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ORIGINAL RESEARCH PAPER

Initiation and development of *Erysiphe necator* chasmothecia and their role in the epidemiology of grapevine powdery mildew in southern Syria

Nujoud Alimad[1], Walid Naffaa[2]*, Fawaz Azmeh[3]

[1] Faculty of Agriculture, Damascus University, Sweida branch, Sweida, Syria
[2] Department of Plant Protection, Faculty of Agriculture, Damascus University, 30621 Damascus, Syria
[3] National Commission for Biotechnology, 30621 Damascus, Syria

* Corresponding author. Email: walid1851966@yahoo.com

Abstract

Powdery mildew caused by *Erysiphe necator* is the most important fungal disease of grapevine in southern Syria. The purpose of this study was to determine the development of chasmothecia and their role as a primary inoculum in spring. Leaves and/or branches were examined by a stereo binocular from July to December 2014 and 2015. The number of chasmothecia was estimated on both surfaces of the leaves, and their viability was estimated by microscopic examination. During 2 years of survey chasmothecia were detected in 45.5% of vineyards. The initial development of chasmothecia on infected leaves was observed in the second half of July. Their numbers increased from July to October, and the sudden reduction at the beginning of November was noted. Chasmothecia were formed on 38.7% of infected leaves, with 12.5%, 18.4%, and 7.5% on the upper, under and on both surfaces of infected leaves respectively. Chasmothecia were more frequent on the leaf under side (0.6 / leaf) than on the leaf upper side (0.4 / leaf), but their occurrence on both sides together was relatively low (0.2 / leaf), and their numbers were highly variable between vineyards and years. Microscopic examination showed that chasmothecia contained 1–5 (usually three) asci with 1–4 (usually three) ascospores in each asci, and 65.6% of chasmothecia were empty. Their viability decreased between December and February, with an average viability of 1.2% and 0.2% in March and April, respectively. Chasmothecia were not detected on bark and ascospores were not trapped at the beginning of the season. These results indicate that the ascospores have no or little role in the initiation of spring infection. To the best of our knowledge, this is the first report of *E. necator* chasmothecia development and their role in the initiating infection on grapevine in Syria.

Keywords

grapevine; powdery mildew; chasmothecia; primary inoculum; *Erysiphe necator*; Syria

Introduction

Powdery mildew, caused by the fungus *Erysiphe necator* Schwein., is one of the most economically important grapevine diseases, and it causes heavy yield losses as well as a reduction in the quality of the produced wine [1]. In Syria, because of the presence of favorable environmental conditions for the development of grapevine powdery mildew, an intense application of fungicides from bud break to the end of season is necessary to control this disease (Naffaa, unpublished data).

Erysiphe necator produces sexual fruiting bodies (chasmothecia) only on the surface of heavily infected tissues of grapevine [2,3]. Chasmothecia are produced mainly on leaves, but also on shoots, branches, and berries [4]. The formation of chasmothecia begins within 48 hours when uninucleate hyphae of two compatible mating types come in contact [5,6]. In the early stages of development, chasmothecia are white translucent, turn to light yellow or cream, and finally to dark brown or black at maturation [7]. During this time, the ascocarp also increases in size.

The principal sources of primary inoculum of powdery mildew on grapevine are mycelium within dormant buds, and/or ascospores borne in chasmothecia [8]. During winter, *E. necator* survives as mycelium in dormant buds of grapevine until the following season, where shoots emerged from infected buds are covered with white to grayish mycelium with abundant sporulation, and appear stunted with wrinkled and deformed leaves [9]. These infected shoots are known as "flag shoots", and carry abundant conidia that cause secondary infections [10]. In most viticultural areas, chasmothecia are the main source of primary inoculum of powdery mildew [4], while they serve as an additional source of primary inoculum when the flag shoots are common [11].

In Syria, the first study conducted about the biology of *E. necator* showed that the flag shoots were the main source of primary inoculum (unpublished data), but the role of chasmothecia in initiating the infection remained unclear. So, the objective of this research was to study the formation, development and occurrence of *E. necator* chasmothecia, and to highlight their potential contribution to the onset of the disease in southern Syria.

Material and methods

Chasmothecia on leaves and branches

During the period from June until the leaf fall in 2014 and 2015, leaves, branches, and berries showing powdery mildew symptoms were collected and placed in plastic bags from 11 vineyards located in five different sites in Sweida (southern Syria), where several local grape varieties are grown (Tab. 1).

In spring, just before bud break, 50 samples of branches were collected, brought to the laboratory, and examined for the chasmothecia formation on the bark [10]. Leaves and branches were examined in a stereo binocular for the chasmothecia observation, and the number of chasmothecia was estimated on the upper and under surfaces of the leaves.

Samples of 30 g of bark were placed in 2-L Erlenmeyer flasks containing 1.5 L of water. The flasks were shaken vigorously by hand for 3 min and the suspension was filtered through 120- and 150-mesh Cobb sieves. The content of the 150-mesh sieve was recovered in 25 mL of water, and the suspension was examined under a light microscope [12].

In fall, senescent leaves still on vines or fallen to the ground were collected, placed in 50 × 50 × 50 cm iron cages covered with polyethylene, and kept in the vineyard during winter to study the development and maturation of chasmothecia and their viability. To evaluate viability, 50 chasmothecia collected from infected leaves of each of five surveyed vineyards, where chasmothecia were observed, were examined in light microscope. Chasmothecia were considered viable when they contained at least one viable ascospore. Viability of ascospores was evaluated using fluorescein diacetate staining [13].

Trapping the released fungal spores

In this experiment, for the determination of primary and secondary infection sources, in each vineyard, a 125 × 10 × 2.5 cm wooden stand was excavated at 25, 50, 75, and 100 cm from the soil surface by length of 75 mm (glass slide length) and depth of 2.5 mm. At the beginning of March, three stands were placed between the rows in

Tab. 1 Grapevine varieties and vineyard's location area where the study was conducted.

Vineyard No.	Location	Cultivar	Training system	Observed area (m^2)	Altitude
1	Kanawat 1st	Balady	Earth-trellised "Jui" system	4000	1270
2	Kanawat 1st	Black	Earth-trellised "Jui" system	2000	1270
3	Kanawat 2nd	Balady	Arbour vineyard	2000	1250
4	Kanawat 2nd	Black	Arbour vineyard	1000	1250
5	Kanawat 2nd	Helwani	Arbour vineyard	1000	1250
6	Kanawat 2nd	Salty	Arbour vineyard	400	1250
7	Daher Aljabel (Research Center)/1	Black	Lateral cordon	5000	1500
8	Daher Aljabel (Research Center)/2	Balady	Head-trained vine	5000	1500
9	Daher Aljabel (Research Center)/3	Black	Earth-trellised "Jui" system	100	1500
10	Daher Aljabel (Albassa)	Salty	Earth-trellised "Jui" system	300	1450
11	Salkhad	Balady	Arbour vineyard	200	1350

each of four selected locations (Kanawat 1st, Daher Aljabel/1, Daher Aljabel/2, and Daher Aljabel/Albassah). Then, four microscopic slides covered with Vaseline were horizontally fixed on every level of each stand for spore trapping. Slides were carried to the laboratory every 7 days. Then, 192 slides were stained with cotton blue – lacto-phenol solution, and the number of trapped spores were determined and recorded every week using a light microscope.

Statistical analysis

One-way analysis of variance and Tukey's test were done using SPSS15 statistical software at ($p \leq 0.05$) to compare differences in chasmothecia formation during the period of observation in the same year and between years, in the same vineyard and between vineyards, on the upper, under and both sides of the leaves.

Results

Leaves and/or branches were examined for chasmothecia formation from July to December in 2014 and 2015. Chasmothecia were detected in 45.5% of vineyards in the 2 years of survey. Observation of 110 branches of 'Balady' and 'Black' cultivars, severely infected by powdery mildew in 2015, showed the presence of *E. necator* mycelium and conidia on the surface of infected branches, but chasmothecia were not detected on 1- and 2-year old branches. At the time of bud break, in March–April 2014 and 2015, chasmothecia were not found on vine bark, nor ascospores were trapped by the slide traps at the beginning of the season.

Development of *E. necator* chasmothecia

Almost identical results were obtained during the two years of the survey. Therefore, only data collected in 2015 are presented here. The initial development of chasmothecia on powdery mildew infected leaves was observed on 20 July 2015 in four vineyards

Tab. 2 Number of grapevine leaves on which chasmothecia were formed from July to December 2015.

Date	No. of examined leaves	No. of leaves with chasmothecia only on upper surface	% of leaves with chasmothecia only on upper surface	No. of leaves with chasmothecia only on under surface	% of leaves with chasmothecia only on under surface	No. of leaves with chasmothecia on both surfaces	% of leaves with chasmothecia on both surfaces	Total number of leaves with chasmothecia	% of leaves with chasmothecia
8/7/2015	20	0	0.0	0	0.0	0	0.0	0	0.0
20/7/2015	10	1[a]	10.0	1[a]	10.0	2[b]	20.0	4	40.0
30/7/2015	50	10[a]	20.0	15[b]	30.0	5[c]	10.0	30	60.0
6/8/2015	89	10[a]	11.2	16[b]	18.0	15[b]	16.1	41	46.1
18/8/2015	99	4[a]	4.0	21[b]	21.2	1[a]	1.0	26	26.3
30/8/2015	81	16[a]	19.8	13[a]	16.0	6[b]	7.4	35	43.2
9/9/2015	57	11[a]	19.3	8[b]	14.0	3[c]	5.3	22	38.6
25/9/2015	21	1[a]	4.8	11[b]	52.4	1[a]	4.8	13	61.9
20/10/2015	30	7[a]	23.3	0[b]	0.0	1[b]	3.3	8	26.7
11/11/2015	50	1[a]	2.0	2[a]	4.0	0[a]	0.0	3	6.0
9/12/2015	30	0	0.0	0	0.0	0	0.0	0	0.0

Data with similar letters are not significantly different ($p \leq 0.05$) between the number of leaves with chasmothecia on upper, under and both sides for each date.

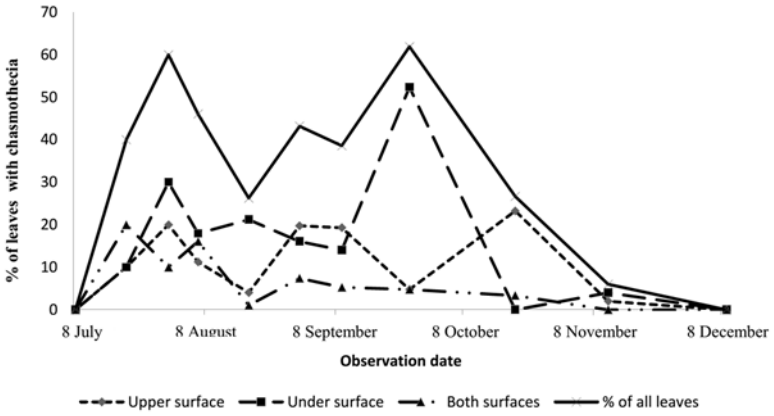

Fig. 1 Number of grapevine leaves on which chasmothecia were formed from July to December 2015.

at Kanawat 1 and Kanawat 2, 2 months after the first observation of the symptoms on the leaves.

Chasmothecia were formed on the upper or/and the under leaf surfaces. The highest percentage of infected leaves, on which chasmothecia were formed, reached 60% and 61.9 on 30 July and 25 September, respectively, with average of 38.7% from 20 July to 11 November. Chasmothecia were formed on the upper surface in 12.5% of infected leaves, with highest occurrence of 23.3% on 20 August, and they were formed on the under surface in 18.4% of infected leaves, with highest occurrence of 52.4% on 25 September (Tab. 2). In contrast, it was noted that the percentage of leaves, on which chasmothecia were formed on both sides, was relatively low with an average of 7.5% (Fig. 1).

Variation in number of chasmothecia

The number of chasmothecia formed on the leaves at the end of the season was estimated just in vineyard No. 1 (Kanawat 1st) in 2014 and 2015, and vineyard No. 4 (Kanawat 2nd) in 2015, but the numbers of chasmothecia on both sides of infected leaves were also estimated only in 2015. Tab. 3 shows that formation of chasmothecia was more frequent on the leaf under side with an average of 0.6 / leaf than on the leaf upper side (0.4 / leaf). The number of chasmothecia formed on both sides together was relatively low with an average of 0.2 / leaf.

Chasmothecia were produced on leaves from July to November. Their number increased slowly between July and September, and it highly increased from September to the end of October, then it had a sudden reduction at the beginning of November . No new formation of chasmothecia was observed in December (Fig. 2).

The results showed also that the number of chasmothecia was variable between vineyards and years. In 2015, their number ranged between 0.2 (vineyard No. 4) to 2.6 (vineyard No. 1) per leaf (Fig. 3).The first detection of chasmothecia on leaves was at the beginning of July in two surveyed vineyards and in both years of survey, but the number of chasmothecia was highly variable between years in the same vineyard (Kanawat 1st). It reached 390 / 100 leaves in October 2014, but it was about 260 in October 2015. Fig. 4 shows the chasmothecia average number in each month.

The initial development of chasmothecia was observed on powdery mildew infected leaves on 20 July. Most of them were immature (61.1%) with cream to yellow color, and 38.9% were semi-mature with brown color (Tab. 4). Yellow and brown chasmothecia had been observed until the beginning of October. Their number quickly increased from mid-July to mid-August, while the amount of dark, mature chasmothecia began to increase rapidly after the middle of October (Fig. 5).

Number of asci and ascospores in chasmothecia

Microscopic examination showed that chasmothecia were 85–180 μm in diameter. Appendages, 7–12 in number, hyaline and sometimes brown at the basal half, sometimes not coiled, 140–300 μm in length, 2–3 μm in width. Asci 1–5 (usually three) in an ascoma, 30–35 × 45–55 μm, and 65.6% of them were empty. Ascospores 1–4 (usually three) in an ascus, 10–25 × 10–15 μm (Tab. 5).

During winter, the number of chasmothecia on the infected leaves decreased slowly, and their viability decreased suddenly between December and February (Fig. 6). In March, at the time of bud break, chasmothecia were still present in small numbers on the leaves, but they had an average viability of 1.2% and 0.17% in April.

Tab. 3 Variation in number of chasmothecia on upper or/ and under leaf surfaces at Kanawat 1st from July to December 2015.

Date	Number of examined leaves	No. of chasmothecia formed only on the upper surface	Average of chasmothecia No. / leaf upper surface	No. of chasmothecia formed only on the under surface	Average of chasmothecia No. / leaf under surface	No. of chasmothecia formed on both surfaces	Average of chasmothecia No. / both leaf sides	Total number of produced chasmothecia	Average of chasmothecia No. / leaf
8/7/2015	20	0	0.00	0	0.00	0	0.00	0	0.00
20/7/2015	10	6[a]	0.60	3[b]	0.30	9[c]	0.90	18	1.80
30/7/2015	50	24[a]	0.48	20[ab]	0.40	15[b]	0.30	59	1.18
6/8/2015	89	40[a]	0.45	35[a]	0.39	18[b]	0.20	93	1.04
18/8/2015	99	39[a]	0.39	53[a]	0.54	14[b]	0.14	106	1.07
30/8/2015	81	32[a]	0.40	35[a]	0.43	25[a]	0.31	92	1.14
9/9/2015	57	36[a]	0.63	19[b]	0.33	11[c]	0.19	66	1.16
25/9/2015	21	8[a]	0.38	43[b]	2.05	2[c]	0.10	53	2.52
20/10/2015	30	24[a]	0.80	51[b]	1.70	3[c]	0.10	78	2.60
11/11/2015	50	1[a]	0.02	1[a]	0.02	0[a]	0.00	2	0.04
9/12/2015	30	0	0.00	0	0.00	0	0.00	0	0.00
Average		19.09[a]	0.38	23.64[a]	0.56	8.82[b]	0.20	51.55	1.14

Data with similar letters are not significantly different ($p \leq 0.05$) between the number of chasmothecia on upper, under and both sides for each date.

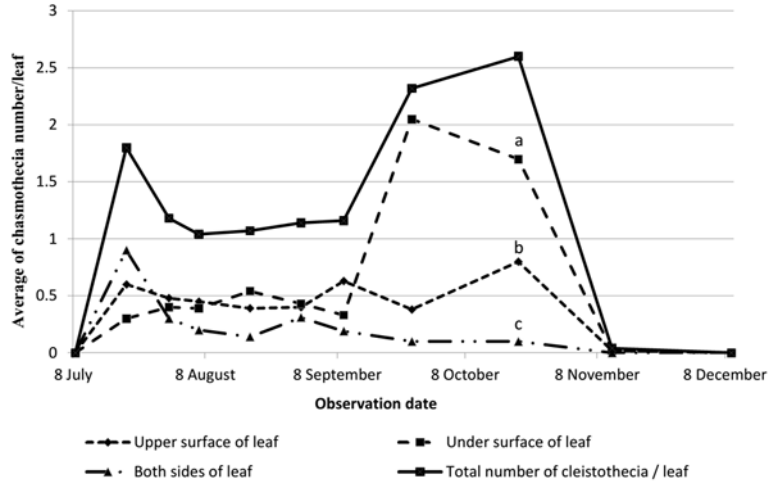

Fig. 2 Variation in number of chasmothecia on upper or/and under leaf surfaces at Kanawat 1st from July to December 2015. Data with similar letters are not significantly different ($p \leq 0.05$) between the number of chasmothecia on upper, under and both sides of leaves at the end of the growing season (October to November).

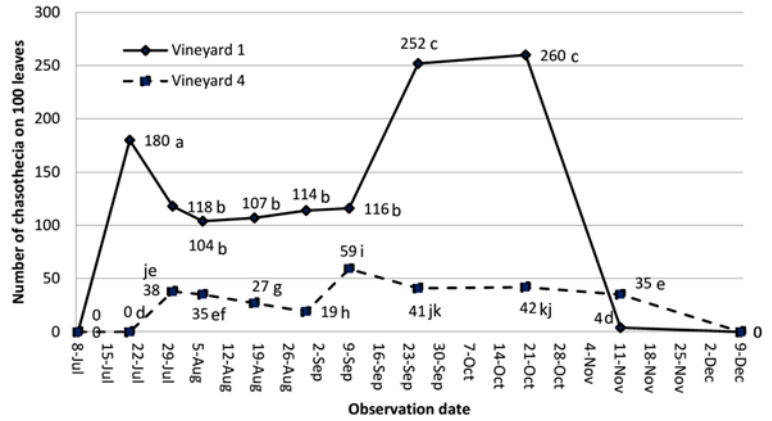

Fig. 3 Number of chasmothecia observed on 100 leaves in vineyards No. 1 (Kanawat 1st) and No. 4 (Kanawat 2) from July to December 2015. Numbers with similar letters are not significantly different ($p \leq 0.05$) between observations in the same vineyard and between vineyards.

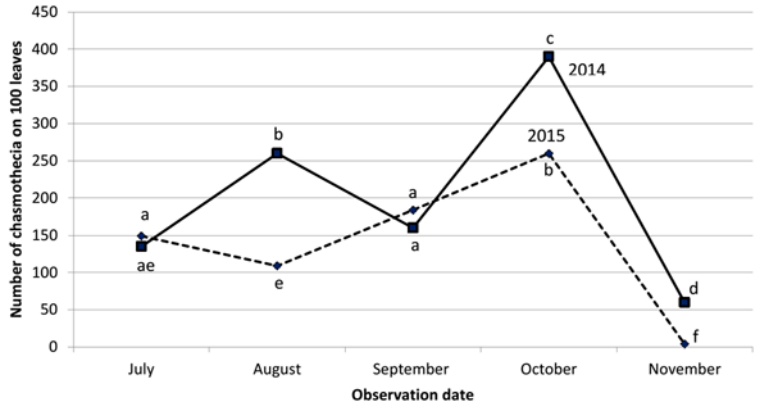

Fig. 4 The number of chasmothecia observed on 100 leaves in vineyard No. 1 (Kanawat 1st) from July to November of 2014 and 2015. The number of chasmothecia was calculated as the average of observations made in each month. Data with similar letters are not significantly different ($p \leq 0.05$) between months in the same year and between years.

Tab. 4 Development of chasmothecia of *Erysiphe necator* on leaves in Kanawat 1st site in 2015. The percentages of young (cream or yellow), semi-mature (brown), and mature (dark brown to black) chasmothecia on infected leaves were determined.

Date	Chasmothecia color (%)			Appendage (%)		
	cream/beige or yellow	orange/brown	black/dark brown	absent	initiation	present
8/7/2015	0.0	0.0	0.0	0.0	0.0	0.0
20/7/2015	61.1	38.9	0.0	100.0	0.0	0.0
30/7/2015	81.4	13.6	5.0	55.9	0.0	44.1
6/8/2015	42.8	48.4	8.8	78.1	8.1	13.8
18/8/2015	30.2	55.7	14.1	76.5	13.2	10.4
30/8/2015	42.1	35.0	22.9	0.8	80.4	13.1
9/9/2015	39.3	40.8	19.9	66.7	24.2	9.1
25/9/2015	39.0	21.9	39.1	37.7	5.7	56.6
20/10/2015	18.5	7.8	73.6	57.1	14.3	28.6
11/11/2015	0.0	0.5	99.6	0.0	0.0	100.0

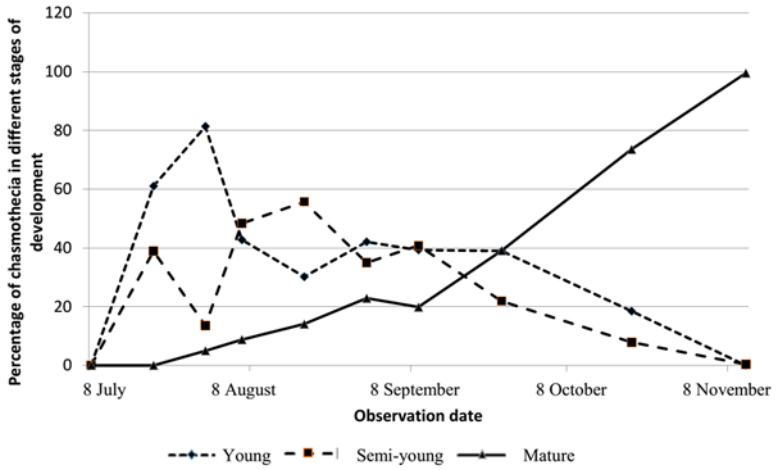

Fig. 5 The percentages of young (cream or yellow), semi-mature (brown), and mature (dark brown to black) chasmothecia observed on infected leaves from July to November 2015.

Tab. 5 Number of asci and ascospores in chasmothecia of *Erysiphe necator*.

Location	Number of examined leaves	Number of examined chasmothecia	Number of asci / ascocarp						Number of ascospores/ascus			
			1	2	3	4	5	empty	1	2	3	4
Kanawat 1st	100	90	1	2	20	6	2	59	0	6	15	4
Salakad	78	58	1	10	15	2	2	28	2	12	28	2

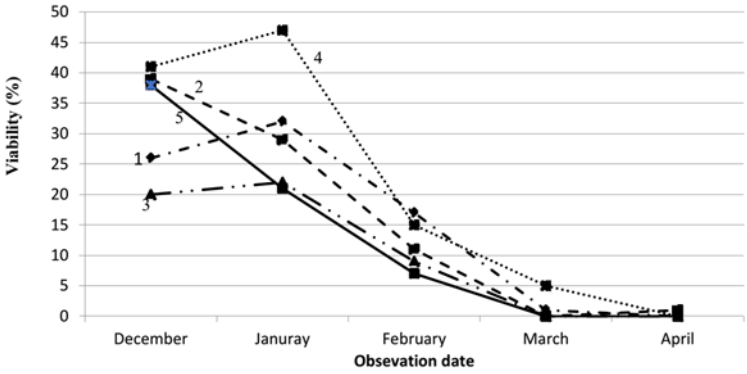

Fig. 6 Viability of chasmothecia on leaves during winter 2015. Numbers indicate the surveyed vineyards.

Discussion

Powdery mildew caused by *E. necator* is an important disease of grapevine in Swieda, southern Syria. The initial development of chasmothecia on infected leaves was observed at the second half of July in 45.5% of studied vineyards. This result is in accordance with those of many previous studies. In New York State, chasmothecia are produced from late July [4]. In Germany, the first yellow fruiting bodies were detected as early as mid-July to mid-August, and the ascocarp formation stops at the beginning of October [2]. In contrast, in Australia chasmothecia are formed during late April and early May [14]. In South Africa, chasmothecia were also observed during April to May on severely infected leaves [15]. Chasmothecia were formed on 38.7% of leaves in average, and they occurred in small number on leaves (1.1 / leaf), and most of them were immature. These results are in according with those of Halleen and Holz [15] who showed that the number of chasmothecia formed on leaves were 1–10 per leaf. No ascospores were trapped by the spore traps, nor found on the bark in spring at the beginning of the season, although they have been observed on the leaves in fall. In contrast, Holb and Füzi [16] showed that ascospores were trapped from early April until end June, where 6.6% of the total ascospores were caught between the initiation of sampling in April and bud break, 62.2% from bud break to bloom, and 31.2% between bloom and the conclusion of sampling at the end of June. In winter and at the beginning of spring, chasmothecia were still present on leaves at a small number and with a very low viability. It seems that the viability of chasmothecia is strongly dependent on the environmental conditions. Corties et al. [10] showed that the favorable climate of southern Italy allowed the survival of the leaf litter until bloom, and leaves seemed to be a potential efficient substrate for overwintering chasmothecia. It seems also that the high temperatures have detrimental effects on the viability of chasmothecia [12]. According to these results, the low viability of chasmothecia may be explained by the high temperature at the beginning of season in southern Syria. All these results confirm those of our previous study that the causal agent of grapevine powdery mildew (*E. necator*) overwinters as mycelium in dormant buds of the grapevines, and the ascospores did not had any role in the initiation of the disease in spring (unpublished data). The number of chasmothecia formed on leaves was highly variable between vineyards and years, these results are in according to those of Hajjeh et al. [12]. The occurrence of *E. necator* chasmothecia were lower in 2015 than they were in 2014. Previous study showed that the flag shoots and the disease severity were also more frequently in 2014 than they were in 2015 because the vineyards had been affected by frost in early spring 2015 when the temperature reached −16°C, which led to the death of most of the branches. Spotts and Chen [17] demonstrated that infected buds infected by powdery mildew were more susceptible to freezing injury and death than healthy buds. When temperatures dropped below −22°C, infected buds were likely to die whereas healthy buds could survive to temperatures dropping to −26°C. Through the above, it seems that there is a very high correlation between the severity of powdery mildew infections on leaves in the vineyards and the number of chasmothecia on leaf area [5,7,18–20].

Conclusion

Although *E. necator* chasmothecia are formed on the infected leaves, it seems that they may not have a role as a primary inoculum of powdery mildew of grapevine at the beginning of season in southern Syria where this disease is very important, especially on 'Balady' and 'Black' grapevine cultivars.

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