Stress-induced development of inflorescence necrosis and bunch-stem necrosis in *Vitis vinifera* L. in response to environmental and nutritional effects

by

M. KELLER and W. KOBLET

Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture, Wädenswil, Switzerland

S u m m a r y : Inflorescences and mature clusters, respectively, of field-grown Müller-Thurgau grapevines (*Vitis vinifera* L.) were immersed in aqueous solutions of the glutamine synthetase (GS) inhibitor phosphinothricin (PPT). Typical symptoms of inflorescence necrosis developed after immersion in 1 or 10 mM PPT, but not in the 0.1 mM treatment. No necrotic symptoms could be induced in mature clusters. In addition, single-node cuttings of field-grown Müller-Thurgau and Pinot noir vines with one cluster, with or without adjacent leaf, were incubated in various solutions with or without PPT at several phenological stages. Necrotic symptoms occurred in all treatments including the H_2O control. However, at early stages of development the symptoms appeared earlier than at later stages, and elevated PPT concentrations reduced the development of necrotic manifestations. The addition of NH_4NO_3 to the solution enhanced the appearance of symptoms both in the presence and absence of PPT, while KNO_3 did not. In incubation solutions without PPT, metallic cations like Mg^{2+}, Ca^{2+} and K^+ reduced the incidence of bunch-stem necrosis. When cuttings were exposed to different environmental conditions, there was no significant light effect, but wind decreased the incidence of both inflorescence necrosis and bunch-stem necrosis. A leaf, attached to the cluster, or the addition of sucrose to the solution effectively delayed the development of necrotic manifestations. These results indicate that GS is present in rachis, flower and berry tissues and that the buildup of toxic NH_4^+ levels is involved in the development of both inflorescence necrosis and bunch-stem necrosis. However, NO_3^- reduction and primary N assimilation appear not to contribute significantly to NH_4^+ accumulation. This buildup may rather be a secondary effect related to senescence of the tissue due to carbon depletion.

K e y   w o r d s : grapevine, phosphinothricin, nitrogen, ammonium, senescence, inflorescence necrosis, bunch-stem necrosis.

Introduction

Inflorescence necrosis (IN) and bunch-stem necrosis (BSN) are two physiological disorders in grapevines, developing similar necrotic symptoms on grape pedicels, rachides and peduncles, that are thought to be induced by NH_3 toxicity (CHRISTENSEN and BOGGERO 1985, SILVA *et al.* 1986, JACKSON and COOMBE 1988). CHRISTENSEN and BOGGERO (1985) reported a close relationship between N nutrition and BSN, and they proposed a threshold of 1.5% total N and 3000 ppm NH_4^+ in the rachis tissue for the occurrence of BSN. JORDAN (1989) found increased NH_3 concentrations in rachis and berries of shaded vines and a positive correlation between NH_3 content and the severity of IN. In addition, both IN and BSN were related to the level of shading in studies of PÉREZ HARVEY and GAETE (1986) and JACKSON (1991).

Ammonia assimilation and NH_3^- releasing reactions have been reviewed by JOY (1988). The glutamine synthetase/glutamate synthase (GS/GOGAT) pathway is considered the primary route for NH_3 assimilation in higher plants. Phosphinothricin (glufosinate, PPT), a phosphinic acid analogue of glutamate, is a widely distributed herbicide in grape production. The mode of action of PPT was extensively studied by WILD and MANDERSCHEID (1984), SAUER *et al.* (1987) and WILD *et al.* (1987). It is now generally recognized that NH_3 accumulates in tissues treated with PPT due to its selective inhibition of GS, leading to a constriction in photosynthetic activity and senescence of the tissue. GU *et al.* (1991, 1994) reported that other GS/GOGAT inhibitors increased NH_4^+ concentrations in grapevine leaves, flowers, fruit and pedicels but not in petioles and rachis.

Apart from NH_4^+, metal cations, like K^+, Ca^{2+} and Mg^{2+} have also been associated with both IN and BSN (ALLEWELDT and HIFNY 1972, FEUCHT *et al.* 1975, STELLWAAG-KITTLER 1975, SCHALLER 1983, JACKSON and COOMBE 1988). However, the relations between cause and effect have been doubted (REDL 1983, CHRISTENSEN and BOGGERO 1985). Nevertheless, foliar treatments with solutions containing Mg and/or Ca are effective in the control of BSN (PERRET and KOBLET 1972, RIZZOTTO 1977, JÜRGENS and BECKER 1987).

The objective of the present investigation was to verify the presence of GS in grape cluster tissues at various phenological stages and to determine the implication of GS inhibition in the occurrence of IN and BSN. In addition, we tested environmental and nutritional effects on the development of IN and BSN, and we also intended to give further evidence for the hypothesis recently proposed by KELLER and KOBLET (1994), that carbon starvation, rather than excessive N nutrition, is triggering these so-called physiological disorders.

Correspondence to: M. KELLER, Food Research Laboratory, Cornell University, Geneva, NY 14456-0462, USA.

Materials and methods

Immersion experiment: The experiment was conducted in a vineyard at the Swiss Federal Research Station in Wädenswil, Switzerland, with 3 years old Müller-Thurgau grapevines (*Vitis vinifera* L.), grafted on SO 4 rootstocks. The vines were cane pruned and trained on a vertical shoot trellis (Guyot). Phosphinothricin (PPT) was applied to 10 randomly selected clusters and 10 leaves adjacent to different clusters by dipping them into aqueous solutions containing 0.1, 1 or 10 mM PPT for 5 s. Clusters were treated at anthesis and maturity in 1991. At maturity the berries were removed from the pedicels of 10 additional clusters before immersion.

Incubation experiments: PPT solution experiments were conducted in the greenhouse in 1991, using clusters from the same vines as in the immersion experiment. Clusters were selected for uniformity of growth and phenological stage at anthesis, berry set, veraison (onset of ripening) and maturity. After excision from the vine, the peduncles were put through rubber caps into 25-ml glass vials containing the PPT solutions made with distilled H_2O. The solutions used at anthesis were: 0, 0.1, 0.5, 1, 5 and 10 mM PPT. Two further treatments were added at the later stages of development: 10 mM PPT plus either 1 mM NH_4NO_3 or 1 mM KNO_3. Eight replications were used. Light intensities were kept at 1-2 % of full sunlight, which is around the light compensation point of photosynthesis in grapevine leaves (Keller and Koblet 1994).

Three experiments were conducted in 1992 with Müller-Thurgau or Pinot noir (clone M1/17, planted in 1983 on 3309 rootstocks) vines from the same vineyard as used in the immersion experiment. Shoots were selected for uniformity of growth and excised at the base. Single-node cuttings with a cluster and adjacent leaf were cut from the shoots. The cuttings were placed in 100-ml glass vials covered with a rubber cap and containing various solutions made with distilled H_2O.

The first experiment was performed with Müller-Thurgau clusters at anthesis and immediately after veraison. The incubation solutions were 0 and 10 mM NH_4NO_3, combined with 3 additional treatment factors: clusters with or without adjacent leaf, full sunlight or deep shade (1-2 % of full sunlight), with or without wind (separate greenhouse cabinets with two opposing windows either open or closed). The leaf was left attached as a carbon source, and the light treatments were imposed to alter gas exchange activities. The wind treatment was used to stimulate a potential NH_3 evolution through the stomata.

The second experiment was conducted with Pinot noir clusters with one leaf at anthesis. The solutions were: 0 or 10 mM NH_4NO_3 combined with 0 or 2 % sucrose as the major transport compound in the phloem.

The third experiment was effected with Pinot noir clusters with two leaves immediately after veraison. The solutions were: 5 or 10 mM NH_4NO_3, 10 mM KNO_3, 10 mM HNO_3, 5 mM $Ca(NO_3)_2$ or 5 mM $Mg(NO_3)_2$. Both, the second and the third experiment were conducted at 1-2 % of full sunlight without wind. Four replications were used

per treatment combination in each experiment. Incubation was continued until symptoms of IN or BSN were visible.

Since NH_4^+ accumulation following PPT applications and the implication of NH_3/NH_4^+ in the occurrence of IN and BSN were confirmed by several researchers (see "Introduction"), tissue-NH_4^+ concentrations were not measured in the present investigation. Symptoms of IN were registered as % necrotic flowers per inflorescence and those of BSN as % necrotic rachis tissue per cluster in all experiments. The data were subjected to Bartlett's test to check homogeneity of variance subsequent to square-root transformation. Analysis of variance, F-test and Duncan's multiple range test were used to examine differences between means.

Results

Immersion experiment: In the field, grapevine leaves treated with 1 and 10 mM PPT became necrotic within 4-6 d after immersion and senesced very rapidly. While there were no differences in the severity of necrotic symptoms between these two PPT concentrations, the 0.1 mM PPT treatment only caused irregular necrotic spots on the leaves, implying that this PPT concentration was insufficient to entirely inhibit GS in the leaf. Inflorescences adjacent to treated leaves showed no necrotic symptoms, suggesting that PPT was not translocated from the leaves. Typical symptoms of IN only developed on the flower clusters that had been immersed and first appeared in the 10 mM PPT treatment, 5-6 d after immersion (Fig. 1: A, B). Necrotic symptoms first occurred on flowers and pedicels and only later on rachis and peduncles. After 30 d, the clusters of both the 1 and 10 mM treatments were completely necrotic (Fig. 1: C, D), but no effect could be observed in the 0.1 mM treatment. Some basipetal translocation of PPT or NH_4^+ must have occurred in the 10 mM treatment, since the shoot internode below the immersed cluster became partly necrotic, too (Fig. 1: D). No symptoms were visible, however, 2 weeks after immersion of mature clusters in PPT solutions, regardless of the presence or absence of berries.
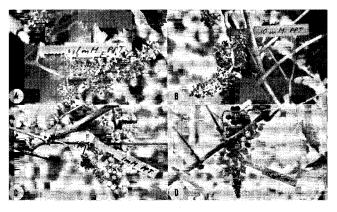

Fig. 1: Occurrence of inflorescence necrosis in response to immersion of Müller-Thurgau grape clusters in phosphinothricin (PPT) solutions: 6 days after immersion in 1 mM PPT (A) or 10 mM PPT (B), and 30 days after immersion in 1 mM PPT (C) or 10 mM PPT (D), respectively.

Incubation experiments: When the peduncles of excised bunches were placed in various PPT solutions, necrotic symptoms and sequential senescence occurred in all treatments (even in the H_2O control) at all developmental stages (Fig. 2). However, in the 5 and 10 mM PPT treatments (Fig. 2: A, C), particularly in combination with NH_4NO_3 but not KNO_3 addition (Fig. 2: B, D, F), the symptoms appeared much earlier and were more intense than at lower PPT concentrations and the H_2O control. The first symptoms were visible after 1 d at anthesis and berry set, after 9 d at veraison and after 5 d at maturity. In contrast to the immersion experiment, symptoms first occurred on peduncles and rachis and only later on flowers or berries (Fig. 2: A, B). At veraison and maturity only peduncles and rachis developed necrotic symptoms, whereas the berries remained green, although a loss of turgor with concomitant dehydration could be observed in all treatments, in particular at veraison (Fig. 2: C - F).

Symptoms of IN or BSN also occurred in all incubation solution treatments conducted with single-node cuttings. Inflorescence necrosis of Müller-Thurgau cuttings was most severe at the combinations +N/-light/±wind/-leaf and least severe at the combination -N/+light/+wind/+leaf (Table). There were, however, significant interactions among treatment factors. When the adjacent leaf had been excised before imposition of the additional treatments, IN severity was always very high. Only if the leaf was left attached, the effects of the other factors became apparent: incubation in water led to low levels of IN, regardless of the presence or absence of wind. If NH_4NO_3 was added to the solution, IN was significantly increased, but wind reduced the N effect (Fig. 3). Bunch-stem necrosis on clus-

Table

Effect of the presence (+) or absence (-) of adjacent leaf, light, wind and NH_4NO_3 (10 mM) on inflorescence necrosis and bunch-stem necrosis of single-node Müller-Thurgau grape cuttings. Values are means ± SE. Within a column section, means followed by the same letter are not significantly different at P < 5 %

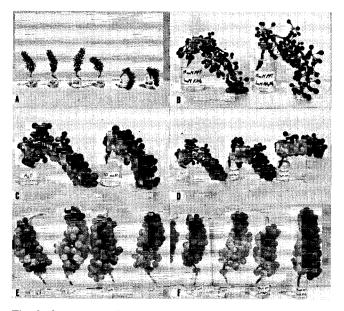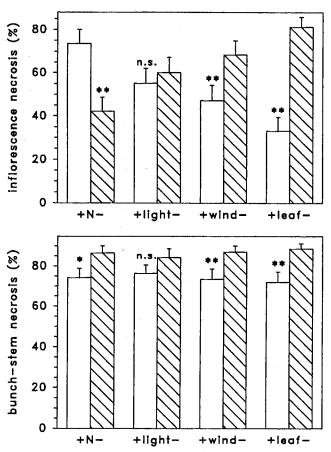| leaf	light	wind	NH_4NO_3	inflorescence necrosis (%)	bunch-stem necrosis (%)
+	+	-	-	20.0±4.6 cd	76.3+16.0 abc
+	+	-	+	98.3±1.7 a	70.0±11.7 abc
+	+	+	-	5.0±0.3 d	62.5±9.7 bc
+	+	+	+	29.5±14.9 cd	48.8±15.3 cd
+	-	-	-	13.3±6.0 d	97.5±1.4 a
+	-	-	+	78.3±14.8 ab	90.0±5.4 ab
+	-	+	-	13.8±3.8 d	97.5±2.5 a
+	-	+	+	30.0±13.7 cd	32.5±11.3 d
-	+	-	-	70.0±10.8 ab	86.3±5.5 ab
-	+	-	+	73.8±16.0 ab	91.3±7.2 a
-	+	+	-	80.0±2.9 a	95.0±3.5 a
-	+	+	+	90.0±10.0 a	80.0±8.4 ab
-	-	-	-	90.0±3.5 a	92.5±6.0 a
-	-	-	+	100.0±0.0 a	92.5±3.2 a
-	-	+	-	60.0±14.1 abc	83.8±16.3 ab
-	-	+	+	100.0±0.0 a	87.5±4.3 ab

Fig. 2: Occurrence of necrotic symptoms in response in incubation of Müller-Thurgau grape clusters in phosphinothricin (PPT) solutions: 1 day in H_2O or 0.1, 0.5, 1, 5, 10 mM PPT at anthesis (A). 1 day in 10 mM PPT plus 1 mM KNO_3 or NH_4NO_3 at berry set (B). 9 days in H_2O or 10 mM PPT at veraison (C). 9 days in H_2O or 10 mM PPT plus 1 mM NH_4NO_3 or KNO_3 at veraison (D). 6 days in H_2O or 0.1, 0.5, 1 mM PPT (E), and in 5 or 10 mM PPT or 10 mM PPT plus 1 mM NH_4NO_3 or KNO_3, respectively, at maturity (F).

Figs. 3 and 4: Effect of N (10 mM NH_4NO_3), light, wind and adjacent leaf on inflorescence necrosis (3, above) resp. bunch-stem necrosis (4, below) of single-node Müller-Thurgau grape cuttings. Left bar of each pair: treatment factor present; right bar: treatment factor absent. Values are means + SE (n = 32). n.s., *, ** non-significant and significant at P < 5 % and at P < 1 %, respectively.

ters from Müller-Thurgau cuttings taken after veraison developed more rapidly than IN. On an average of all treatments, 81% of the rachis tissues were necrotic after 7 d of incubation, as compared to 58 % of the inflorescences after 10 d (Table). However, these clusters had been collected in the vineyard during a period of cool and rainy weather, and only 8 d later a severe incidence of BSN occurred in the field. Therefore, BSN may already have been induced when the cuttings were taken. Nevertheless, as with IN, the percentage of BSN was always very high if the adjacent leaf was excised. When the leaf was left attached, the severity of BSN was increased in the shade, except for the treatment combination +N/-light/+wind/+leaf, where the leaves remained green for a prolonged period. This combination also fully accounted for the statistical significance of the N effect on BSN (Fig. 4).
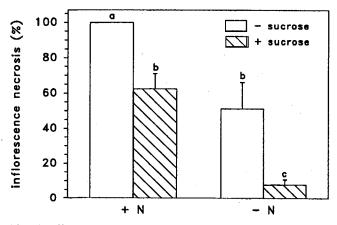

Fig. 5: Effect of the presence (+) or absence (-) of N (10 mM NH_4NO_3) and sucrose (2%) in the incubation solution on inflorescence necrosis of single-node Pinot noir grape cuttings. Values are means + SE (n = 4). Treatments with the same letter are not significantly different at P < 5 %.
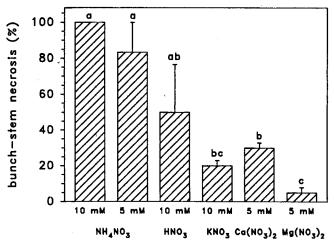

Fig. 6: Effect of N form and concentration and different cations in the incubation solution on bunch-stem necrosis of single-node Pinot noir grape cuttings. Values are means + SE (n = 4). Treatments with the same letter are not significantly different at P < 5 %.

As shown in Fig. 5 for Pinot noir cuttings at anthesis, the adjacent leaf could be replaced by the supply of 2 % sucrose as carbon source to the incubation solution. After 14 d of incubation, sucrose added to the NH_4NO_3 solution

reduced IN to the level of the H_2O control and almost prevented the development of IN in the absence of NH_4NO_3. In this latter treatment, berry development could be sustained until the pea-size stage without further sucrose supply. On Pinot noir cuttings taken after veraison, all leaves senesced and were abscised within one week, while BSN only developed after leaf abortion. The N concentration in the incubation solution did not significantly alter the severity of BSN, while the substitution of NH_4^+ for different cations reduced BSN, although the difference between NH_4^+ and H^+ was not significant. Metal cations however, in particular Mg^{2+}, effectively prevented the development of BSN after 17 d of incubation (Fig. 6).

Discussion

Our results suggest that GS is present and NH_4^+ assimilation may occur in all organs of the grape cluster at any stage of development between anthesis and maturity. Both IN and BSN could be induced by using PPT as GS inhibitor. Thus, the implication of NH_4^+ accumulation in the development of these disorders was confirmed indirectly. When attached grape inflorescences were immersed in PPT solutions, necrotic symptoms first developed on flowers and pedicels, while they first occurred on peduncles and rachis during incubation of detached clusters in PPT solutions. This is not surprising, since PPT is relatively immobile in plants, and it must have been taken up through the stomata and cuticula in the immersion experiment or through the cut end of the peduncle in the incubation experiments.

Necrotic symptoms developed earlier when NH_4NO_3 was added to PPT solutions as compared to KNO_3 plus PPT or PPT alone. Thus, the N effect was due to NH_4^+ supply, and the NO_3^- reduction appears not to contribute significantly to NH_4^+ accumulation, regardless of the light conditions, even if GS is inhibited. This is consistent with our earlier results (KELLER and KOBLET 1994) and with data obtained by WILD et al. (1987), GU et al. (1991) and LÖHNERTZ (1991). Even the glycolate pathway, which was proposed by WILD et al. (1987) as the major source for NH_4^+ after PPT application, may have provided substantial amounts of NH_4^+ in inflorescence tissues only in the immersion experiment, but not in the incubation experiments conducted at light intensities close to the compensation point of photosynthesis, since photorespiration is reduced in proportion to net photosynthesis at low irradiance (SHARKEY 1988). Therefore, the question remains, where the NH_4^+ that is accumulated is derived from.

The leaf area to fruit ratio is a widely used indicator of the source-sink balance, and separation of isolated sink organs from the parent source plant causes a stress itself due to carbon starvation, since interactions with other plant parts are no longer possible. SRIVASTAVA and SINGH (1987) suggested that glutamate dehydrogenase (GDH) may be responsible for NH_4^+ assimilation under conditions of stress or high NH_4^+ levels, and both GS and GDH were reported to be active in grape berries by GHISI et al. (1984). In our

incubation experiments, the inhibition of GS invariably resulted in senescence of the tissue, while in the absence of PPT, i.e. when GS was active, the addition of sucrose to NH_4NO_3 solutions delayed the occurrence of IN, although sucrose has been shown to inhibit GDH (Srivastava and Singh 1987) and to increase NO_3^- reduction (Aslam and Huffaker 1984) under experimental conditions similar to those used in our study.

Thus, GDH does not appear to contribute to NH_4^+ assimilation or detoxification, as described by Walls-grove et al. (1983) and Gu et al. (1994). On the contrary, Robinson et al. (1992) reported that GS activity decreased and GDH activity increased during periods of carbon starvation, due to protein degradation to supply carbon skeletons to the tricarboxylic acid (TCA) cycle. Protein breakdown is related to NH_3 release and senescence (Joy 1988), and Rabe (1990) assumed that any stress causing glucose depletion and/or reduced growth results in NH_3 accumulation.Therefore, we suppose that both IN and BSN are the visible manifestations of senescence processes induced by stress situations leading to carbon starvation. The accumulation of NH_3 may, hence, be a secondary effect of senescence. This hypothesis is supported by the fact that sucrose was equally effective in preventing the development of IN as was the presence of a leaf. Under non-stress conditions, as it was the case in the experiment using attached clusters in the field, there seems to be only insignificant NH_3/NH_4^+ production, since ripe grapes failed to develop necrotic symptoms after immersion in PPT solutions. On the other hand, the large variance within treatments in the incubation experiments may partly be due to different numbers of flowers or berries per unit leaf area. In addition, the flowers of the same inflorescence do not reach the same stage of development simultaneously (Swanepoel and Archer 1988), and thus, some flowers may have gained competitive advantage over the other flowers.

Incubation of excised clusters in PPT solutions took 1 d at anthesis, 9 d at veraison and 5 d at maturity for development of necrotic symptoms. Between berry set and veraison grape clusters accumulate large amounts of malate that is used for gluconeogenesis after veraison (reviewed by Ruffner 1982). Malate, a TCA cycle intermediate, is a mobile storage form of carbon and reducing equivalents (Naik and Nicholas 1986), and thus, to a limited extent grape berries can make use of their own reserves when carbon supply is restricted. An increase in glycolysis was also found in abscised apple fruit, as well as in attached fruit after girdling or excision of adjacent leaves by Blanke et al. (1987). Therefore, senescence processes in flower and fruit tissues may be initiated only after depletion of accessible carbon reserves, which are dependent on the stage of development. This interpretation may also explain earlier results of Koblet and Theiler (1970) and Wienhaus (1975), stating that BSN symptoms were induced by inhibition of enzymes involved in glycolysis and TCA cycle.

Jordan (1989), Jordan et al. (1991) and Gu et al. (1991) also induced IN symptoms by incubating detached grape tendrils or clusters in a solution containing NH_4^+, whereas Holzapfel and Coombe (1991) reported that the responses

to various solutions of inorganic salts were inconsistent. Yet, all these authors used unnaturally high ion concentrations (100 to 330 mM) that may represent osmotic stress leading to dehydration and loss of turgor. Water stress increased IN severity in a study of Jackson (1991), and Stellwaag-Kittler (1975) found BSN symptoms to be identical with those caused by desiccation of detached clusters. In addition, grapevine GS is inhibited at NH_4^+ concentrations above 25 mM (Roubelakis-Angelakis and Kliewer 1983). In contrast, the concentrations used in our study were within physiological ranges.

Metal cations, in particular Mg^{2+}, in incubation solutions without PPT delayed the development of BSN in Pinot noir cuttings. Chang and Kliewer (1991) also observed symptoms of BSN in heavily NO_3^--fed vines only if they were Ca^{2+} deficient, and in vines fed with NH_4^+ as the sole N source. A possible explanation for these results is that GS, which is responsible for reassimilation of released NH_4^+ into glutamine under natural conditions (Kamachi et al. 1991), is activated by relatively high concentrations of divalent cations, whereof Mg^{2+} is almost five times more efficient than Ca^{2+} (Roubelakis-Angelakis and Kliewer 1983). In addition, Mg^{2+} and Ca^{2+} are involved in the control of nitrate reductase by photosynthetic capacity to prevent toxic accumulations of NO_2^- and NH_4^+ in the dark (Riens and Heldt 1992, De Cires et al. 1993), and Brown et al. (1991) reported Ca^{2+} to delay senescence due to its ability to maintain membrane integrity. Besides its role in the regulation of stomatal opening K^+ is an activator of the glycolytic enzyme pyruvate kinase, which is stimulated by NH_4^+ assimilation (Paul et al. 1978, Steer 1979).

In addition to refixation, NH_3 may be emitted from the sub-stomatal cavity to the atmosphere (Farquhar et al. 1980, Schjoerring et al. 1991). The beneficial effect of the wind treatment in the incubation experiments may have been brought about by stimulation of NH_3 emmission due to reduced boundary layer resistance and increased NH_3 vapor pressure gradient. This suggests that the severity of BSN may be reduced by removal of old leaves in the cluster zone to facilitate air movement around grape bunches, which is a common canopy management practice in temperate climate zones.

Acknowledgements

Literature cited

Alleweldt, G.; Hifny, H. A. A.; 1972: Zur Stiellähme der Reben. II. Kausalanalytische Untersuchungen. Vitis 11, 10-28.

Aslam, M.; Huffacker, R. C.; 1984: Dependency of nitrate reduction on soluble carbohydrates in primary leaves of barley under aerobic conditions. Plant Physiol. 75, 623-628.

BLANKE, M. M.; HUCKLESBY, D. P.; NOTTON, B. A.; 1987: Distribution and physiological significance of photosynthetic phosphoenolpyruvate carboxylase in developing apple fruit. J. Plant Physiol. 129, 314-321.

BROWN, J. H.; PALIYATH, G.; THOMPSON, J. E.; 1991: Physiological mechanisms of plant senescence. In: STEWARD, F. C.; BIDWELL, R. G. S. (Eds.): Plant Physiology, a Treatise.Vol. X: Growth and Development, 227-275. Academic Press, San Diego.

CHANG, S. S.; KLIEWER, W. M.; 1991: Effect of nitrogen forms and rates, shading, and presence and absence of Ca++ on the growth, tissue nitrogen compositions, and fruit quality of grapevines. In: RANTZ, J. M. (Ed.): Proc. Intern. Symp. Nitrogen Grapes Wine, Seattle Washington, USA, June 1991, 228-238. Cal Central Press, Sacramento.

CHRISTENSEN, P.; BOGGERO, J.; 1985: A study of mineral nutrition relationships of waterberry in Thompson Seedless. Amer. J. Enol. Viticult. 36, 57-64.

DE CIRES, A.; DE LA TORRE, A.; DELGADO, B.; LARA, C.; 1993: Role of light and CO₂ fixation in the control of nitrate-reductase acitvity in barley leaves. Planta 190, 277-283.

FARQUHAR, G. D.; FIRTH, P. M.; WETSELAAR, R.; WEIR, B.; 1980: On the gaseous exchange of ammonia between leaves and the environment: Determination of the ammonia compensation point. Plant Physiol. 66, 710-714.

FEUCHT, W.; FORCHE, E.; PORSTENDÖRFER, J.; 1975: Ermittlung der Kalium- und Calciumverteilung in Traubenachsen von Vitis vinifera mit Hilfe der Röntgenstrahlenmikroanalyse am Rasterelektronenmikroskop. Vitis 14, 190-197.

GHISI, R.; IANNINI, B.; PASSERA, C.; 1984: Changes in the activities of enzymes involved in nitrogen and sulphur assimilation during leaf and berry development of Vitis vinifera. Vitis 23, 257-267.

GU, S.; LOMBARD, P. B.; PRICE, S. F.; 1991: Effect of glutamine synthetase/ glutamate synthase (GS/GOGAT) inhibitors on ammonium accumulation in Pinot noir leaf and cluster tissues. In: RANTZ, J. M. (Ed.): Proc. Intern. Symp. Nitrogen Grapes Wine, Seattle Washington, USA, June 1991, 262-265. Cal Central Press, Sacramento.

- -; - -; - -; 1994: Inflorescence necrosis induced from ammonium incubation and deterred by α-keto-glutarate and ammonium assimilation in Pinot noir grapevines. Amer. J. Enol. Viticult. 45, 155-160.

HOLZAPFEL, B.; COOMBE, B. G.; 1991: Toxicity effects of perfused inorganic salt solutions on excised grape clusters and tendrils. In: Rantz, J. M. (Ed.): Proc. Intern. Symp. Nitrogen Grapes Wine, Seattle Washington, USA, June 1991, 102-107. Cal Central Press, Sacramento.

JACKSON, D. I.; 1991: Environmental and hormonal effects on development of early bunch stem necrosis. Amer. J. Enol. Viticult. 42, 290-294.

- -; COOMBE, B. G.; 1988: Early bunchstem necrosis in grapes - a cause of poor fruit set. Vitis 27, 57-61.

JORDAN, D. T.; 1989: Ammonium in grapevines: Seasonal levels in tissue and xylem extracts, and a tendril model system. Ph. D. Thesis Oregon State Univ., USA.

- -; BREEN, P.; PRICE, S. F.; LOMBARD, P. B.; 1991: Inflorescence necrosis: Is ammonium the culprit? In: RANTZ, J. M. (Ed.): Proc. Intern. Symp. Nitrogen Grapes Wine, Seattle Washington, USA, June 1991, 102-107. Cal Central Press, Sacramento.

JOY, K. W.; 1988: Ammonia, glutamine, and asparagine: a carbon-nitrogen interface. Can. J. Bot. 66, 2103-2109.

JÜRGENS, G.; BECKER, R.; 1987: Ergebnisse bei der Bekämpfung der Stiellähme. Mitt. Klosterneuburg 37, 135-139.

KAMACHI, K.; YAMAYA, T.; MAE, T.; OJIMA, K.; 1991: A role for glutamine synthetase in the remobilization of leaf nitrogen during natural senescence in rice leaves. Plant Physiol. 96, 411-417.

KELLER, M.; KOBLET, W.; 1994: Is carbon starvation rather than excessive nitrogen supply the cause of inflorescence necrosis in Vitis vinifera L.? Vitis 33, 81-86.

KOBLET, W.; THEILER, R.; 1970: Vorläufige Resultate zur künstlichen Auslösung der Stiellähme. Wein-Wiss. 25, 429-446.

LÖHNERTZ, O.; 1991: Soil nitrogen and the uptake of nitrogen in grapevines. In: RANTZ, J. M. (Ed.): Proc. Intern. Symp. Nitrogen Grapes Wine, Seattle Washington, USA, June 1991, 1-11. Cal Central Press, Sacramento.

NAIK, M. S.; NICHOLAS, D. J. D.; 1986: Malate metabolism and its relation to nitrate assimilation in plants. Phytochem. 25, 571-576.

PAUL, J. S.; CORNWELL, K. L.; BASSHAM, J. A.; 1978: Effects of ammonia on carbon metabolism in photosynthesizing isolated mesophyll cells from Papaver somniferum L. Planta 142, 49-54.

PÉREZ-HARVEY, J.; GAETE, L.; 1986: Efecto del microclima luminoso sobre la calidad de la uva Sultanina en sistema de parronal español. II. Desgrane, palo negro y pudricion gris. Cienc. Invest. Agrar. 13, 113-120.

PERRET, P.; KOBLET, W.; 1972: Versuche zur Bekämpfung der Stiellähme 1971. Schweiz. Z. Obst- Weinbau 108, 10-15.

RABE, E.; 1990: Stress physiology: The functional significance of the accumulation of nitrogen-containing compounds. J. Hort. Sci. 65, 231-243.

REDL, H.; 1983: Verlauf der Makro- und Mikronährstoffgehalte in Traubengerüst, Beeren und Blättern während der Reifephase in Beziehung zum Auftreten der Stiellähme. Mitt. Klosterneuburg 33, 39-59.

RIENS, B.; HELDT, H. W.; 1992: Decrease of nitrate reductase activity in spinach leaves during a light-dark transition. Plant Physiol. 98, 573-577.

RIZZOTTO, N.; 1977: Untersuchungsergebnisse über die Bekämpfung der Stiellähme. Mitt. Klosterneuburg 27, 187-190.

ROBINSON, S. A.; STEWART, G. R.; PHILLIPS, R.; 1992: Regulation of glutamate dehydrogenase activity in relation to carbon limitation and protein catabolism in carrot cell suspension cultures. Plant Physiol. 98, 1190-1195.

ROUBELAKIS-ANGELAKIS, K. A.; KLIEWER, W. M.; 1983: Ammonia assimilation in Vitis vinifera L.: II. Leaf and root glutamine synthetase. Vitis 22, 299-305.

RUFFNER, H. P.; 1982: Metabolism of tartaric and malic acids in Vitis: A review - Part B. Vitis 21, 346-358.

SAUER, H.; WILD, A.; RÜHLE, W.; 1987: The effect of phosphinothricin (glufosinate) on photosynthesis. II. The cause of inhibition of photosynthesis. Z. Naturforsch. 42, 270-278.

SCHALLER, K.; 1983: Die Rolle von Mineralstoffen, insbesondere Calcium und Magnesium, beim Auftreten der Stiellähme der Rebe. Mitt. Klosterneuburg 33, 116-121.

SCHJOERRING, J. K.; KYLLINGSBÆK, A.; MORTENSEN, J. V.; 1991: Ammonia exchange and nitrogen dynamics of barley crops at different levels of N supply. In: ANGELETTI, G.; BEILKE, S.; SLANINA, J. (Eds.): Air pollution research report 39, 383-393. Joint Workshop COST 611/Working Party 3 and EUROTRAC. TNO, Delft, The Netherlands. October 28-30, 1991. Commission Europ. Communities.

SHARKEY, T. D.; 1988: Estimating the rate of photorespiration in leaves. Physiol. Plant. 73, 147-152.

SILVA, H.; GIL, G.; RODRIGUEZ, J.; 1986: Desecación del escobajo de la vid (palo negro): ¿Un exceso de nitrógeno amoniacal? Aconex 14, 9-10.

SRIVASTAVA, H. S.; SINGH, R. P.; 1987: Role and regulation of L-glutamate dehydrogenase activity in higher plants. Phytochemistry 26, 597-610.

STEER, B. T.; 1979: Integration of photosynthetic carbon metabolism and nitrogen metabolism on a daily basis. In: MARCELLE, R.; CLIJSTERS, H.; VAN POUCKE, M. (Eds.): Proc. Conference Photosynthesis and Plant Development, Diepenbeek, Belgium. July 1978, 185-192. Dr. W. Junk bv Publishers, The Hague.

STELLWAAG-KITTLER, F.; 1975: Untersuchungen zur Aufklärung des Auslösungsmechanismus der Stiellähme an Trauben. Mitt. Klosterneuburg 25, 3-18.

SWANEPOEL, J. J.; ARCHER, E.; 1988: The ontogeny and development of Vitis vinifera L. cv. Chenin blanc inflorescences in relation to phenological stages. Vitis 27, 133-141.

WALLSGROVE, R. M.; KEYS, A. J.; LEA, P. J.; MIFLIN, B. J.; 1983: Photosynthesis, photorespiration and nitrogen metabolism. Plant Cell. Environ. 6, 301-109.

WIENHAUS, H.; 1975: Die Auslösung der Stiellähme durch Hemmung der Zellatmung. Mitt. Klosterneuburg 25, 49-56.

WILD, A.; MANDERSCHEID, R.; 1984: The effect of phosphinothricin on the assimilation of ammonia in plants. Z. Naturforsch. 39, 500-504.

- -; SAUER, H.; RÜHLE, W.; 1987: The effect of phosphinothricin (glufosinate) on photosynthesis. I. Inhibition of photosynthesis and accumulation of ammonia. Z. Naturforsch. 42, 263-269.