EFFECT OF NITROGEN FERTILIZATION, GREEN PRUNING AND FUNGICIDE TREATMENTS ON BOTRYTIS BUNCH ROT OF GRAPES

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SUMMARY

Grape vineyard culture practices including nitrogen fertilization, removal of leaves, and thinning of clusters, as well as fungicide treatments were evaluated for their effect on the development of Botrytis bunch rot. High nitrogen fertilization predisposed grapevines to infection by *Botrytis cinerea* and increased disease severity. Latent infection of cups and berries as well as visible infection of clusters increased as the rate of ammonium nitrate amendment increased. Conversely, removal of leaves around clusters, when practiced two or three times during the season, and thinning of berries significantly reduced Botrytis bunch rot development and resulted in less latent and visibly infected clusters and berries. These green pruning practices also attenuated the beneficial effects of nitrogen fertilization on disease development. *In vitro* experiments revealed that among the tested fungicides, Vinchlozoline, Chlorothalonil, and Dichlofluanide were effective in completely inhibiting the germination of conidia. Iprodione and Procymedone were apparently confronted to the problem of fungal resistance. Folpel, Copper and Chlorothalonil were not able to control mycelial growth as effectively as conidial germination. Field experiments showed that all tested fungicides significantly reduced disease compared to the untreated control, but Methyl-thiophanate, Dichlofluanide and Vinchlozoline provided the best control and gave a more than 60% reduction in infected clusters and approximately 40% increase in yield as compared to the control. Incidence of *Botrytis* infection was significantly influenced by the period of fungicide application during bloom. The best results are obtained with the fungicide applied early during bloom. Based on these results, a strategy combining cultural management techniques and reasonable use of fungicides is recommended to control Botrytis bunch rot.

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RIASSUNTO

Effetti della fertilizzazione azotata, della potatura verde e dei trattamenti fungicidi sul marciume del grappolo causato da botrite su vite. Pratiche colturali quali la fertilizzazione azotata, lo sfoltimento fogliare ed il diradamento dei grappoli, come pure i trattamenti fungicidi sono stati valutati per i loro effetti sullo sviluppo del marciume del grappolo causato da botrite. Alte fertilizzazioni azotate hanno predisposto la vite all'infezione da *Botrytis cinerea* ed hanno aumentato la gravità della malattia. Le infezioni latenti nei peduncoli e negli acini come pure le infezioni visibili dei grappoli sono aumentate con l'aumentare della quantità di nitrato di ammonio. Al contrario, l'asportazione di foglie attorno ai grappoli, quando praticata due o tre volte durante la stagione, ed il diradamento degli acini hanno ridotto significativamente lo sviluppo della botrite diminuendo il numero dei grappoli e degli acini con infezione latente e visibile. Queste pratiche di potatura verde hanno anche attenuato gli effetti della fertilizzazione azotata sullo sviluppo della malattia. Gli esperimenti *in vitro* hanno mostrato che tra i fungicidi saggiati, Vinclozolin, Chlorothalonil e Dichlofluanid avevano efficacia nell'inibire completamente la germinazione dei conidi. Iprodione e Procymidone erano apparentemente affetti dal problema della resistenza acquisita. Folpet, Rame e Chlorothalonil non erano in grado di controllare la crescita miceliare tanto efficacemente quanto la germinazione dei conidi. Gli esperimenti in campo hanno mostrato che tutti i fungicidi saggiati hanno significativamente ridotto la malattia in confronto al controllo non trattato, ma Thiophanate-methyl, Dichlofluanid e Vinclozolin hanno fornito il miglior controllo con una riduzione maggiore del 60% di grappoli infetti ed approssimativamente un incremento produttivo del 40% rispetto al controllo. L'incidenza delle infezioni di *Botrytis* erano significativamente influenzate dal momento di applicazione del fungicida durante la fioritura. I risultati migliori sono stati ottenuti con il fungicida applicato appena all'inizio della fioritura. Sulla base di questi risultati, è raccomandata una strategia di difesa che combini tecniche di gestione colturale ed un ragionevole uso di fungicidi per il controllo del marciume del grappolo causato da botrite.

Key words: *Botrytis cinerea*, culture practices, disease, leaf removal, thinning of clusters.

INTRODUCTION

Botrytis bunch rot or gray mold caused by *Botrytis cinerea* Pers.:Fr. is one of the most economically important diseases of grapes throughout the world (McClellan and Hewitt, 1973). The pathogen may induce important yield losses as a result of early invasion of inflorescences, premature drop of bunches due to stalk rot and/or desiccation of infected berries (McClellan and Hewitt, 1973; Bullit and Dubos, 1982; 1988). The most destructive Botrytis bunch rot has been associated with mature grapes subjected to late season rains or prolonged periods of high humidity (McClellan and Hewitt, 1973). Losses are particularly severe on cultivars with dense canopies and compact clusters where maturing berries are compressed together (Thomas *et al.*, 1988). Nitrogen fertilization has reportedly been considered as an enhancer of Botrytis bunch rot development (Ribereau-Gayen, 1970). Different investigations have revealed that judicious use of nitrogen fertilization, appropriate trellis systems and removal of leaves around the fruit slow the development of the disease (Savage and Sall, 1983, 1984; Gubler *et al.*, 1987).

Attempts to control Botrytis bunch rot of grapes using chemicals have met with varied success (Bolay and Rochaix, 1976; Leroux and Moncomble, 1993). A program of four applications, developed by Lafon and Couillard (1970), in France, referred to as the standard method, has given satisfactory results. In that program, the first treatment was scheduled at the end of bloom and the beginning of fruit set, the second treatment just before berry touch, the third treatment at the beginning of véraison (beginning of berry ripening; beginning of loss of green color) and the last treatment three weeks before harvest. Although the majority of researchers recommended starting chemical control at the end of bloom, McClellan and Hewitt have demonstrated that *B. cinerea* infect grape floral parts early at bloom time. The fungus then becomes latent and remains in the stigma and style tissue until later in the season, at which time it renews growth and rots the berries. Therefore, earlier fungicide applications during the season may presumably result in a better control of Botrytis bunch rot. Another problem related to chemical treatments is the development of resistance by strains of *Botrytis* to the fungicide, which becomes ineffective. This phenomenon was frequently reported in the case of fungicides belonging to benzimidazoles and dicarboximides (Bulit and Dubos, 1988).

Since it is generally believed that a combination of cultural practices and chemical control measures is necessary to minimize losses caused by *B. cinerea* on grapes (Bulit and Dubos, 1988), the objectives of the present studies were to first determine the incidence of nitrogen fertilization alone and in combination with removal of leaves and/or berries (clusters) on the severity of *B. cinerea*, and second to evaluate the effectiveness of different chemical programs with selected fungicides on bunch rot development.

MATERIALS AND METHODS

Plant material. Field experiments were conducted in vineyards, located in the region of Takelsa (North-East of Tunisia), with previous incidences of bunch rot caused by *B. cinerea*, during the 1995 growing season. These studies were performed with the table variety 'Muscat d'Italie' trained either according to the pergola or the pergolette trellising system.

Nitrogen fertilization and green pruning. The first experiment was carried out in a pergolette vineyard to evaluate the effects of nitrogen fertilization on latent and visible infection of grapes by *B. cinerea*. Nitrogen was used in the form of ammonium nitrate (NH_4NO_3) and the trial comprised five treatments: nitrogen amendment with 200, 300, 450, or 700 Kg ha^{-1} of NH_4NO_3, and the control without nitrogen amendment. Ammonium nitrate was applied to the soil at three different periods: the first part was applied in February before bud swell, the second in early June at the fruit set stage and the last part was incorporated by mid July at the fruit touch stage. Treatments were arranged according to a completely randomized design with ten replications. Each replication consisted of three rows of ten vines.

The second experiment was undertaken in a pergola trained vineyard, at the same location, to evaluate the combined effects of nitrogen fertilization and green pruning (removal of leaves and berries) on Botrytis bunch rot incidence. In this trial a Split-Block experimental design, with three replications, was adopted in which nitrogen fertilization was considered as the main factor and green pruning as a secondary factor. Four levels of nitrogen were used: 0 (control), 225 (D1), 345 (D2), and 763 Kg ha^{-1} of ammonium nitrate (D3). Ammonium nitrate was applied at three different periods as mentioned above (February, early June, and mid-July). Six green pruning treatments were considered: a single leaf-removal in early June (1LR); two leaf-removals, the first in early June and the second four weeks later (2LR); two leaf-removals as in (2LR) plus one thinning of berries at the fruit touch stage (2LR+TH); Three leaf-removals at 4 week-intervals (3LR); three leaf-removals as in (3LR) plus one thinning of berries at the fruit touch stage (3LR+TH); and a control treatment without leaf-removal and without thinning of berries (C). Thus each of the three replications was comprised of 24 treatments (4 doses x 6 green pruning treatments), and each treatment plot consisted of two rows of ten vines (480 vines per replicate). Fruit- and leaf-removal was carried out by hand.

In both experiments we determined the incidence of each treatment on latent and visible levels of infection by *B. cinerea.* Presence of latent mycelium in caps and berries was followed by random sampling every 15 days from May to August. The samples were collected throughout this period, surface sterilised in 70% ethanol for 30 s, and plated on 2% Malt agar containing 100 ppm chloramphenicol. Tissue platings were incubated in a growth chamber at 20°C for 48 h, and then examined microscopically for fungal growth. Calculated means are based on the examination of 100 cups or berries. Visible infections caused by *B. cinerea* were rated based on the method used by Jermini and Jelmini (1986), which is based on the level and intensity of rot development. There were five disease classes based on percent berries rotted: I=0-5%; II=5-25%; III=25-50%; IV=50-95%; and V=95-100%. Rating for visible disease symptoms was performed at véraison and maturity stages, and samples were analysed for *Botrytis* propagules using the Malt-chloramphenicol medium mentioned above.

Chemical control experiments. Different fungicides were assayed for their activity against *B. cinerea in vitro* and *in planta.* Evaluated fungicides are: Iprodione (Rovral 50 WP, Rhodiagri-Littorale, SEPCM, TUN), Vinchlozolin (Ronilan 50 WP, BASF France, STIMA, TNU), Procymidone (Sumisclex 50 WP, ICI Spora, Agrochimie, TUN), Dichlofluanide (Euparene 50 WP, Bayer France, Agrochimie, TUN), Thiophanate-methyl (Pelt 44, 70 WP, Procida, STEC, TUN), and Cymoxanil + Folpel + Copper (Anteor C3, 4-16.7-25 WP, Procida, STEC, TUN). All fungicides were suspended in distilled water and tested at 1, 1/5, 1/10, and 1/100 of the registration dose (RD). For the first three fungicides RD = 75 g hl^{-1} and for the others the value of RD is respectively 200, 140 and 600 g hl^{-1}.

Laboratory studies. *Botrytis* isolate used for laboratory studies was from infected grapes of the cultivar 'Muscat d'Italie'. It was grown from a single spore and maintained on Potato Dextrose Agar (PDA) medium. Conidia were collected from 12-day-old cultures, suspended in 0.001% Tween 20 (polyoxyethylene sorbitan monolaurate). They were then filtered through glass wool, washed three times with distilled water, and resuspended in water to give a final concentration of $5x10^4$ spores ml^{-1}, estimated by counting with a hemacytometer. Germination essay was carried out in cavity slides. One hundred µl of the test suspension and 50 µl of the conidial suspension were pipetted onto cavity slides and then placed on wet Whatman N°1 filter papers in Petri dishes and held at 22°C, with a 12-h-photoperiod of approximately 145 µE m^{-2} sec^{-1}. Counts of germinated conidia were taken 48 h post-incubation, and calculated means were based on 100 measurements. Four replicates of each concentration, and two separate tests were performed.

Fungicides were also tested for their activity against mycelial growth. Suspension of fungicides were prepared in sterile distilled water and added to Fababean Dextrose Agar (FDA; 200 g of fababean grains, 20 g of dextrose and 18 g of agar l^{-1} of distilled water) medium at approximately 50°C to yield the appropriate concentration. After mixing, the amended FDA was dispensed into 9-cm-diameter Petri dishes and allowed to cool. Six-mm-diameter plugs of agar from actively growing mycelium of *B. cinerea*, grown on PDA, were placed with the surface mycelium face down on the test FDA medium. The plates were then incubated at 22°C, and mycelial growth was measured at 24h intervals. Four replicates of each concentration were used, and three separate tests were performed.

Field studies. In a first field trial, carried out in a pergola trained vineyard, we tested the effects of the above mentioned fungicides on Botrytis bunch rot development. Fungicides were applied according to the standard program (end of bloom; before berry touch; beginning of véraison; 3 weeks before harvest), at label rates. Treatments were randomized in a complete block design with three replicates of four vines. Fungicide effects, based on disease severity, were assessed at harvest. The latter was estimated by the percentage of decayed clusters and yield, which was determined by weighing all apparently uninfected clusters.

In a second trial we compared four different application programs using the fungicide Iprodione: in program 1 (P1), Iprodione was applied at the beginning of bloom, at véraison and at berry maturation; in program 2 (P2) treatments were applied at full flowering (approximately 50% of caps fallen), at véraison, and at berry maturation; in program 3 (P3), treatments were applied at late bloom, at véraison, and at berry maturation; and in program 4 (P4), Iprodione was applied based on the standard program. This assay was conducted according to a completely randomized block design with four replicates. After bloom treatments, we monitored latent cap and berry infections, as previously described, and at harvest we determined the percentage of visible infection by *B. cinerea*.

Data analysis. Data for yield and Botrytis bunch rot variables were analysed by ANOVA, means were separated by Newman-Keuls test, and responses were judged significant at the 5% level (P=0.05). The software SAS (SAS Institut, Cary, N.C.) was used for statistical analysis.

RESULTS

Nitrogen fertilization and green pruning. Fig. 1 shows, the incidence of nitrogen fertilization on the percentage of cups and berries latently infected by *B. cinerea*. All cups and berries were symptomless at the time of sampling. From Fig. 1 it appears that *Botrytis* infection of cups and berries was low, relatively stable and similar in all treatments from may to early July. Past this period, the percentage of latent infection of cups and berries shows a significant increase in the nitrogen treated vines as compared to the controls (Fig. 1). These levels of infection increased as the rate of nitrogen amendment was higher. At the last sampling date, while nearly 45% of cups and 35% of berries were infected in the highest nitrogen treatment, only approximately 10% of these organs were contaminated in the controls (Fig. 1). Determination of the percentage of clusters visibly infected by *B. cinerea*, at véraison (Fig. 2A) and maturity (Fig. 2B), revealed significant increases in rot development following nitrogen fertilization. Decayed clusters increased as the level of nitrogen fertilization increased and also as berries neared maturity (Fig. 2). The application of nitrogen increased not only the percentage of clusters infected, but also the percentage of decayed berries per cluster (Fig. 2). In fact, at the maturity stage, only vines receiving the doses 450 Kg ha^{-1} and 700 Kg ha^{-1} yielded clusters with class III (25-50%) and/or class IV (50-95%) decayed berries (Fig. 2B). Many almost completely decayed clusters were obtained with the highest nitrogen dose (700 kg ha^{-1}). On the other hand, the untreated control vines showed less than 5% of *Botrytis*-infected clusters, with less than 5% decayed berries during all the experimental period (Fig. 2).

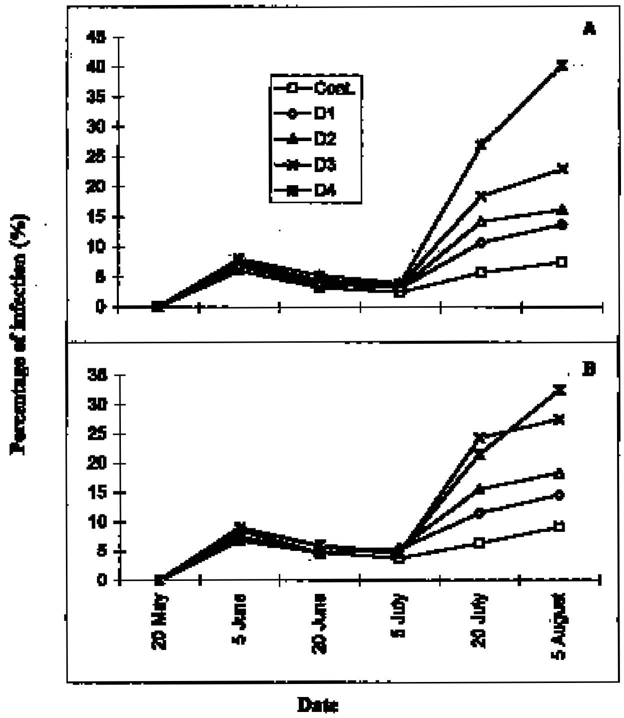

Fig.1. Effect of nitrogen fertilization on the percentage of cups (**A**) and berries (**B**) latently infected by *Botrytis cinerea*. D1: 225; D2: 345; D3: 763 kg ha^{-1} of ammonium nitrate.

Results relative to the combined effects of nitrogen fertilization and green pruning (leaf and berry removal), determined at the maturity stage, are presented in Fig. 3. At the véraison stage, we obtained the same tendency of differences among green pruning treatments, for all nitrogen doses tested (not shown). Statistical analysis of these results showed highly significant effects of nitrogen fertilization as well as green pruning on Botrytis bunch rot development. We also found a significant interaction between both treatments. In agreement with the previous results obtained with pergolette-trained vines, increased nitrogen fertilization enhanced the percentage of infected clusters and disease development (Fig. 3A, D). For example, application of 763 Kg ha^{-1} of ammonium nitrate to controls (without green pruning) (Fig. 3D) more than doubled the percentage of infected clusters as compared to their non-treated counterparts (Fig. 3A). Moreover, the former treatment resulted in the appearance of class II, III, and IV clusters with more severe *Botrytis* attacks.

In terms of green pruning incidence on Botrytis bunch rot development, the beneficial effects of leaf-removal and berry-thinning were evident in the present experiment, as far as the percentage of infected clusters

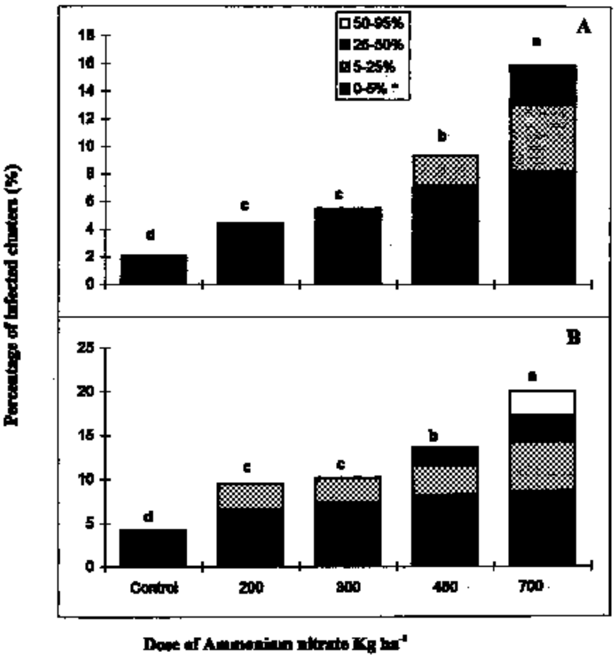

Fig.2. Effect of nitrogen fertilization on the percentage of clusters visibly infected by *Botrytis cinerea* at véraison stage (**A**) and maturity stage (**B**). Bars with the same letter are not significantly different at the 5% level (P=0.05). * rotted berries.

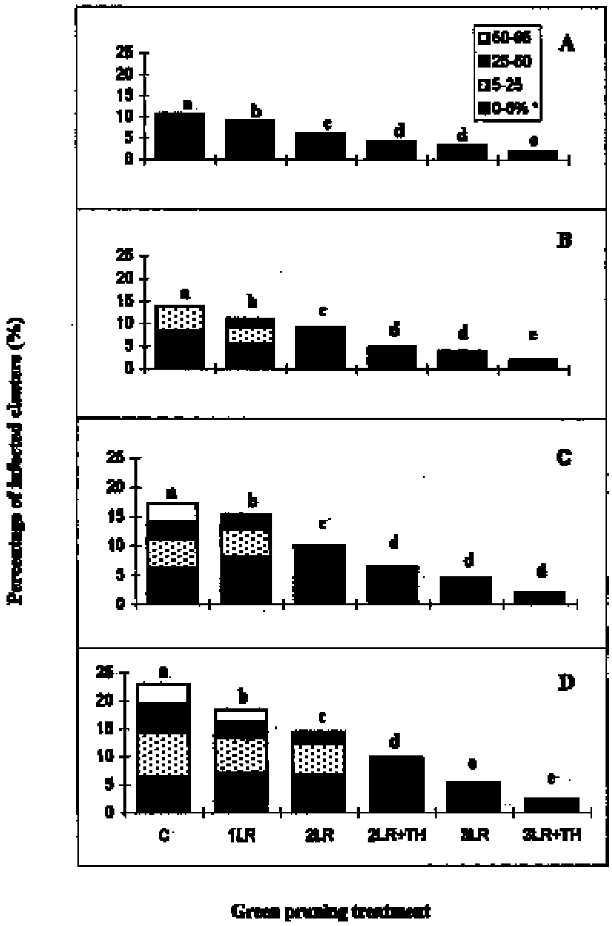

Fig.3. Effect of nitrogen fertilization (**A**: control; **B**: 225 kg ha^{-1}; **C**: 345 kg ha^{-1}; **D**: 763 kg ha^{-1}) and green pruning (C: control; LR: leaf removal; TH: thinning of berries) on the percentage of clusters infected by *Botrytis cinerea*. Within each treatment bars with the same letter are not significantly different at the level 5% (P=0,05). * rotted berries.

is concerned. In fact, from a percentage mean of infected clusters of 16.2% obtained in the controls, we observed 13.4% of infection after one leaf-removal, 9.9% after two leaf-removals, and only 4.3% following three leaf-removals. A supplementary thinning of berries further decreases the percentage of infected clusters. As a matter of fact, combination of this treatment with two or three leaf-removals resulted in approximately 33% and 50% decrease in mean percentage of infected clusters, respectively, as compared to the same berry-non-thinned treatments. Interestingly, the beneficial effects of green pruning practices are not limited to only a decrease in the percentage of infected clusters, but generally reduced Botrytis bunch rot severity (Fig. 3A, D). This appears clearly in the treatments: (2LR+TH), (3LR) and (3LR+TH), where less than 5% (class I) of berries were rotted, regardless of nitrogen treatment (Fig. 3).

Chemical control experiments.

Effect of fungicides on germination of B. cinerea *conidia.* From Fig. 4 it can be seen that germination of conidia was completely inhibited by Chlorothalonil and Diclofluanide at their RD and even at 1/5 RD. These active ingredients as well as Vinchlozoline were very active against conidial germination even at very low concentrations (15-20 ppm). Copper and Folpel showed the same activity against germination of conidia of *B. cinerea*. They completely inhibited the germination of conidia at RD, but they were ineffective at lower doses. On the other hand, Iprodione and Procymidone were unable to inhibit germination completely even at the highest dose.

Effect of fungicides on mycelial growth of B. cinerea. In tests using fungicide-amended FDA, the most effective active ingredient was Methyl-thiophanate, which completely inhibited mycelial growth at the highest doses, RD and 1/5 RD (Fig. 5). This fungicide caused approximately 70% inhibition of mycelial growth, 11 days after incubation of the pathogen, on plates amended with Methyl-thiophanate at 1/10 and 1/100 RD (Fig. 5).

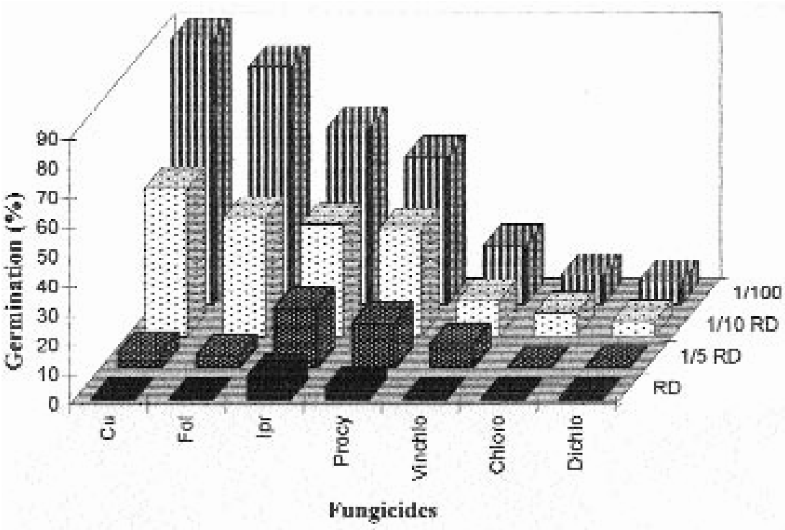

Fig.4. Effect of fungicides on conidial germination of *Botrytis cinerea*. Counts of germinated conidia were taken 48 h post-incubation. Each value is the mean based on 400 measurements. (RD: Registration Dose; Co: Copper; Fol: Folpel; Ipr: Iprodione; Procy: Procymidone; Vinchlo: Vinchlozoline; Chloro: Chlorothalonil; Dichlo: Dichlofluanide).

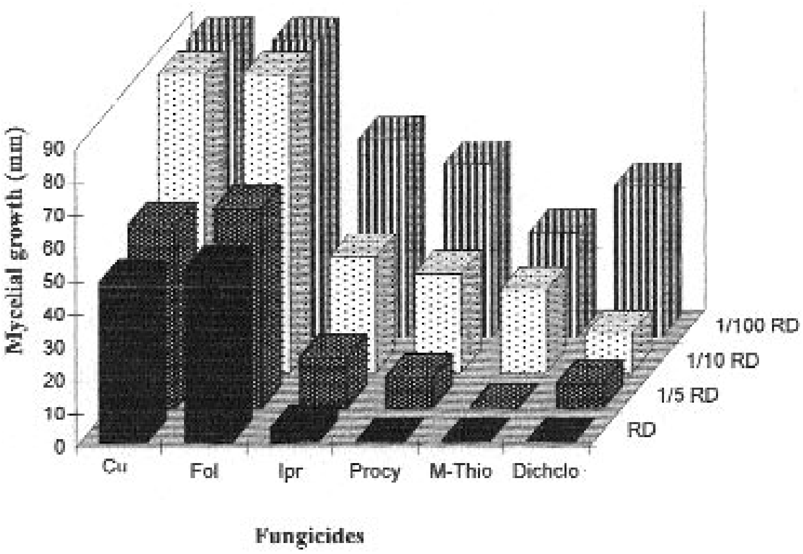

Fig.5. Effect of fungicides on mycelial growth of *Botrytis cinerea*. Measurements were taken 11 days post-incubation. Each value is the mean based on 4 measurements in triplicate experiments. (RD: Registration Dose; Co: Copper; Fol: Folpel; Ipr: Iprodione; Procy: Procymidone; M-Thio: Methyl-Thiophanate; Dichlo: Dichlofluanide).

Complete inhibition of mycelial growth was also maintained during all the experimental period with Diclofluanide and Procymidone, when used at RD. At lower doses, these fungicides generally caused more than 50% inhibition of mycelial growth. In contrast, complete inhibition of mycelial growth did not occur even at the registration dose with Folpel, Copper and Iprodione. Nevertheless, Iprodione was more active than Folpel and Copper against fungal growth, at all tested doses.

Field studies. From the results illustrated in Fig. 6A, related to field studies, it appears that all tested fungicides reduced (P=0.05) disease compared with the nontreated control, but Methyl-thiophanate, Dichlofluanide and Vinchlozoline provided the best control as compared to the other active ingredients. Methyl-thiophanate and Dichlofluanide were equally effective in controlling Botrytis bunch rot and showed more than 70% reduction in infected clusters compared to the nontreated control. Besides the observed reduction in the percentage of clusters attacked following treatment with either of these two fungicides, they reduced the percentage of infected berries as well as the number of lesions observed on attacked berries (not shown). Although Procymidone, Iprodione, and Anteor (Cymoxanil + Folpel + Copper) reduced (P=0.05) the percentage of infected clusters by approximately 50%, 30%, and 15%, respectively, compared with the nontreated control (Fig. 6A), important yield losses were noted on vines receiving these treatments as compared to those treated with Dichlofluanide, Methyl-thiophanate, or Vinchlozoline (Fig. 6B). Incidentally, we found a highly significant negative correlation between the percentage of infected clusters and the average yield per vine (r=-0.97). Yield was reduced from approximately 50 kg/vine with Methyl-thiophanate treatment to less than 34 kg/vine with Anteor treatment and the nontreated control (Fig. 6B).

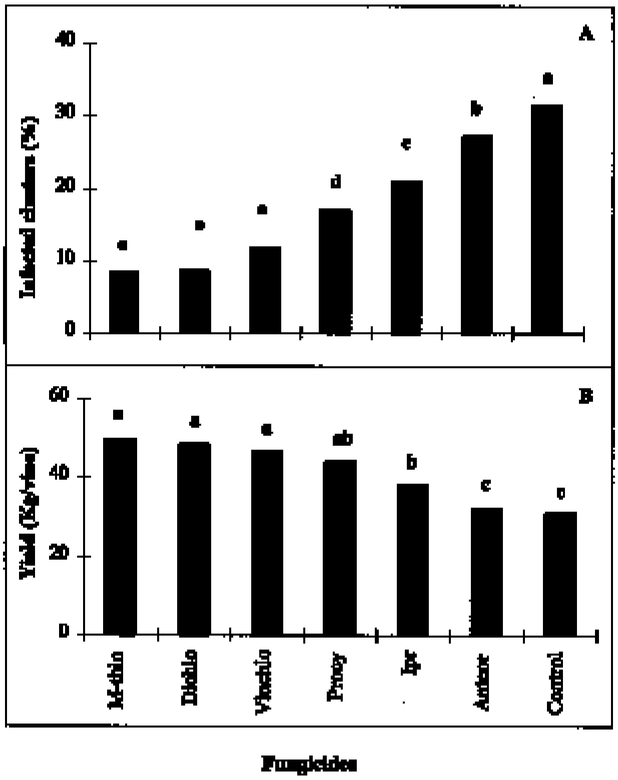

Fig.6. Effect of fungicides on the percentage of clusters visibly infected by *Botrytis cinerea* (**A**) and on yield (**B**). Fungicides were applied according to the standard program at label rates. Bars with the same letter are not significantly different at the level 5% (P=0,05). (M-Thio: Methyl-Thiophanate; Dichlo: Dichlofluanide; Vinchlo: Vinchlozoline; Procy: Procymidone; Ipr: Iprodione).

Comparison of treatment programs. Fig. 7 shows the effect of the stage at which Iprodione is applied, during bloom, on the percentage of cups and berries latently infected with *B. cinerea* as a function of time. Results revealed that application of Iprodione at early and mid-bloom significantly reduced the percentage of infected cups during all the experimental period (Fig. 7A). From late bloom to early véraison, the percentage of infected berries remained more or less constant, regardless of Iprodione treatment (Fig. 7B). By July, the number of infected berries dramatically increased in the late bloom treatment and the nontreated control, but to a much less extent when Iprodione was applied at early and mid-bloom (Fig. 7B). The last two treatments resulted in significantly less infected cups (Fig. 7A) and berries (Fig. 7B) by the end of the experiment.

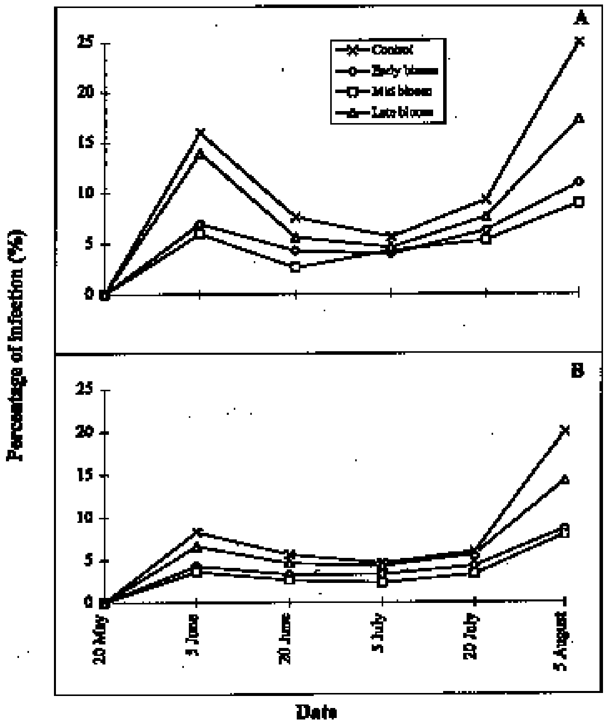

Fig.7. Effect of Iprodione treatment on the percentage of cups (**A**) and berries (**B**) latently infected by *Botrytis cinerea*.

Visible infection of clusters was significantly reduced when Iprodione was applied according to programs P1 and P2 compared to P3 and P4 (Fig. 8). More than 30% increase in the percentage of visibly attacked clusters was observed in the last two programs (Fig. 8).

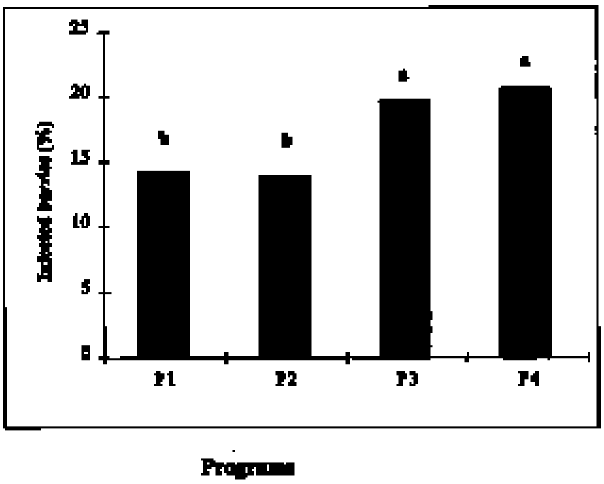

Fig.8. Comparison of the different programs of Iprodione application against Botrytis bunch rot development. P1: fungicide applied at beginning bloom, at véraison and at berry maturation; P2: fungicide applied at mid-bloom, at véraison and at berry maturation; P3: fungicide applied at late bloom, at véraison, and at berry maturation; P4: fungicide applied based on the standard program. Bars with the same letter are not significantly different at 5% level (P=0.05).

DISCUSSION

Cultural control measures, although sometimes expensive, not only result in a decrease in pathogen attacks, but also enable reduction in the use of chemicals. Several studies have investigated the role of climatic factors, such as temperature, relative humidity, and wind speed (Thomas *et al.*, 1988), as well as the effect of cultural practices, such as nitrogen fertilization, pruning, thinning, and training systems (Bulit and Dubos, 1988), on the development of Botrytis bunch rot. In line with those studies, our experiments demonstrated that high nitrogen fertilization predisposed grapevines to *Botrytis* infection and increased the severity of the disease. Latent infection of cups and berries as well as visible infection of clusters increased as the rate of ammonium nitrate amendment increased. The increased disease incidence of *B. cinerea* by nitrogen may be explained by the probable direct stimulation of fungal growth, as a consequence of soluble nitrogen increase within berries, or indirectly by affecting the thickness and anatomy of the berry epidermis and cuticle (Galet, 1982). According to Gartel *et al.* (1977), grapevines receiving high rates of nitrogen may show a defective blooming, which results in a heavy and effortless colonization of necrotic flower remnants. On the other hand, nitrogen excess results generally in excessive vegetation and leafy canopies providing a microclima favourable to bunch rot development.

Our studies indicate that removal of leaves around clusters significantly influences Botrytis bunch rot and results in less infected clusters and berries. Leaf removal was highly effective when practiced two or three times during the season, and this effect was further improved by a supplementary thinning of berries. Leaf removal and berry thinning result in less shaded interior canopies, well aerated clusters, and less moistened berries (English *et al.*, 1989). These conditions are less conducive to *Botrytis* mold development, and may affect fungal growth by limiting mycelial growth, conidiation, and conidium survival (Thomas *et al.*, 1988). Additionally, beneficial effects of these conditions may be attributed in part to a reduction in time necessary for berries to mature due to increased light levels and a subsequent increase in assimilated carbohydrates available to the developing berries. In this context, we found that green pruning increased the levels of soluble sugars and pH (unpublished). Consequently, grapes with earlier maturation would escape late season rains which are very favorable to *Botrytis* attacks. Moreover, good green pruning practices almost completely attenuated the beneficial effects of nitrogen fertilization on disease development.

Iprodione, Procymidone, and Vinchlozoline, among dicarboximides, are known as effective against both conidial germination and mycelial growth (Leroux and Moncomble, 1993). Our results show that only Vinchlozoline, among the tested dicarboximides, was effective in completely inhibiting the germination of conidia. While Dichlofluanide and Chlorothalonil were the most efficacious against the germination of conidia, Iprodione and Procymidone were apparently subjected to the problem of fungal resistance. From these experiments it also appears that effectiveness of fungicides as inhibitors of conidial germination is not always indicative of their performance against mycelial growth. This situation is illustrated in the case of Folpel, Copper, and Chlorothalonil, which were unable to control mycelial growth as effectively as conidial germination. On the other hand, although ineffective against germination of conidia, Procymidone gave a complete inhibition of mycelial growth at the highest rate. This was not the case of Iprodione, where the phenomenon of fungal resistance was observed in both conidial germination and mycelial growth tests. Resistance to benzimidazoles and/or dicarboximides was reported in Switzerland (Bolay and Pezet, 1987), India (Wang *et al.*, 1986; Locke and Fletcher, 1988), France (Groupe de travail, 1990), and Germany (Locker *et al.*, 1987). In our *in vitro* and *in planta* studies, Methyl-thiophanate was very effective against mycelial growth and bunch rot development. Field experiments revealed that Methyl-thiophanate together with Dichlofluanide and Vinchlozoline significantly reduced the percentage of infected clusters and resulted in an important increase in yield compared to the other treatments and the control. Nevertheless, according to Northover and Matteoni (1986), Benzimidazoles are very susceptible to fungal resistance. Therefore, these chemicals must be used as mixtures, intermittently, or alternately in sequence to avoid selection of resistant strains. In addition to resistance related to dicarboximides and benzimidazoles, this phenomenon was also observed, in several countries, in the case of Dichlofluanide (Rewal *et al.*, 1991).

Based on field experiments with Iprodione, comparison of results obtained with the different treatment programs revealed excellent protection from bunch rot with the fungicide applied at early bloom or at midbloom. By contrast, application of the first Iprodione treatment at late bloom resulted in more severely attacked clusters and in a lower level of disease control, regardless of the number of treatments carried out subsequently during the season. Therefore, application of protectants must be carried out at early bloom to maximize efficacy, to reduce the number of sprays from 4 to 3, and to minimize the threat of resistance. Data from our study demonstrated a lower percentage of latently infected cups and berries, when the treatment was executed early during bloom. Studies of artificial inoculation and natural infection in vineyards, reported by McClellan and Hewitt (1973), showed that *B. cinerea* penetrates the grape flower through the stigma end and that maximum infection take place at early bloom and during bloom. That study also showed that the fungus becomes unable to colonize the decayed stigmatic portion during growth stages following the bloom period, presumedly because of the lack of moisture in this tissue and the inhibiting effect of extracts from young berries. In this paper it is demonstrated that the percentage of latently infected berries remains more or less constant, in the different treatments, from late bloom to véraison. In line with these findings, many investigations showed that during this period the fungus remains in a quiescent state until later in the growing period, at which time it renews growth (McClellan and Hewitt, 1973; Jermini and Jelmini, 1986; Pezet and Pont, 1988). That investigation further showed that while pollen and stigma extracts enhance germination of conidia and stimulate growth of germ tubes, tissue extracts of immature berries have an inhibitory effect on germ tube growth.

In conclusion, to reduce the incidence of *B. cinerea* on grapes, control measures combining cultural management techniques and a reasonable use of chemicals can reduce both the initial percentage of infected clusters and berries that result from infection by germinating conidia and the extent of mycelial growth of the pathogen from véraison to harvest. We recommend the following strategies for effective control of *Botrytis* infection: (*i*) the first chemical treatment should be carried out at early bloom with a protectant such as Chlorothalonil, Dichlofluanide, or Folpel; (*ii*) two leaf-removals have to be achieved, one in early June, and the second 4 weeks later; (*iii*) berry thinning should be realized at least once at the beginning of berry touch, in the case of cultivars with compact clusters; (*iv*) if canopy vegetation is still excessive, a third leaf removal may be done in early August; (*v*) two additional chemical treatments, at véraison and maturity stages, with one of the eradicants (Methyl-thiophanate, Dichlofluanide) are necessary. Nevertheless, before this program becomes commercially feasible, economical thinning and leaf-removal methods must be developed.

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