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Infection of *Botrytis cinerea* in Different Fungicide Application Programs in Semillon Grape

Nagehan Desen KÖYCÜ[1]* **Cengiz ÖZER[2]** **Erhan SOLAK[3]** **Nafiz DELEN[4]**

[1]Department of Plant Protection, Faculty of Agriculture, University of Tekirdağ Namık Kemal Süleymanpaşa/Tekirdağ 59030, Turkey;

[2]Viticulture Research Institute Süleymanpaşa/Tekirdağ 59100, Turkey;

[3]Republic of Turkey in Ministry of Food Agriculture and Livestock Süleymanpaşa/Tekirdağ 59100, Turkey;

[4]Department of Plant Protection, Faculty of Agriculture, University of Eagean Bornova/İzmir 35040, Turkey (Retired)

*Corresponding author E-mail: dkoycu@nku.edu.tr

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Botrytis cinerea can lead to reduction in the yield and quality of table and wine grapes, with high economic loses in the world and also Turkey. In this work was compared fungicide applications in trial vineyard of Semillon cv. with that of several grower vineyards the effectiveness to fruit infection of *Botrytis cinerea* at harvest. Studies were conducted at five sites in Tekirdağ. All fungicide treatments reduced mean *B. cinerea* berry infection when compared to the unsprayed control treatment. Trial programme resulted in at least disease severity (1.46%) and incidence (5.83%) and this programme was used fungicide at flower stage for *B. cinerea*. The highest disease severity (11.46%) and incidence (31.67%) was noted in Grower I Programme. This is followed by Grower III programme, Grower II programme and Grower IV programme, respectively. The recommendation for control of *B. cinerea* in the Trakya region is to apply one spray at bloom.

Key Words: Gray mold chemical control, berry infection, vine

Farklı Fungisit Uygulama Programlarında Semillon Üzümünde *Botrytis cinerea*'nın Enfeksiyonu

Botrytis cinerea dünyada olduğu gibi aynı zamanda Türkiye'de de sofralık ve şaraplık üzümlerde önemli ekonomik kayıplara neden olabilmektedir. Bu çalışmada, Semillon şaraplık üzüm çeşidinde *B. cinerea*'nın hasat döneminde meydana getirdiği meyve enfeksiyonlarına karşı fungisit deneme programı ile üreticilerin uyguladığı fungisit programları karşılaştırılmıştır. Uygulanan tüm fungisit programları kontrol üretici bağı ile karşılaştırıldığında *B. cinerea*'nın tane enfeksiyonlarını azalttığı tespit edilmiştir. En az hastalık şiddeti (%1,46) ve hastalık oranı (%5.83) çiçeklenme döneminden itibaren başlatılan deneme programında tespit edilmiştir. En yüksek hastalık şiddeti (%11.46) ve hastalık oranı (%31.67) I. üreticinin uyguladığı programda tespit edilmiştir. Bunu sırasıyla III. üretici programı, II. üretici programı ve IV. üretici programı takip etmiştir. Trakya Bölgesi'nde *B. cinerea*'nın kontrolü için çiçeklenme döneminde fungisit uygulamalarının başlatılması önerilmektedir.

Anahtar Kelimeler: Kurşuni küf, kimyasal kontrol, tane enfeksiyonu, bağ

Introduction

Gray mold is caused by the fungus *Botrytis cinerea*. The disease can lead to reduction in the yield and quality of table and wine grapes, with high economic losses in the world (Leroux et al., 1999) and also Turkey (Burçak and Delen, 2000; Koplay, 2003; Özer et al., 2004; Köycü et al. 2005). The disease appears as shoot blight or blossom blight following spring rains; flowers also become infected during bloom but the pathogen becomes latent until later in the season (McClellan and Hewitt 1973). The fungus growths and infects the entire fruit often resulting in berry-to-berry contact, where the cuticle is absent or very thin, increases the susceptibility of berries (Elmar and Michailides, 2004) and varieties within grape cultivars (e.g. Barış, Zinfandel Semillon) characterized by thin cuticle (Köycü et al. 2005). An understanding of the life cycle in grapes can increase the ability to reduce disease expression in bunches at harvest. The morphological, anatomical of cultivars with a range of resistance to *B. cinerea* is different (Elmar and Michailides, 2004). Therefore, disease prediction models rely on in field environmental monitoring stations that predict when conditions appropriate to infection (Nair and Allen 1993; Broome et al. 1995). A properly timed spray programme is essential for managing Gray mold in the vineyard. Since the disease spreads very fast especially in coastal region due to high humidity, successful control depends on controlling the flower infections (Nair et al. 1995). Trakya is a region in north-west Turkey where table and especially wine grapes are cultivated about 6800 hectares. During winter years with warmer temperatures and more than average humidity, bunch rot is likely to predominate in the Trakya region while cooler and wetter years are likely to lead to a higher occurrence of gray mold. Trakya Province and especially coastal areas are most vulnerable to bunch rot. In grapes, cultivar is one of the most important variables affecting grey mold epidemics (Özer et al., 2004). Estimations of the amount of *B. cinerea* occurring at different years in vineyard in the Trakya Province showed high infection of *B. cinerea* in wine grape cultivars. Disease incidence was determined as 38.5% in Semillon cultivars (Köycü et al. 2005). Fungicide applications in flower stage reduced *B. cinerea* fruit infections in Zinfandel and Emir wine grape cultivars at harvest. Therefore, the relationship between early infection and latency are important for disease control in Trakya region vineyards. The recommendation for control of *B. cinerea* in the Trakya region is to apply botryticide at flowering (Köycü, 2007). The growers in Trakya region use predominantly chlorothalonil+carbendazim (non-side specific organochlorin; benzimidazole), cyprodinil+fludioxonil (anilinopyrimidine; phenylpyrrole), fenhexamid (hydroxyanilide), iprodione (dicarboximide, pyrimethanil(anilinopyrimidine) a.i. botryticide fungicides.

Treatments, however, with these fungicides rapidly became inefficient because of reduced sensitivity to fungicides of isolates on grapevines (Köycü et al., 2012) and resistance to these fungicides have been reported in *B. cinerea* worldwide (Gullino et al., 1989; Latorre et al., 1994; Hilber and Hilber-Bodmer, 1998; Ziogas and Klamarakis, 2001; Baroffio et al., 2003; Leroux, 2004; Walker et al., 2012).

Table 1: Fungicides used to disease control in vineyards.

Active Ingredient	Commercial Product	Supplier	Disease *
Azoxystrobin 250g/L	Quadris SC,	Syngenta Turkey Ltd	DA, PM, DM
Bordo mixture (2%)	2000+1000 gr	-	DM
Captan 50%	Captan WG	Bayer Turkey Ltd.	DM
Carbendazim 50%	Deresol WP	Bayer Turkey Ltd.	PM
Chlorothalonil+Carbendazim 450+100 g/L	Multyfix SC	Hektaş Ltd.	GM
Copper oxychlorid 50%	Cupravitob 21 WP	Bayer Turkey Ltd.	DM
Cymoxonil+Propineb 6%+70%	AntracolCombi 76 WP	Bayer Turkey Ltd.	PM
Cyprodinil+Fludioxonil 37.5%+25%	Switch 62.5 WG	Bayer Turkey Ltd.	GM
Fenhexamid 500 g/L	Teldor SC	Bayer Turkey Ltd.	GM
Iprodione 50%	Rovral WP	Bayer Turkey Ltd.	GM
KresoximMethyl+Boscalid 100+200 g/L	Collis SC	Basf Ltd	PM
Myclobutanil+Quinoxyfen 45+45 g/L	Porter Super 90 SC	Dow Agro Ltd.	PM
Penconazole 100 g/L	Topas 100 EC	Syngenta Turkey Ltd.	PM
Propineb 70%	Antracol 70 WP	Bayer Turkey Ltd.	DM, DA
Pyrimethanil 300 g/L	Mytos SC	Bayer Turkey Ltd.	GM
Sulphur 80%	Thiovit Jet WP	Syngenta Turkey Ltd	PM
Triadimenol 50 g/L	Bayfidan EW	Bayer Turkey Ltd.	PM
Trifloxystrobin 50 g/L	Flint WG	Bayer Turkey Ltd.	PM

*:fungicides registered to diseases in Turkey

DA: Dead Arm (*Phomopsis viticola*)
DM: Downy Mildew (*Plasmopara viticola*)
GM Gray Mold (*Botrytis cinerea*)
PM Powdery Mildew (*Erysiphe necator*)

The use of pyrimethanil, tebuconazole in Trakya region began in 1996. cyprodinil+fludioxonil, fenhexamid have been using since 1998 and 2001, respectively. No information is available on effectiveness against to *B. cinerea* of different fungicide application programming in vineyard in Turkey. The aim of this work was to compare fungicide applications in trial vineyard with that of several grower vineyards the fruit infection of *Botrytis cinerea* at harvest.

Materials and Methods

Vine plot and spray program

The experiment was conducted in Tekirdağ (40^o 58.8'- 27^o 30.0') Viticulture Research Station in northeast Trakya. The cultivar was *Vitis vinifera* cv. Semillon on rootstock 5BB, trellis system with 1.5 m between vines and 3.5 m between rows. The area used for the experiment was 45 stocks for each line. The vine-stocks inspected were all of approximately the same age and development. Fungicides were applied at the BBCH stages 68, 77, 81, 83, 85. Other cultural practices and insecticides for insects were done in accordance to the standard practices used in that part of the vineyard. Trial applications were arranged in a randomized block, with three replicates. Fungicides using in this study and dates are showed table 2.

Grower trials

The grower evaluations (Gr I, Gr II, Gr III, Gr IV, Gr control) (Table 2) , which were cultivated with the same cultivar, are located in Tekirdağ location (Trakya region, Turkey). Each grower vines were same standard with trellis system with 1.5 m between vines and 3.5 m between rows. Studies were conducted at five sites in same location (40^o 58.8'-27^o 30.0') with Tekirdağ Viticulture Research Station in Tekirdağ. In the grower evaluations, the effect of the fungicides (Table1) using at different date for downy mildew, powdery mildew and *B. cinerea* was evaluated in a grower situation compared to a Vine-trial. The grower control consisted of sulphur and copper applications as described in Table2. The grower control did not receive any botryticides application. Each evaluation consisted of three replicates and a randomized block design.

Field Monitoring of *B. cinerea*

Fungicide applications of four grower practices and control grower were evaluated with the compare Vine-trial the following season at harvest to determine the degree of infection by *Botrytis cinerea*. Field sampling was carried out on 30 vine-stocks in plot distributed along three lines. Seventy clusters were evaluated in each plot for bunch rot symptoms (including visible mycelia or slip skin). Disease severity (percentage of symptomatic berries per cluster) was assessed for each plot by averaging severity estimates for each rated cluster. Disease was assessed on 12 September, three weeks following the last fungicide application. Disease severity (average estimated diseased bunch surface in %) was evaluated according to the following 0-4 scale: 0, no infection: 1, infected 5 fruit on clusture: 2, less than 20; 3, 21%-40%; 4, infected more than 40% of clusture (Anonymous, 1996). Disease incidence was assessed average percentage number of infected bunch at harvest. The efficacy (%) of fungicide was calculated according to Abbott formula=100*[Untreated control grape cultivars (A)-treated grape cultivars/A] (Abbot, 1925). The untreated cultivar was assessed for gray mold in the Grower.

Table 2. Fungicide spray schedules applied to each of the grower and trial vineyards for controlling gray mold caused by *Botrytis cinerea*.

Grower	Application number	Application dates	Active Ingredient %
Gr I	I	May 13	Sulphur; Copper-oxychlorid
	II	June 1	Trifloxystrobin; Propineb
	III	June 17	Triadimenol
	IV	June 22	Propineb
	V	July 6	Triadimenol
	VI	July 14	Triadimenol; Copper-oxychlorid
	VII	August 10	Triadimenol; Cyprodinil+Fludioxonil
	VIII	August 26	Iprodione
Gr II	I	April 15	Bordo mixture
	II	May 15	Sulphure; Copper-oxychlorid
	III	May 30	Copper-oxychlorid
	IV	June 14	Propineb
	V	June 16	Triadimenol
	VI	June 29	Pyrimethanil
	VII	July 15	Pyrimethanil
	VIII	August 1	Pyrimethanil
	IX	August 17	Fenhexamid
Gr III	I	April 2	Copper-oxychlorid
	II	April 12	Propineb
	III	April 27	Sulphure
	IV	May 12	Triadimenol
	V	May 28	Propineb+Cymoxonil
	VI	June 07	Triadimenol
	VII	June 15	Pyrimethanil
	VIII	July 03	Cymoxonil+Propineb
	IX	July 25	Fenhexamid
	X	August 15	Fenhexamid
Gr IV	I	April 30	Propineb; Triadimenol
	II	May 10	Sulphur
	III	May 16	Bordo mixture
	IV	May 30	Penconazole; Propineb
	V	June 15	Carbendazim; Propineb
	VI	June 22	Trifloxystrobin
	VII	June 29	Sulphur
	VIII	July 03	Propineb
	IX	July 11	Iprodıone
	X	July 30	Myclobutanil+Quinoxyfen; Pyrimethanil
	XI	August 10	Cyprodinil+Fludioxonil
	XII	August 28	Fenhexamid
Trial	I	April 28	Captan
	II	May 15	Azoxystrobin
	III	June 9	Chlorothalonil+Carbendazim; Captan
	IV	June 24	Tebuconazole
	V	July 28	KrexoximMethyl+Boscalid
	VI	August 11	Cyprodinil+Fludioxonil
	VII	August 26	Fenhexamid
Gr control	I	May 17	Sulphur; Copper-oxychlorid
	II	May 26	Sulphur; Copper-oxychlorid
	III	June 10	Sulphure; Copper-oxychlorid
	IV	June 26	Sulphure; Copper-oxychlorid

Results

This experiment revealed valuable information about apparent difference between grower programme and trial programme effectiveness Fig.1. The trial programme treatment resulted in significantly decreasing disease severity and incidence compared to the grower control and grower programmes.

The results demonstrate the importance of controlling *Botrytis* infections at flowering, with all fungicides reducing *Botrytis* bunch rot at harvest. Despite no significant differences between disease severity for each of grower and trial programmes, there were differences in disease incidence (%). Disease incidence ranged from 5,83% to 66,67% across all programmes, indicating wide-spread occurrence of the disease. Disease severity reached high levels, reaching 42% under natural epidemics (unsprayed) grower control. All fungicide treatments reduced (P<0.05) mean *B. cinerea* berry infection when compared to the unsprayed control treatment. Trial programme resulted in at least disease severity (1.46%) and incidence (5.83%) and this programme was used chlorothalonil+carbendazim at the end of bloom for *B. cinerea*. Among the grower application the highest disease severity (11.46%) and incidence (31.67%) was noted in Gr I Programme, due to application fungicide for *B. cinerea* at veraison and before harvest. The less effective fungicides for control of gray mold disease in grower fungicide application programme were Gr I programme. This is followed by Gr III programme, Gr II programme and Gr IV programme, respectively.

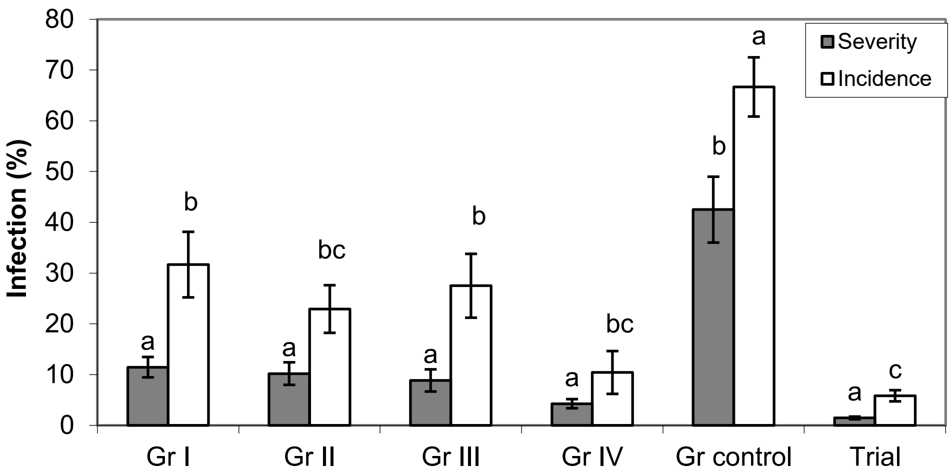

Fig 1. Disease severity and incidence of *Botrytis cinerea* in Grower and trial programmes.

Values are means ± standart error (n=3).

Discussion

B. cinerea represents a classic 'high risk' pathogen, its abundant sporulation, the polycyclic nature of the disease it causes, its wide host range, and high the number of fungicide applications required for its successful control (Leroux, 2004). The key tool developed by the research programme was targeted spraying for diseases. This involved field monitoring for diseases and the use of decision support software, which contained the Bacchus *Botrytis cinerea* risk model. Adoption of target based spraying resulted in a reduction in fungicide usage of up to 50% (Agnew et al., 2004). The vineyard monitoring developed of *B. cinerea* disease severity and incidence as part of the research programme has contributed to the development in spraying time of the sustainable vine growing in Tekirdağ (Köycü et al., 2007). The adoption of the *B. cinerea* disease management system as industry best practise in Trakya region has had economic benefits, with reduced fungicide and application costs, as well as environmental benefits, through reduced fungicide usage. The timing of fungicide sprays for *Botrytis* control is unclear on many fungicide labels, but it is generally considered that sprays are most effective when applied at the end of bloom (BBCH 68) (Petit et al., 2011) in this location (Köycü, 2007). The relative importance of each of these spray timings will vary depending on the time of a rain event and the amount of rain, as well as the number of *Botrytis* spores available to infect at each period. July was sometimes characterized by heavy rainfall, so the *Botrytis* attacks, normally occurring from the veraison, were not present. In August, hot weather (mean temperature 20^{o}C above seasonal average over August) with high humidity (>85%) was responsible for the appearance of the first visible rot. At the middle of the July, the week before veraison, heavy rainfall caused a very rapid spread of *Botrytis* (Köycü, 2007).

The efficacy of any program is further complicated by the presence of resistant strains of *Botrytis* in a vineyard particularly with fungicides of the anilinopyrimidines (pyrimethanil), dicarboximides (procymidone), azoles (imazalil, myclobutanil, penconazole, triadimenol) (Leroux, 2004; Delen, 2016). Resistance to this the fungicides are also present in Tekirdağ location (Köycü et al., 2012). Cyprodinil+fludioxonil, tebuconazole and fenhexamid were effect control of berry infection in laboratory tests (Köycü et al., 2012). Suppression of gray mold is usually achieved by a programme of three to six fungicidals sprays, applied to the crop between flowering and harvest (Shtienberg, 2004). On the other hand, the poor fungicide efficacy at Grower vineyards may be due to timing of applications and fungicides used. Therefore, our results showed that the trial program of eight fungicide applications provided the best control of *B. cinerea* in the Tekirdağ location in comparison with the other grower programmes, while a significant reduction of both disease severity and incidence was demonstrated when trial program was applied at stage at the end of bloom (BBCH 68). This indicates that application at BBCH 68 is decisive for the most effective control of grey mould disease (Nair et al., 1995; Petit et al., 2011). Our findings in Tekirdağ location have confirmed the efficacy of timing in reducing *B. cinerea* infections in vineyards. In vineyards, alternation botriticides recommended at the end of the bloom (BBCH 68). In addition, botriticides used at the end of bloom seems to have a greater effect on disease incidence than on disease severity, indicating that this fungicide may act by diminishing the size of fungal infection foci rather than in reducing the number of foci. Selection pressure exerted by fungicides on *B. cinerea*strains and defence responses of grapevine to fungicides were then tested to evaluate potential interactions between these factors and effectiveness of fungicide treatments

Conclusion

These results suggest that *B. cinerea* can be a major problem in vineyards in Tekirdağ/Turkey, causing flower infections, flower blight and thereby reducing fruit set and yield. Prediction of inoculation and infection events, tailored to vineyards, may aid timing of fungicide application to protect flowers from *B. cinerea* infections. However any Turkey studies have been carried out to determine which are the more effective material and how they perform in different spray programs. Differences among treatments were highly significant, the trend toward better control was evident with fungicides applicating trial vine. The late rains caused there to be a significant increase in disease and at the same time we did not make additional fungicide applications immediately after the rain.

The failure of many fungicides spray programs has likely been misuse and inappropriate spray timing. A better understanding on fungicide properties and activity will improve decision making in relation to spray timing and reduce the losses due to *Botrytis* bunch rot. Therefore, inoculum-focused management options for the control of botrytis bunch rot in grapes under Tekirdağ conditions are important for minimizing overwintering inoculum sources, flower infections, infection of other vine tissues and berry infections.

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