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Investigations on the influence of N fertilizer on resistance to powdery mildew *(Oidium tuckeri)* downy mildew *(Plasmopara viticola)* and on phytoalexin synthesis in different grapevine varieties[1])

by

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Effetto della concimazione azotata sulla resistenza all'oidio *(Oidium tuckeri)*, alla peronospora *(Plasmopara viticola)* e sulla sintesi di fitoalexine in alcuni ibridi e varietà di vite

Riassunto: Talee di vite di un anno (delle varietà Gf Ga-58-30, Fr 993-60, Riesling e Kerner), coltivate in vaso all'interno di una serra, sono state sottoposte ad una concimazione diversificata con quattro dosi di azoto, al fine di valutare la resistenza allo oidio, alla peronospora e la possibile sintesi di fitoalexine (resveratrolo ed ε-viniferina).

Dallo studio è emerso che:

— dosi crescenti di azoto hanno determinato una diminuzione della resistenza all'oidio, alla peronospora e della sintesi di fitoalexine;
— gli ibridi (Gf Ga-58-30 e Fr 993-60) presentano una più elevata resistenza all'oidio, alla peronospora ed una più elevata sintesi di fitoalexine, rispetto alle varietà di *Vitis vinifera* (Riesling e Kerner);
— la sintesi di fitoalexine aumenta durante il ciclo vegetativo annuale.

Key words: variety of vine, Plasmopara, oidium, Botrytis, nitrogen, fertilizing, resistance, phytoalexin.

Introduction

Mineral nutrition of the plant is one of the several factors affecting the physiological behaviour of a genotype. It influences not only the balance of yield : quality, but it determines also in large measure the resistance or susceptibility to diseases, by controlling the plant's biochemical mechanisms which hasten or slow pathogenesis, and the virulence and ability of pathogens to survive (HUBER 1980).

Mineral elements are, in fact, directly involved in all mechanisms of defense as integral components of cells, substrates, enzymes, and electron carriers, or as activators, inhibitors and regulators of metabolism (MENGEL and KIRKBY 1978; FREGONI 1980).

Since REMY in 1898 observed the potassium effect in decreasing diseases of barley, a lot of researchers improved the knowledge of the role of mineral nutrition in defence mechanisms (TROLLDENIER 1969).

The greatest response to mineral elements is often with tolerant or moderately resistant plants, while disease reactions of highly resistant or highly susceptible plants are not as readily altered by nutrition (MÜLLER 1959; HUBER 1980).

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Macronutrients normally increase resistance to disease, if not at all, only in the deficiency range; supraoptimal amounts of nutrients are commonly without effect on disease or may encourage pathogens in the case of nitrogen (YARWOOD 1959).

Nevertheless, the effects of nitrogen on increasing or decreasing diseases change, depending on the pathogen and the host, the nitrogen form (HUBER and WATSON 1974), the time of application (LAST 1954), the age of the plant (JONES and HAYES 1971), the period of the seasonal growing cycle (MCLAUGHLIN and SHRINER 1980), or the interaction with other elements (LAST 1962).

The aim of the present paper was to evaluate the influence of N fertilization on the resistance against the three most important fungus diseases. It was of further interest to study the reactions of different genotypes which are known from the field to have different degrees of resistance against these diseases.

Materials and methods

1-year-old grapevine cuttings were grown in a greenhouse in metal pots (6 l volume) containing a mixture of sand and compost (wastes) in the ratio 2 : 1. Before adding basic nutrients, the main soil characteristics were: pH 6.4, total N = 0.4 ‰; P_2O_5 = 31 mg/100 g soil; K_2O = 32 mg/100 g soil; MgO = 29 mg/100 g soil.

The varieties tested were: Riesling and Kerner (Trollinger × Riesling) as *Vitis vinifera* cultivars, and Gf Ga-58-30 (Optima × S. V. 12-375) and Fr 993-60 (S. V. 5-276 × [Riesling × Ruländer]) as interspecific hybrids.

Nitrogen treatments were 0.2 g N/pot (dose 1), 0.5 g N/pot (dose 2), 0.8 g N/pot (dose 3), 1.1 g N/pot (dose 4), added as NH_4NO_3 in solution to the soil surface, when average shoot length was about 80 cm. 2 months later, when the shoots were twice as long, nitrogen treatments were repeated as follows: 0.2 g N/pot (dose 1), 0.7 g N/pot (dose 2), 1.2 g N/pot (dose 3), 1.7 g N/pot (dose 4). Each treatment was replicated eleven times.

10 d after the first N treatment (1st sampling date), resistance to powdery mildew and stilben synthesis were tested; 50 d later (2nd sampling date), resistance to *Plasmopara viticola* was analysed, and 70 d later (3rd sampling date) — 10 d after the second N treatment — stilben synthesis was analysed again.

Before the tests, the leaves were washed by using a 8 % 'Domestos' solution (containing NaOCl).

The severity of infection for each fungus was recorded for the 5th leaf (beginning from the tip of the shoot) using the *in vitro* method of STEIN *et al.* (1985).

The nitrogen content of the plants was measured by chemical analysis of leaves, using the colorimetric method, after digestion of oven-dry leaves in H_2SO_4/H_2O_2 (COTTENIE 1980).

The severity of powdery and downy mildew infection was recorded by evaluating the conidiophores and zoosporangia covering of the discs and screened from 1 to 9 by increasing resistance. Class 1 meant 100 % of the disc surface covered by fungus, class 9 quite no infection.

The stilbene synthesis was obtained by exploiting 'elicitor' characteristics of mucic acid (method of STEIN and HOOS 1984).

Leaf discs (17 mm ∅) were placed on filter cardboard imbued with mucic acid solution (0.01 %). After phytoalexin extraction and distillation, the stilbenes resveratrol and ε-viniferin were identified by using quantitative thin-layer chromatography; the amount of each sample was 2 µl. The resveratrol and ε-viniferin values were expressed as scan units (s. u.), using a Shimadzu-Chromato-Scanner CS 920, under 325 nm UV radiation.

Results

1. Powdery mildew

Nitrogen supply and the genotype influence the severity of infection in a significant way. The resistance decreases (from 7.0 to 5.5) when increasing N from dose 1 to dose 4 (Table 1).

The hybrids have a higher average value of resistance (8.5) than the *V. vinifera* varieties (3.7); Gf Ga-58-30 shows the highest resistance (8.8), followed by Fr 993-60 (8.2), Riesling (4.6) and Kerner (2.8) (Table 2).

Table 1

Effect of N fertilization on the degree of resistance against powdery and downy mildew and the resveratrol synthesis (average values from the varieties)

Effetto della concimazione azotata sulla resistenza all'oidio, alla peronospora e sulla sintesi di resveratrolo

N-doses	*Oidium* resistance	*Plasmopara* resistance	Resveratrol (s.u.)	
			1st sampling date	3rd sampling date
1	7.0	7.6	1130	4110
2	5.9	7.1	710	4970
3	5.9	6.2	620	4030
4	5.5	6.0	430	2720
LSD 0.05	0.28	0.30	270	1050

Table 2

The degree of resistance against powdery and downy mildew and the stilbene synthesis of different grape varieties (average values from the N doses)

Livelli di resistenza all'oidio, alla peronospora e sintesi di fitoalexine nei diversi genotipi

Varieties	*Oidium* resistance	*Plasmopara* resistance	Resveratrol (s.u.)		Viniferin (s.u.)
			1st sampling date	3rd sampling date	
Riesling	4.6	5.0	350	1480	230
Kerner	2.8	6.1	370	2390	260
Fr 993-60	8.2	7.4	870	1430	230
Gf Ga-58-30	8.8	8.5	1290	10520	780
LSD 0.05	0.28	0.30	270	1050	170

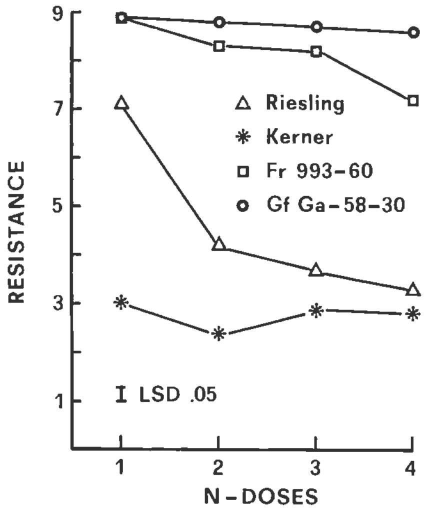

Fig. 1

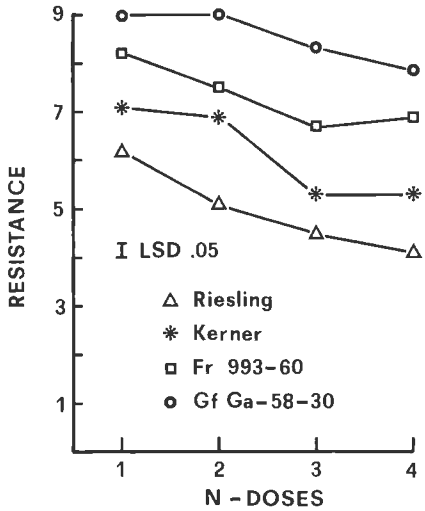

Fig. 2

Fig. 1: The effect of nitrogen supply on the degree of powdery mildew resistance of different grape varieties.

Effetto delle diverse dosi di azoto sul livello di resistenza all'oidio, nei quattro genotipi.

Fig. 2: The effect of nitrogen supply on the degree of downy mildew resistance of different grape varieties.

Effetto delle diverse dosi di azoto sul livello di resistenza alla peronospora, nei quattro genotipi.

The high resistance of Gf Ga-58-30 and the low one of Kerner are independent of the N fertilization, while Fr 993-60 and Riesling show a lowering of resistance due to increasing nitrogen supply (Fig. 1).

2. Downy mildew

The resistance to the fungus is affected by the rates of nitrogen and the genotype.

By increasing the nitrogen supply, the resistance decreases from 7.6 to 6.0 (Table 1).

The hybrids show a high resistance (8.5 in Gf Ga-58-30 and 7.4 in Fr 993-60), while the *V. vinifera* varieties have a medium one (5.0 in Riesling and 6.1 in Kerner) (Table 2).

All genotypes react in a similar way, when nitrogen supply is changed (Fig. 2).

3. Stilbene synthesis

By increasing the nitrogen supply, the resveratrol synthesis decreases, at the 1st sampling time changing from 1130 s. u. to 430 s. u.; at the 3rd sampling time the highest resveratrol content is shifted onto 2-N dose (4970 s. u.) and than it decreases up to 4-N dose (2720 s. u.) (Table 1).

The highest resveratrol synthesis at the 1st sampling time occurs in Gf Ga-58-30 (1290 s. u.), the lowest one in Riesling (350 s. u.); the differences among the genotypes are higher at the 3rd sampling time, where the extreme values are 10 520 s. u. for

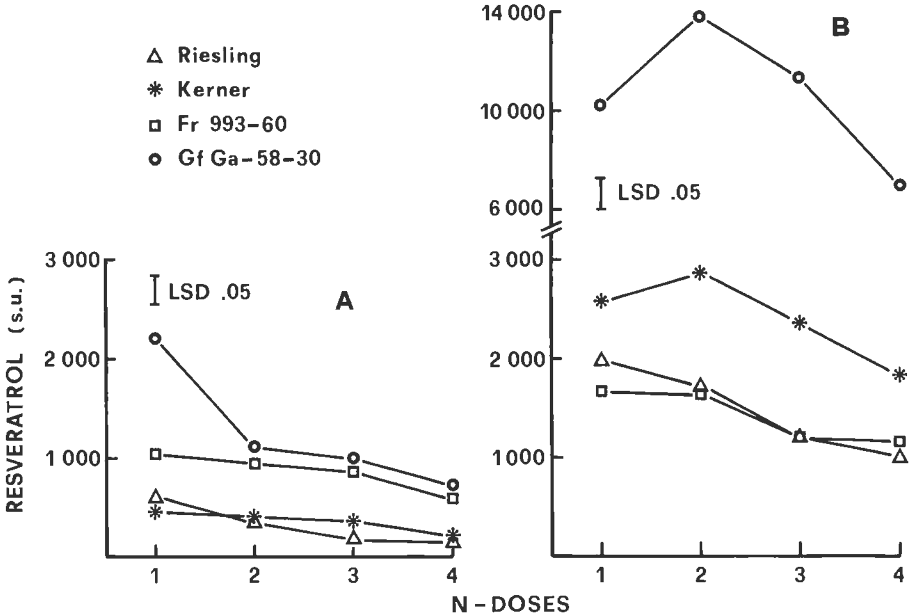

Fig. 3: The effect of nitrogen supply on leave resveratrol synthesis of different grape varieties. — A = 1st sampling date, B = 3rd sampling date.

Effetto delle diverse dosi di azoto sulla sintesi di resveratrolo nei quattro genotipi. — A = 1° epoca di campionamento, B = 3° epoca di campionamento.

Gf Ga-58-30 and 1430 s. u. for Fr 993-60 (Table 2). Gf Ga-58-30 synthetizes the highest ε-viniferin amount (780 s. u.), Fr 993-60 and Riesling the lowest ones (230 s. u.).

At the 1st sampling time each genotype has the same resveratrol progress depending on nitrogen supply, while at the 3rd one a different behaviour is observed, when changing from 1-N dose to 2-N dose (Fig. 3).

The phytoalexin ε-viniferin (synthetized only at the 3rd sampling time) has a significant response to the rates of nitrogen supply just in Gf Ga-48-30. (Fig. 4).

At the 3rd sampling date a higher resveratrol synthesis is observed than at the 1st one.

Discussion

The causes of grape resistance to powdery mildew are — up to now — unknown, even if some authors tried to give possible explanations (BOUBALS 1961; PRATT *et al.* 1984; DOSTER and SCHNATHORST 1985; HEINTZ 1985). According to DANAILOV *et al.* (1970), the vine susceptibility to powdery mildew increases by increasing the nitrogen supply. This statement can be proved in the present study, save for the highly resistant variety (Gf Ga-58-30) and the highly susceptible one (Kerner).

The severity of disease is related — by negative correlations — with the N content of the leaves, above all in Riesling (Table 3).

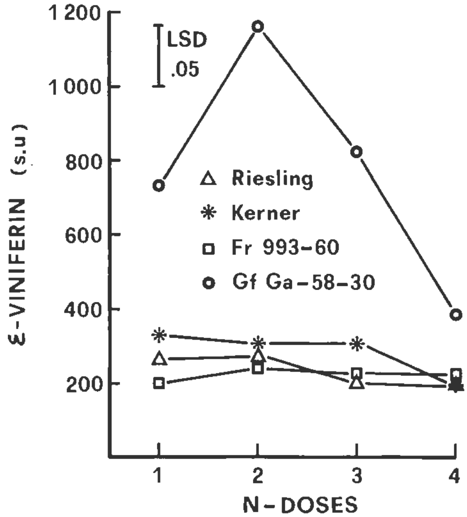

Fig. 4: The effect of nitrogen supply on ε-viniferin synthesis of different grape varieties.

Effetto delle diverse dosi di azoto sulla sintesi di ε-viniferina nei quattro genotipi (3° epoca di campionamento).

The causes of grape resistance to downy mildew are also unknown. Authors agree about the hypersensitivity response of the resistant plant (HUSFELD 1931; BOUBALS 1959; LANGCAKE and LOVELL 1980; ABADZHYAN 1983).

An influence of N supply upon the severity of downy mildew has not been reported in literature. In this research, a clear effect of nitrogen in decreasing the degree of resistance can be observed, in each genotype, even if Gf Ga-58-30 and Fr 993-60 preserve their high resistance characteristic, while Riesling and Kerner their medium one. As well as in *Oidium*, negative and significant correlations between the N content of the leaves and the severity of infection occur (Table 3).

Phytoalexin (resveratrol and viniferins) synthesis has been implicated in the limitation of lesions produced by *Botrytis cinerea* in Vitaceae, including *V. vinifera* (LANGCAKE and MCCARTHY 1979; BLAICH and BACHMANN 1980; POOL *et al.* 1981; BLAICH *et al.* 1982; STEIN and HOOS 1984; STEIN and BLAICH 1985).

Table 3

Correlation coefficients between the N content of the leaf and the degree of resistance against powdery and downy mildew and the resveratrol synthesis

Coefficienti di correlazione tra il contenuto di azoto nelle foglie ed i livelli di resistenza all'oidio, alla peronospora e la sintesi di resveratrolo

Varieties	*Oidium* resistance	*Plasmopara* resistance	Resveratrol 1st sampling date	Resveratrol 3rd sampling date
Riesling	−0.89**	−0.75**	−0.88**	−0.71**
Kerner	−0.11	−0.67**	−0.22	−0.63**
Fr 993-60	−0.36	−0.71**	−0.19	−0.58*
Gf Ga-58-30	−0.55**	−0.73**	−0.80**	−0.23

* Significant for P = 0.05.
** Significant for P = 0.01.

Besides phytoalexins, some morphological and histological factors play an appreciable role in defence against *Botrytis cinerea,* as perforations in the cuticle of berries (BLAICH *et al.* 1984) and cuticle thickness (BERNARD 1976; KARADIMCHEVA 1981).

Some authors observed a negative effect of nitrogen excess on grape resistance to the fungus, by evaluating the degree of attack against the bunch (RIBÉREAU-GAYON 1960; CHABOUSSOU 1970; DONCHEV *et al.* 1975; MAROCKE *et al.* 1977; DELAS *et al.* 1982). Nevertheless, evidence has not yet been obtained that nitrogen supply influences phytoalexin synthesis in grapes, save a note of STEIN (1984).

The results of this research mean that the plant needs an optimum of N supply to synthetize the highest amount of resveratrol (1-N dose in the 1st sampling date, 2-N dose at the 3rd sampling date); at higher N doses, the synthesis of resveratrol as well as of ε-viniferin decreases.

According to GRAHAM (1983), under nitrogen limiting conditions the balance between primary and secondary metabolic pathways is probably shifted onto the shikimate pathway, providing for a large pool of phenolics and alkaloids. These are the basis for many of the defence mechanisms of plant: physical, chemical, phytoalexins.

A rise of resveratrol synthesis, changing from the 1st sampling time to the last one (observed by STEIN and BLAICH 1985, too), could be explained by the seasonal allocation of carbon to protection. In the last part of the growing cycle, there are no great demands on carbon for structure or metabolism and the production of phenols occurs much more (McLAUGHLIN and SHRINER 1980).

Conclusions

1. N fertilization influences the resistance to powdery mildew; the degree of resistance decreases when changing from the lowest N-dose to the highest one.
   There are big differences among the genotypes; hybrids are much more resistant than *V. vinifera* varieties.
2. N fertilization influences the resistance to downy mildew; the severity of disease increases by increasing the nitrogen supply. — Hybrids are more resistant than *V. vinifera* varieties.
3. N fertilization influences the stilbene (resveratrol and ε-viniferin) synthesis; high rates of N fertilizer decrease the stilbene synthesis. Gf Ga-58-30 synthetizes the highest amounts of resveratrol and ε-viniferin, while Riesling the lowest ones.

Summary

1-year-old grapevine cuttings (cvs. Gf Ga-58-30, Fr 993-60, Riesling, Kerner) were grown in a greenhouse in pots at four levels of nitrogen supply, in order to test their resistance to powdery mildew, downy mildew, and their possible stilbene synthesis.

Our most significant findings are that:

a) increased rates of nitrogen supply decrease the degree of resistance against powdery and downy mildew and phytoalexin (stilbene) synthesis;
b) the hybrids (Gf Ga-58-30 and Fr 993-60) show a higher powdery and downy mildew resistance and a higher stilbene synthesis than the *V. vinifera* varieties (Riesling and Kerner);
c) the amount of stilbene (resveratrol and ε-viniferin) increases during the growing cycle.

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