Study on the biology and epidemiology of Uncinula necator the causal agent of grape powdery mildew disease

*Hossein Karbalaei Khiavi[1], Haji Shikhlinskiy[2], Asadollah Babaei Ahari[3] & Mohammad Akrami[4]

[1]Agriculture and Natural Resources Research Center of Ardabil Province, Ardabil, Iran.
[2]Azerbaijan National Academy of Sciences, Baku, Azerbaijan.
[3]Department of Plant Protection, College of Agriculture, University of Tabriz, Tabriz, Iran.
[4]University of Peyam Noor, Tabriz, Iran.
* Corresponding Author: hossein_karbalaee@yahoo.com

Abstract: This study was carried out during 2007-2009 in the vineyards of Ardabil province of Iran to study the biology and the epidemiology of Uncinula necator the causal agent of grape powdery mildew disease. The study concentrated on the survival and the initiation of primary inoculum of the fungal causal agent. Results of histopathological experiments indicated that U. necator survived as mycelium in the dormant buds of the grapes during winter season. Results of study on the effect of environmental factors on fungus biology showed that the pathogenic activity of the fungus began when the temperature was between 16-19°C with a relative humidity more than 50%. It was also found that optimum temperature and relative humidity for the sporulation of U. necator was 20-25°C and 50-100% respectively. According to the results, fungal conidia were trapped during formation of 5-6 true leaves and first disease symptoms were observed on the clusters on late June after fruit formation. Fungal cleistothecia were observed abundantly at the end of season on the leaves, petioles and twigs but they were not able to survive during winter. Formation of ascospores on young leaves was proved but their role as the primary inoculum was not supported by the results of this study. Results of this study and the new findings on the biology and epidemiology of U. necator may be of national and international interests for the management of powdery mildew disease which is one of the most destructive diseases around the world including Iran.

[H. Karbalaei Khiavi, H. Shikhlinskiy, A. Babaei Ahari & Mohammad Akrami. **Study on biology and epidemiology of Uncinula necator the causal agent of grape powdery mildew disease.** *Life Sci J* 2012;9(3):1787-1792] (ISSN:1097-8135). http://www.lifesciencesite.com. 258

Key Words: Grape, Powdery mildew, Uncinula necator, Biology, Epidemiolog

1. Introduction:

Grape is one of the most important fruits around the world and it is believed that has been cultivated in many regions of the world since thousands years ago. Over the time different pests and diseases have been spread to grape vineyards and have become one of the most important yield reducing factors in the world vineyards **[3]**.

Powdery mildew disease caused by plant pathogenic fungus, Uncinula necator is one of the most important and destructive diseases of grape in many countries of the world including Iran **[1, 3, 5, 9, 15, 20]**. It can cause serious damages and loses to grape production in conducible environmental conditions, affect the grape production and the yield quantitatively and qualitatively and increase the production cost significantly **[3, 4, 7, 13]**. The disease was reported from Iran grape vineyards in 1946 for the first time and since then it has been observed in many grape growing provinces of the country **[3]**. It is believed that U. necator the causal agent of the disease has first been identified in North America and then it has been spread to Europe before 1840,s and has officially been reported from Europe in 1945. In general almost all Vitis vinifera cultivars and its hybrids are susceptible to powdery mildew disease **[13]**. Studies on the life cycle of the fungal agent of the disease have shown that it usually survives in the dormant buds of the plant as mycelium **[12, 14, 16]**. Most researchers believe that the sexual stage of the fungus does not play an important role in disease cycle **[12, 14, 16]**. Sexual form of the fungus as cleistothecium on older leaves and twigs has been reported from Iranian vineyards **[1]**. However, their roles and importance in disease biology and epidemiology have not studied yet. Some studies have investigated the biology and epidemiology of U. necator previously. For example **Brunelli [2]** reported that the fungus has two biotypes and is capable of overwintering as cleistothecium and mycelium. Formation of cleistothecium at the end of season on grape branches and leaves have been reported by several studies conducted in USA **[2]**, France **[18]**, Australia **[17]** and Iran **[1]**. In Italy and USA, researchers have reported cleistothecia as the primary inoculum of the disease **[2, 14]**. Germination of ascospores of U. necator was reported for the first time in 1895 **[9]**. **Galloway [10]** reported that fungal ascospores did not play important roles in powdery mildew disease incidence. They also believed that

production of ascs in disease cycle was not pathologically important.**Cortesi et al. [5]** reported that fungus survival in the winter (overwintering) was not well known and it may survive as ascocarp or mycelium.

In Aedebil province of Iran where this study was carried out, primary inoculum of U. necator for disease occurrence in vineyards is not well known. This study was therefore conducted and executed during 2007–2009 to study and investigate the biological and epidemiological aspects of the disease including survival, overwintering and primary inoculums formation in relation with environmental conditions and plant phenology.

2.Materials and Methods:

2.1.Visiting provincial vineyards for disease monitoring

During March-November 2007-2009, different vineyards in the province were visited routinely and fungus survival (overwintering), disease incidence and symptoms development in relation with plant phenology were investigated. Major grape varieties grown in these regions included Keshmeshi, Rasmi and Shahani belonging to Vitis vinefera species.

2.2.Investigation of fungal conidia and ascosporic release

In this experiment, for determination of primary and secondary infection sources, in each vineyard, 20 wooden stands with the height of 25, 50, 75 and 100 cm respectively were placed between the rows in five selected locations and microscopic slides covered with Vaseline were carefully placed on the upper and side surface of each stand for spore trapping. Slides were carried to the laboratory every 3 days, were stained with cotton blue-lacto-phenol solution and the number of trapped spores were determined and recorded using a light microscope.

2.3.Examination of dormant twigs for survival of fungal mycelium

During March 2007-2009, and before budding stage, grape dormant buds were collected in experimental sites and were carried to the laboratory for further processing. Collected samples were then examined for the presence of U. necator mycelium by fixation, staining, molding in paraffin and microtome profiling using procedure described by **Gee et al. [11]**. Permanent profiles were then prepared from leaf fragments using glycerol gel and were examined under a light microscope for the detection of fungal mycelium.

2.4. Investigation of fungal ascospore release

During the month of October 2007-2009, infected grape leaves containing fungal cleistothecia were collected from experimental vineyards, were cut to 5-cm pieces, were placed in cloth bags and were hanged on woody stand for ascospore release. During November through May 2007-2009 the samples were examined every two weeks for the release of ascospores according to the procedures described by Pearson and Gaudry **[13]**. For the collection of cleistothecia, 10 peaces of the leaves were placed in a flask containing 100 ml of distilled water and were shaken for release of the cleistothecia. Distilled water in the flask containing cleistothecia was then screened twice using micro screens and fungal ascocarps were collected and were placed on paper disks. Paper disks were then placed in a moist petri dish containing a microscope slide. Release of fungal ascospores was determined after 24 hours by examining the slides under a light microscope.

2.5.Investigation of cleistothecia survival during the season

During October to May 2007-2009 infected twigs, leaves and surface soil were collected every months using the procedure described by **Cortesi et al. [5]**. Fungal ascocarps were examined under a light microscope and viability of ascospores was determined in fluorecin diacetate solution after 5 minutes according to the method described by **Gee et al. [11]**. Viability determination of ascocarps was based on the production of green fluorescent pigment by at least 50% of the ascospores **[11]**.

2.6.Evaluation of pathogenicity of fungal ascospores

To investigate the pathogenicity of U. necator, infected leaves revealing disease symptoms were collected from experimental vineyards and were taken to the laboratory for further processing. Infected leaves were placed in 9-cm diameter Petri dishes, were surface sterilized and were then transferred into other Petri dishes containing 25 ml water agar (WA) medium and 100 ppm benzimidazole fungicide. Four moist paper disks each containing 20 fungal cleistothecia were placed inside the lid of each Petri dish. Leaves were then examined for the infection caused by fungal ascospores **[8]**.

2.7.Investigation of the effect of environmental factors on U.necator sporulation

The impact of environmental factors including temperature and moisture on the germination of fungal conidia in invitro condition was evaluated according to the procedure described by Spencer **[19]**. The impact of weather conditions including rain and

air temperature on the release of fungal spores and disease incidence were also investigated using data obtained from provincial metrological service.

Results

Powdery mildew disease symptoms on the grape plants did not appear in the experimental vineyards until mid June. However, various disease symptoms including fungal conidia, tissue discoloration and fruit deformation appeared on the upper surface of the leaves, on the underside of the leaves, on the fruit and on the shoot. Fungal asexual and sexual structures are are on the leaves, fruits and twigs. According to the results of cytological tests in our study, it was shown that the survival of the causal agent of the disease (U. necator) during winter (overwintering) took place as mycelium in the dormant buds of the grape plants (Fig. 1). Results of the study also indicated that fungal conidia were released from late May to early September. These conidia were shown to be the primary inoculum of the disease. First conidia were trapped around late May in our experiments.

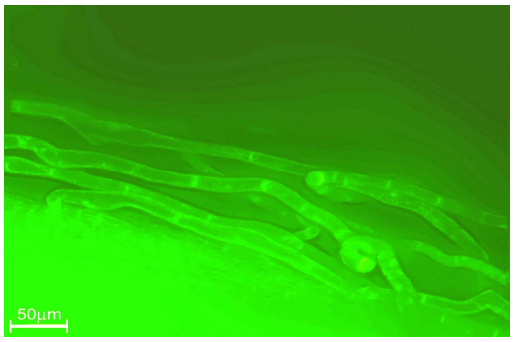

Figure 1. Hyphae of powdery mildew in h of a grape dormant bud

According to the results, first disease symptoms were observed on leaf and fruit in mid June and sexual forms of the fungus (ascocarps) were detected on the infected plants in early October (Fig. 2).

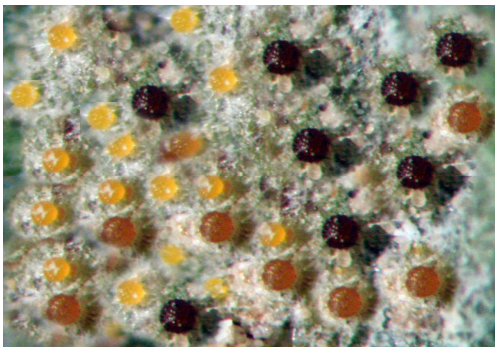

Figure 2. Cleistothecia of Uncinula necator in various stages of maturity on grape leaf

Results of the study on the effects of environmental factors on sporulation of U. necator indicated that in experimental vineyards conidia release began in late May when temperature was about 16-19°C and the maximum conidia release was observed when air temperature reached 20-25°C (Table 1). The optimum temperature and relative humidity for fungal conidia germination were 25°C and 40-100% respectively (Table 1 and 2). In temperature below 20°C conidia release was gradually reduced. The maximum temperature for conidia germination was found to be 34°C and it was stopped when temperature exceeded 34°C (Table 2).

Table 1. Effects of temperature in the germination of Uncinula necator conidia at different time intervals

No.	Temperature (°C)	Time before germination begins (hr)	Germination (%)	Maximum germination (%)	Time before maximum germination occurs (hr)
1	7	19	0.24	5	29-34
2	10	18	1.23	7	29-34
3	13	11	1.60	11	27-29
4	16	11	2.95	13	22-25
5	19	2	5.30	28	21-25
6	22	2	15.00	62	18-22
7	25	2	18.50	78	14-18
8	28	2	15.75	63	11-15
9	31	2	6.10	27	6-10
10	33	2	0.65	-	0-2
11	34	-	-	-	-

Table 2. Effects of relative humidity on the germination of Uncinula necator the causal agent of powdery mildew disease

No.	Relative humidity (%)	Conidia germination (%)
1	10	5
2	20	13
3	40	68
4	60	75
5	80	76
6	100	79

4. Discussion:

Results of our study in the role of cleisthothecia in the survival and overwintering of U. necator showed that cleistothecia were formed abundantly on the leaves and branches at the end of season, but these cleistothecia could not resist and survive during the winter. These findings did not support the roles of cleistothecia in fungal survival and pathogenesis. Our results agree with those of previous studies which have indicated that cleistothecia do not play important roles in the occurrence of powdery mildew disease **[9, 11, 18]**. In these studies winter surviving cleistothecia on the leaves were tested for the pathogenicity, but they failed to cause disease by lack of symptoms induction **[8, 9, 11,18]**. We obtained the similar results in this study and our test cleistothecia did not induce any disease symptoms on the young leaves when the leaves were inoculated with these cleistotehcia.

In a previous study, **Gadouri and Pearson [14]** proved that when grape leaves carrying ascocarpes were buried in the soil, all ascocarps lost their viability **[8]**. The results of our experiments in this regard agree with those of above-mentioned study. In our study it was also found that no viable ascocarp was detected in the leaves which were collected from the vineyards soil in early spring.

The results of our study on the impact of environmental factors on fungal sporulation agree with those of previous studies carried out by **Delp [6], Built and Lafon [3], Pearson and Goheen [15] and Spencer [19]**. According to our results and their findings environmental factors including temperature and relative humidity are very important and play critical roles in fungal conidia and ascospore release.

The overall results of this study on biology and epidemiology of U. necator the causal agent of grape powdery mildew disease provide some novel information for better understanding of the interactions among environmental factors, the host plant and the pathogen. To select and choose more effective control methods for a certain disease on a given plant and in a given area, study of the interactions among the above factors is very important. The outcomes and findings of this study may therefore be used effectively in formulation of management strategies to combat and overcome the powdery mildew disease which is one of the most destructive and damaging diseases of the grape around the world including Iran.

5. Conclusion:

Generally, Results of histopathological experiments indicated that U. necator survived as mycelium in the dormant buds of the grapes during winter season. Results of study on the effect of environmental factors on fungus biology showed that optimum temperature and relative humidity for the sporulation of U. necator was 20-25°C and 50-100% respectively. According to the results, fungal conidia were trapped during formation of 5-6 true leaves and first disease symptoms were observed on the clusters on late June after fruit formation. Fungal cleistothecia were observed abundantly at the end of season on the leaves, petioles and twigs but they were not able to survive during winter.

Acknowledgement:

This work was supported scientifically by Dr. N. Sokhandan and Dr. M. Arzanlu, College of Agriculture, University of Tabriz, Hereby author is grateful of them.

Corresponding author:

Dr. Hossein karbalaei Khiavi, Agriculture and Natural Resources Research Center of Ardabil Province, Ardabil, Iran, Telephone: 09141513647, Fax: 0452-5220834,

E-mail: hossein_karbalaee@yahoo.com

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9/24/2012