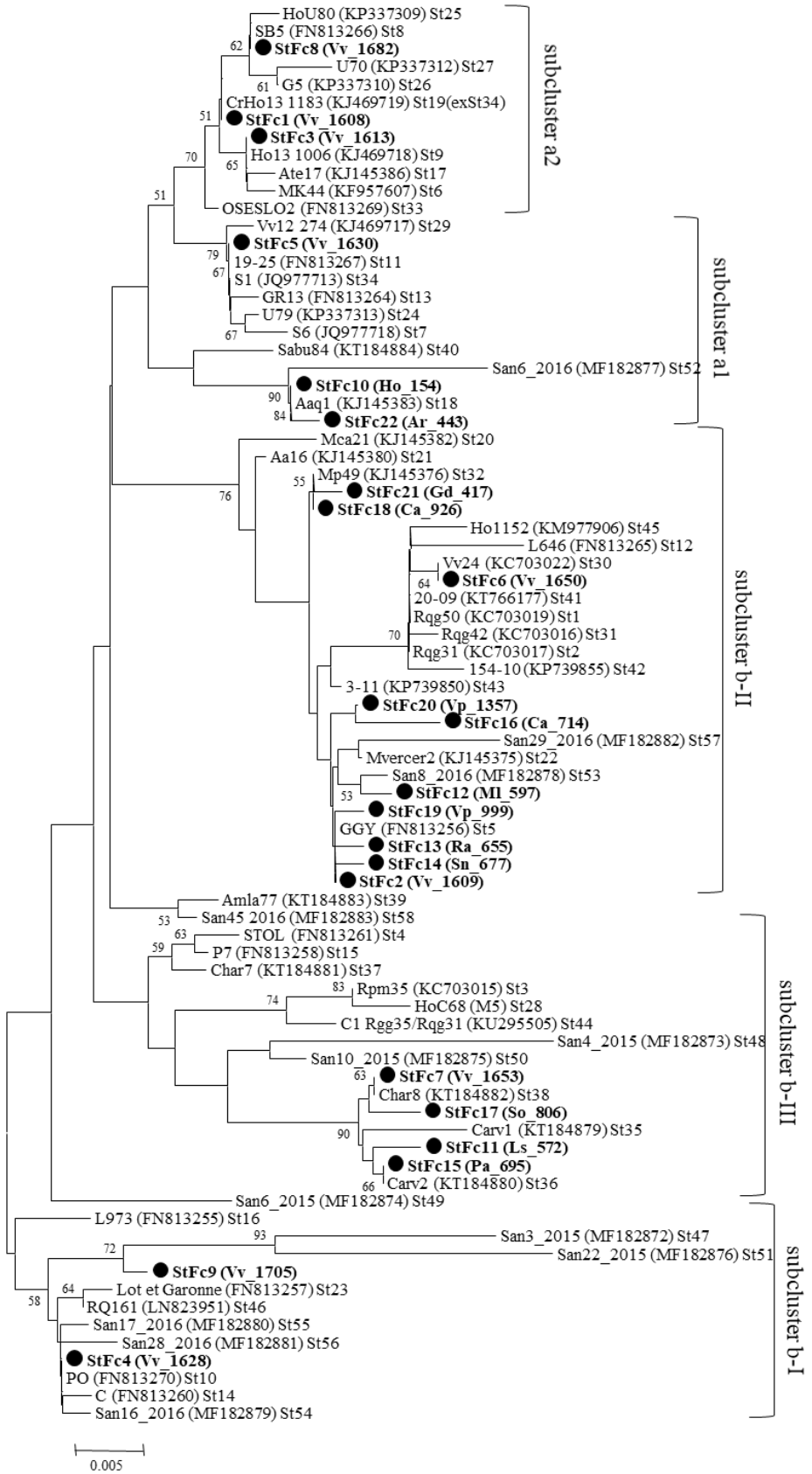
829

830

Figure 4.


HoU80 (KP337309) St25
SB5 (FN813266) St8
StFc8 (Vv_1682)
U70 (KP337312) St27
G5 (KP337310) St26
CrHo13 1183 (KJ469719) St19(exSt34)
StFc1 (Vv_1608)
StFc3 (Vv_1613)
Ho13 1006 (KJ469718) St9
Ate17 (KJ145386) St17
MK44 (KF957607) St6
OSESLO2 (FN813269) St33
subcluster a2
Vv12 274 (KJ469717) St29
StFc5 (Vv_1630)
19-25 (FN813267) St11
S1 (JQ977713) St34
GR13 (FN813264) St13
U79 (KP337313) St24
S6 (JQ977718) St7
Sabu84 (KT184884) St40
San6_2016 (MF182877) St52
StFc10 (Ho_154)
Aaq1 (KJ145383) St18
StFc22 (Ar_443)
subcluster a1
Mca21 (KJ145382) St20
Aa16 (KJ145380) St21
Mp49 (KJ145376) St32
StFc21 (Gd_417)
StFc18 (Ca_926)
Ho1152 (KM977906) St45
L646 (FN813265) St12
Vv24 (KC703022) St30
StFc6 (Vv_1650)
20-09 (KT766177) St41
Rqg50 (KC703019) St1
Rqg42 (KC703016) St31
Rqg31 (KC703017) St2
154-10 (KP739855) St42
3-11 (KP739850) St43
StFc20 (Vp_1357)
StFc16 (Ca_714)
San29_2016 (MF182882) St57
Mvercer2 (KJ145375) St22
San8_2016 (MF182878) St53
StFc12 (Ml_597)
StFc19 (Vp_999)
GGY (FN813256) St5
StFc13 (Ra_655)
StFc14 (Sn_677)
StFc2 (Vv_1609)
subcluster b-II
Amla77 (KT184883) St39
San45 2016 (MF182883) St58
STOL (FN813261) St4
P7 (FN813258) St15
Char7 (KT184881) St37
Rpm35 (KC703015) St3
HoC68 (M5) St28
C1 Rgg35/Rqg31 (KU295505) St44
San4_2015 (MF182873) St48
San10_2015 (MF182875) St50
StFc7 (Vv_1653)
Char8 (KT184882) St38
StFc17 (So_806)
Carv1 (KT184879) St35
StFc11 (Ls_572)
StFc15 (Pa_695)
Carv2 (KT184880) St36
subcluster b-III
San6_2015 (MF182874) St49
L973 (FN813255) St16
San3_2015 (MF182872) St47
San22_2015 (MF182876) St51
StFc9 (Vv_1705)
Lot et Garonne (FN813257) St23
RQ161 (LN823951) St46
San17_2016 (MF182880) St55
San28_2016 (MF182881) St56
StFc4 (Vv_1628)
PO (FN813270) St10
C (FN813260) St14
San16_2016 (MF182879) St54
subcluster b-I
0.005