O4: Role of *Colomerus vitis* (Pagenstecher) in the epidemiology of grapevine leaf mottling and deformation in North-eastern Italy

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INTRODUCTION

Grapevine Pinot gris virus (GPGV) was discovered in Italy in 2012 (Giampietruzzi et al., 2012) and successively in several grape-growing regions worldwide infecting different varieties. Studies associating GPGV with symptoms of leaf mottling and deformation (GLMD) showed that different strains of the virus responsible for eliciting or not the symptoms exist (Saldarelli, 2015) and that *Colomerus vitis* (Pagenstecher) collected from infected grapes were able to transmit GPGV to healthy grapevines (Malagnini et al., 2016). GPGV represents a potential threat for grapevine production in Europe and elsewhere (e.g., Beber et al., 2013; Beuve et al., 2015; Raiola et al., 2013; Morelli et al., 2014; Fan et al., 2015; Al Rwahnih et al., 2016). Acquisition and transmission by an arthropod vector is central to the infection cycle of the majority of plant pathogenic viruses. Filling the gap of information of epidemiological aspects of GPGV strains/*C. vitis* interactions would help in implementing efficient strategies of control of the associated GLMD disease. The current study was aimed at identifiying the main drivers of GPGV spread and define the epidemiology of GLMD disease in North-eastern Italy vineyards. In particular, the spatio-temporal distribution of GPGV symptomatic grapes and *C. vitis* was studied in two vineyards between 2013 and 2015 growing seasons. Later, GPGV and *C. vitis* distributions were coupled assuming the existence of a potential relationship between eriophyoid mites and GPGV spread. Moreover bait grapevine plants were used in a symptomatic vineyard to verify the progress of natural infection.

MATERIAL AND METHODS

Acquisition and transmission trials were carried out under controlled conditions (22±2°C, 70 % RH±10, 16:8 L:D). In transmission GPGV infected buds and leaf erinea infested by *C. vitis* were placed onto GPGV free vines and left until tissue desiccation. After transmission trials, the presence of GPGV in single individuals of C. vitis and in grapevine plants was ascertained by RT-PCR. Total RNAs of single *C. vitis* specimens were extracted using a modified Trizol (Malagnini et al., 2016) method and GPGV detection was carried out using RT-PCR (Glasa et.al. 2014; Saldarelli et al. 2015) and real time RT-PCR (Ratti personal communication 2015). PCR products were both sequenced and compared with GenBank.
In acquisition trials eriophyid mites collected from healthy plants were put on infected grapevine leaves and resampled after 1, 4 and 8 hours for GPGV detection.
The spatial distribution of GPGV symptomatic grapes was studied in three subsequent growing seasons (2013, 2014 and 2015) in two vineyards located in the Trento Province, North-eastern Italy. In 2015 the spatial distribution of *C. vitis* was also assessed. Indices of local aggregation were interpolated by Kriging analysis and were mapped. The degree of spatial association among the different considered variables was also quantified. Each vineyards was subdivided in plots of 5 plants using a regular grid and the number of plants with symptoms of *C. vitis* and GLMD was counted. Spatial Analysis by Distance IndicEs (SADIE) were performed to map the spatial distribution pattern of plants with symptoms of *C. vitis*, GLMD and new GLMD symptomatic plants occurring during the trial. Using SADIE red – blue methods, we evaluated the local contribution to a group (cluster) of relatively high-density (patch) or to a group of zero or relatively small counts (gap) over the period of study. Tests of non-randomness based on overall index of aggregation (Ia) were performed ($\alpha = 0.05$). The similarity between the spatial patterns of *C. vitis*, GLMD and new GLMD symptomatic plants was also quantified by estimating the spatial association index and its associated probability (Px, two-tail test $\alpha = 0.05$).

To assess GPGV natural infection bait grapevine plants, previously tested free of GPGV, were placed close to symptomatic vines in an infected vineyard. Each group of bait vines consisting of ten plants were exposed for about one month and then were placed in a screen house to avoid subsequent infection. The trial started on May 2016 and stopped on October 2016. One group of vines was left in the vineyard from October 2016 until the spring 2017. The same experiment was repeated during 2017. Each bait vine was inspected for the presence of leaf erinea and eriophyid mites were collected. All bait vines and mites were tested to assess the presence of GPGV as above described.

RESULTS AND DISCUSSION

Acquisition trials showed that *C. vitis* can assume GPGV from both symptomatic and asymptomatic-infected leaves in four hours. Transmission trials confirmed that *C. vitis* can transmit GPGV to healthy grapevine plants under controlled condition as described in Malagnini et al (2016), moreover these data suggest that *C. vitis* can transmit GPGV from infected symptomatic and asymptomatic vines to healthy grapevine plants under controlled condition.

An aggregated distribution was found in both vineyards for GLDM while the distribution of plants bearing *C. vitis* galls was aggregated only in one vineyard. The distributions of GLDM symptomatic plants (= GPGV symptomatic grapevines) observed in the three growing seasons (2015, 20016 and 2017) were always associated among each other's. In one vineyard the distribution of *C. vitis* and of plants showing "new occurring" GLMD symptoms was associated. Spatial distributions of GLMD symptomatic plants over the seasons were substantially stable showing a slight increase of symptoms. However, *C. vitis* distribution was only partially associated with that of GLMD symptomatic plants and further studies are required to clarify this aspect.

GPGV was detected in bait plants placed in infected vineyards. Eriophyid mites collected from leaf erinea and buds of these vines were positive to GPGV.

These results represent an important milestone to understand the spread of GPGV in field.

However further studies are requested to improve our knowledge on relationships between vectors and virus spread.

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