

EFFECT OF DIFFERENT LEVELS OF NITROGEN FERTILIZER, LEAF REMOVAL AND A FUNGICIDE TREATMENTS ON BUNCH ROT DISEASE OF GRAPES

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ABSTRACT

Two field trials with 10 years old grapevines c. v. Red globe (seeded cultivar) were conducted during 2003 and 2004 at Menia governorate, Egypt. In these trials, treatments consisted of N1 at 30 unit of Nitrogen (Ammonium sulphate), N2 at 45 N unit , N3 at 60 N unit , N4 at 75 N unit , N5 at 90 N unit and control (without adding N) were applied to determinate the effect of different levels of Nitrogen on bunch rot disease incidence and severity. An additional trial was to compare the leaf removal treatment with non managed control. All plots were established in a split-plot design with or without using fungicides. The above mentioned treatments were applied during each growing season starting at full bloom till veraison stage (the beginning of ripening) in order to control bunch rot disease. The obtained results showed that incidence and severity of bunch rot disease were significantly reduced when the treatment of nitrogen fertilizer 30 N unit was used while the high levels of nitrogen fertilizer increased incidence and severity of the bunch rot.

Leaf removal also reduced disease incidence and severity in nonsprayed control compared with the in tact nonsprayed control. The highest reduction in disease incidence and severity was in the treatments with three sprays of fungicide Euparen M at bloom, pre-close and veraison stages. Also, the produced fruit yield from treated vines was significantly higher during the first and the second seasons in comparison with that of untreated vines.

Keywords: bunch rot, disease incidence, disease severity, leaf removal, intact veraison stage, ammonium sulphate, fungicide.

INTRODUCTION

Grape (*Vitis vinifera* L.) is the leading fruit crop all over the world. In Egypt, grapevine occupies the second rank among fruit crops after citrus. However, the area under this economic crop in 2003 reached about 155143 feddans and the average of grape production was about 1196852 tons. (Anonymous 2003).

Under the Egyptian environmental conditions grapevines are attacked by several diseases, among which powdery and downy mildews and fruit rot diseases are of great economic importance.

Bunch rot under Menia governorate environmental conditions is a serious disease of grapes (*Vitis vinifera* L.) caused by *Alternaria geophila*, *Aspergillus niger*, *Cladosporium herbarum*, and *Rhizopus stolonifer*. Bunch rot infection of grape berries is of common incidence in cultivars with dense canopies or tight berry clusters. First symptoms of the disease on susceptible cultivars are, generally, evident when fruit sugar levels begin to increase (veraison).

Fertilization is a cultural practice which is considerably important to agricultural sustainability in vineyards. Also, it is undoubtedly one of the factors which conditions the productivity and quality of the vine yield

Vineyard pest management can clearly be influenced by the nutritional status of grapevines.

Incidence of grape disease can be influenced by vineyard fertilization practices. Excessive use of nitrogen fertilizer often results in overly vigorous canopy development and increased incidence and severity of the bunch rot complex (Arden-Clarke and Hodges. 1988, Pearson and Goheen 1988). Houma *et al* (1998) found that high N predisposed grapevines to infection by *B. cinerea* and increased disease severity

In general, disease pressures increase when high levels of synthetic N fertilizer are applied to grapevines (Kast,1991) . This relationship also occurs in nectarines (Daane *et al.* 1995, Ramirez 1993). Other negative effects of excessive use of N fertilizer in vineyards may include groundwater pollution, reduced productivity, and reduced fruit quality (Schaller 1991, Spayd *et al.* 1991, Weinbaum *et al.* 1992). Some of the negative aspects of high levels of N fertilization may be due to indirect effects(shading) rather than direct N effects (Smart 1991). Chambers *et al* (1993) found that the bunches from vigorous vines grown with excess N could develop high levels of *B. cinerea* rot

On the other hand, when leaves are removed from the base of shoots, grapevine canopy microclimate is modified (English *et al.* 1990, Zoecklein *et al.* 1992). Radiation levels and evaporation rates within the canopy are increased. Consequently, the incidence and severity of grape fungal diseases are reduced by leaf removal (Perez-Munoz 1993, Bettiga *et al.* 1989, Chellemi *et al.* 1992, Gubler *et al.* 1987, Biedose *et al.* 1988, English *et al.* 1990, English *et al.* 1989, Hunter and Visser 1988, Hunter and Visser 1990, Zoecklein *et al.* 1992). Wind speed through grapevine canopies was shown to increase markedly after leaf removal (English *et al.*, 1989) and development of *B. cinerea* was decreased inversely with wind speed (Thomas, 1988). Research into other potential means of canopy management has shown positive effects of increased yield and higher quality fruit resulting from changes in canopy microclimate, (Smart, 1985). Also integrating leaf removal with chemical control may reduce the need for multiple fungicide applications (Bettiga *et al.*, 1989). Rot reduction after leaf removal was greatest when the leaves were pulled from the fruit zone on both sides of cordon - trained vines (Stapleton and Grant, 1992, Candolfi-Vasconcelos and Koblet. 1990).

The present study was planned to investigate the effect of different levels of nitrogen fertilization on the bunch rot disease. It was also expanded to highlight on the use of leaf removal technique alone or combined with a fungicide applications for potential control of bunch rot.

MATERIALS AND METHODS

A field trial was conducted in two successive seasons, 2003 and 2004 in a 10-years-old grapevines cv. Red Globe (seeded) commercial vineyard (grown in clay soil) in Menia governorate, Egypt. Vines on this site were moderately vigorous, cordon – trained, spur – pruned and planted on a spacing of 3.5 × 1.5 m. supported on Y shape.

Methods of irrigation and other cultural practices for grapevine were as recommended to commercial vineyards in this site. The vines were distributed in a completely randomized block design at which twelve vines as a treatment with 3 replicates have received either of the following treatments: (1) Control (without adding N fertilizer) (2) N_1 at the rate of 30 Unit of Nitrogen (ammonium sulfate) (3) N_2 at the rate of 45 Unit of Nitrogen (4) N_3 at the rate of 60 Unit of Nitrogen (5) N_4 at the rate of 75 Unit of Nitrogen (6) N_5 at the rate of 90 Unit of Nitrogen were used.

Another trial was conducted in the same field to investigate the use of leaf removal alone or in combination with a fungicide applications (subplot). Spray timings were established according to growth stages of the grapevine. Treatments consisted of single application of Euparen M (Tolylfluanid) at 200g/100L.W. at each of bloom, preclose, and veraison stage. A fourth treatment included three sprays at the aforementioned times, and fifth treatment was a nonsprayed control. The spray treatments were applied to the two inside rows. Bunch rot and yield weight were evaluated at harvest. Three randomly vines from each treatment in each replicate were hand harvested and evaluated for incidence & severity of bunch rot and yield.

Bunch rot incidence:

Bunch rot incidence was evaluated by counting diseased clusters per vine.

Disease severity:

Disease severity was determined by counting rotted berries and converting these figures to a percent rot per cluster based on the average number of berries per cluster according to (Gubler *et al.*, 1987).

Leaf removal:

Leaves and laterals located opposite, one node above, and one node below each of flower cluster were removed by hand at late bloom, resulting in window of exposed clusters.

Evaluation of the yield:

Yield was obtained by taking clusters weight per vine.

Data were statistically analyzed according to Snedecor and Cochran (1972) and treatment averages were determined according to Duncan's Multiple Rang test (1955).

RESULTS

The incidence and severity of grape bunch rot disease were estimated under the effect of different levels of nitrogen fertilizer as mentioned before. Data presented in Table (1) clearly show that the disease incidence percentage of bunch rot reached 30, 87, 40.33, 52.83, 67.33, 80.67 and 35.67% for N_1 , N_2 , N_3 , N_4 , N_5 and the control, respectively in the first season (2003). The disease severity percentage of bunch rot was also influenced by the different levels of nitrogen treatment. Bunch rot disease severity was significantly reduced by reducing the levels of nitrogen. The respective averages recorded 3.63, 4.18, 5.38, 6.47, 7.67 and 4.00% for N_1 , N_2 , N_3 , N_4 , N_5 and the control, respectively.

In the second season (2004), it is clear from the data in Table (1) that all treatments showed the same trend for disease incidence and severity. The

obtained results from Table (1) indicate that the disease incidence recorded 28.33, 41.33, 54.67, 69.67, 83.33 and 32.67% for N₁, N₂, N₃, N₄, N₅ and the control, respectively. In general, incidence and severity of bunch rot disease were relatively low in both seasons of 2003 and 2004. Orthogonal contrasts identified significant differences resulting from application of Nitrogen fertilizer in the first season (Table 1). The respective averages of the disease severity of bunch rot reached 3.12, 4.63, 5.14, 6.80, 7.27 and 3.87%, respectively.

The yield/vine was significantly increased in the treatments that showed reduction in the infection with bunch rot. The averages of clusters weights harvested from vines treated with N₁, N₂, N₃, N₄, N₅ and the control were 13.63, 12.97, 11.43, 10.18, 8.37 and 9.57Kg/vine, respectively in the first season (2003), while these figures were 12.67, 11.73, 10.53, 10.00, 8.77 and 8.93Kg/vine, respectively in the second season (2004).

Table (1): Effect of different levels of nitrogen fertilizer treatments on bunch rot disease of grapes ,cv. Red Globe (During the seasons 2003,2004)

Treatments	2003			2004		
	Disease incidence (%)	Disease severity (%)	Yield/vine (kg)	Disease incidence (%)	Disease severity (%)	Yield/vine (kg)
N ₀ (without N)	35.67	4.00 c	9.57 c	32.67	3.87 c	8.93 d
N ₁ 30 unit N	30.87	3.63 d	13.63 a	28.33	3.12 d	12.67 a
N ₂ 45 unit N	40.33	4.18 c	12.97 a	41.33	4.63 b	11.73 ab
N ₃ 60 unit N	52.83	5.38 b	11.43 b	54.67	5.14 b	10.53 bc
N ₄ 75 unit N	67.33	6.47 a	10.18 c	69.67	6.80 a	10.00 c
N ₅ 90 unit N	80.67	7.67 a	8.37 d	83.33	7.27 a	8.77 d

a,b,c Values in the same column with different superscripts are differed significantly at P<0.01.

Data of 2003 season indicate that the trial of leaf removal method significantly reduced the incidence and severity of bunch rot (Table 2). Orthogonal contrast analysis of the data indicated that disease incidence was significantly reduced from 47.57% in the control treatment to 33.00% when the leaves were removed. Leaf removal also significantly decreased disease severity. Data also show a reduction in disease severity from 43.87% in the control treatment to 30.87% in the leaf removed treatment. Single fungicide (Euparen M) application at bloom, preclose and veraison resulted in significant reduction in both disease incidence and severity in the vines managed by leaf removal (Table 2) compared with vines of intact leaves. Similarly, Euparen M applications at bloom, preclose and veraison stages significantly reduced bunch rot incidence on leaf removed vines. A fungicide (Euparen M) application on intact vines resulted in better disease control compared to unsprayed control vines.

In the second season, it is clear from the obtained data in Table (3) that all treatments showed the same trend of the data were obtained in the first season.

Table (2): Effect of leaf removal and using the fungicide Euparen M on the incidence and severity of bunch rot disease and yield of grapevine cv. Red Globe, during season 2003.

Leaf treatment	Timing of a fungicide application					Mean
	Control	Stages			Bloom+ Preclose+ Veraison	
		Bloom	Preclose	Veraison		
Disease incidence (Disease clusters %) ^a						
Leaf removal	33.00	21.17	23.97	26.18	12.93	23.45 ^a
Leaves intact	47.57	34.18	35.17	36.43	28.36	36.34
Means	40.29	27.68	29.57	31.31	20.65	
Disease severity (Percent rot per cluster) ^a						
Leaf removal	30.87	5.87	7.00	7.97	4.33	11.21 ^a
Leaves intact	43.87	7.37	8.93	9.83	6.18	15.24
Means	37.37	6.62	7.97	8.90	5.26	
Yield/Vine (Kg) ^a						
Leaf removal	6.84	8.67	8.13	8.00	11.37	8.60
Leaves intact	5.14	6.87	6.67	6.37	8.16	6.64
Means	5.99	7.77	7.40	7.19	9.77	

^a Results are expressed as an average of three replicates. Mean differences were determined with orthogonal contrasts.

* Figures followed by an asterisk denote a significant ($P < 0.01$) effect from that treatment Sprayed with Euparen M at 200g / 100 L.W

The obtained results (Table 3) indicate that disease incidence was significantly reduced from 48.76% in the control treatment to 34.63% when leaves were removed. Leaf removal also significantly decreased disease severity. Data show a reduction in disease severity from 42.14% in the control treatment to 31.97% in the leaf removed treatment.

Table (3): Effect of leaf removal and using the fungicide Euparen M on the incidence and severity of bunch rot disease and yield of grapevine cv. Red Globe, during season 2004.

Leaf treatment	Timing of Fungicide application					Mean
	Control	Stages			Bloom+ Preclose+ Veraison	
		Bloom	Preclose	Veraison		
Disease incidence (disease clusters %) ^a						
Leaf removal	34.63	22.43	24.76	26.73	12.14	24.14
Leaves intact	48.76	35.43	35.83	36.37	27.67	36.81
Means	41.70	28.93	30.30	31.55	19.91	
Disease severity (Percent rot per cluster) ^a						
Leaf removal	31.97	5.93	7.18	7.53	4.97	11.52
Leaves intact	42.14	7.43	8.78	9.63	7.14	15.02
Means	37.06	6.68	7.98	8.58	6.06	
Yield/Vine (Kg) ^a						
Leaf removal	6.12	7.95	8.00	7.00	11.65	8.14
Leaves intact	4.67	6.43	6.53	5.95	8.48	6.41
Means	5.40	7.19	7.27	6.48	10.07	

^a Results are expressed as an average of three replicates. Mean differences were determined with orthogonal contrasts.

* Figures followed by an asterisk denote a significant ($p < 0.01$) effect from that treatment. Spray with Euparen M (Tolylfuanid at 200g/100L.W.

The yield/vine was significantly increased in the treatments that showed reduction in the infection with bunch rot. The average weights of clusters harvested from vines treated by a fungicide at Bloom, Preclose, Veraison, Bloom + Preclose+ Veraison and from the unmanaged control vines with leaf removal were 8.67, 8.13, 8.00, 11.37 and 6.84Kg/vine, respectively in the first season (2003). The corresponding values obtained during the second season (2004) were 7.95, 8.00, 7.00, 11.65 and 6.12Kg/vine on the average, respectively.

DISCUSSION

Data of the present study demonstrated that bunch rot disease incidence and severity were significantly reduced by reducing the levels of nitrogen. Data from field trials showed that nitrogen fertilizer at 30 N unit resulted in excellent disease control. Also, the use of high level of nitrogen fertilizer increased disease incidence and severity of the bunch rot complex.

The obtained results are in accordance with the findings of Pearson and Goheen (1988), Houma *et al* (1998), Daane *et al* (1995), Ramiraz (1993), Chambers *et al* (1993) and Smart (1991), who showed that the use of high levels of nitrogen fertilizer increased disease incidence and severity of the bunch rot complex.

Controlling bunch rot of grape through the use of canopy management is a viable alternative to repeated fungicide applications. Data from field trials showed that leaf removal resulted in excellent disease control even under conditions otherwise causing severe rot. Other treatments used in this study also reduced the incidence and severity of bunch rot but less than using the leaf removal technique.

Nowadays, fungicides are used widely in controlling bunch rot disease on grapes, but generally become less effective because of heavy canopy growth and bunch closing as the grapevine matures. Usually, by the three fungicide application at or near veraison stage, it becomes virtually impossible to penetrate the canopy with enough volume to adequately protect the cluster targets (Gubler *et al.*, 1987).

Results of fungicide timing trials also lead to the need for a fungicide application at bloom stage. The obtained data showed significant differences in disease control between single fungicide applications made at bloom or preclose or veraison and the three sprays at the timing described, these results are in line with those obtained by McClellan and Hewitt, (1973) who reported that applications at the bloom stage were most effective. They based this on the ability of *B. cinerea* to infect immature grape berries via senescing flower parts resulting in latent infection (Savage and Sall, 1983). However, they were unable to detect the presence of the fungus in the immature berries. Fungicides alone do not provide adequate protection against bunch rot during severe disease pressure. Integrating the cultural control practice; i.e. leaf removal with the chemical control will provide an adequate protection against bunch rot disease.

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تأثير مستويات مختلفة من النيتروجين ، إزالة الأوراق والمبيدات على مرض عفن
عنقود العنب

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أجريت تجربتين حقليتين على شجيرات عنب عمرها ١٠ سنوات صنف الرد جلوب
(صنف بذري) في محافظة المنيا - مصر. في هاتين التجربتين كانت المعاملات تشمل أضافه ٣٠
وحده نيتروجين (سلفات النشادر) ، ٤٥ وحدة نيتروجين ، ٦٠ وحدة نيتروجين ، ٧٥ وحدة
نيتروجين ، ٩٠ وحدة نيتروجين و معاملة الكنترول (بدون إضافة نيتروجين) ، استخدمت هذه
المعاملات لتقييم تأثير المستويات المختلفة من النيتروجين على نسبة وشدة الإصابة بعفن العنقود
أظهرت النتائج المتحصل عليها أن نسبة وشدة الإصابة بعفن ١ لعنقود قلت بدرجة عالية
عندما استخدمت معاملة النتروجين بمعدل ٣٠ وحده بينما أدى استخدام المستويات العالية من السماد
النيتروجيني إلى زيادة نسبة وشدة الإصابة بعفن العنقود.

كما أجريت تجربة إضافية لمقارنة معاملة إزالة الأوراق بمعاملة الكنترول (بدون إدارة
رأس الشجرة). تم تصميم هذه التجربة بنظام القطع المنشقة سواء المرشوشة أو غير المرشوشة
بالمبيد الفطري . المعاملات المذكورة عالياه استخدمت خلال موسم النمو ابتداء من مرحلة التزهير
الكامل حتى مرحلة بداية النضج لمقاومة عفن العنقود .

أدت إزالة الأوراق أيضاً إلى خفض نسبة وشدة الإصابة في معاملة الكنترول غير
المرشوشة مقارنة بمعاملة الكنترول ذات الأوراق السليمة وغير المرشوشة. أظهرت المعاملة ذات
الثلاث رشات بمبيد اليوبارين- إم عند التزهير ، وقبل تلامس الحبات ، و عند مرحلة بداية النضج
انخفاضاً ملحوظ في نسبة وشدة الإصابة بالمرض. وكان محصول الثمار المنتج من شجيرات العنب
المعاملة أعلى معنوياً في كلا موسمي الدراسة مقارنة بمحصول شجيرات العنب غير المعاملة.