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Changes in the Activity of Catalase (EC 1.11.1.6) in Relation to the Dormancy of Grapevine (*Vitis vinifera* L.) Buds[1, 2]

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ABSTRACT

Catalase activity in grapevine (*Vitis vinifera* L.) buds cv. 'Perlette.' increased to a maximum in October and thereafter decreased within 3 months to less than half its maximal rate. The decrease in catalase activity coincided with the decline in temperature during winter. The rate of sprouting of buds forced at 23°C was negatively related to the activity of catalase. Artificial chilling of grapevine canes at 5°C resulted in a 25% decrease of catalase activity in the buds after 3 days and 31% after 17 days. The activity of catalase increased to the control level only 96 hours after removing canes from 5°C to room temperature. Efficient bud-dormancy breaking agents, such as thiourea and cyanamide decreased catalase activity to 64 and 50% of the controls respectively, while the activity of peroxidase remained the same under those conditions. A less efficient dormancy breaking agent dinitro-*ortho*-cresol, did not decrease catalase activity.

The control of dormancy in perennial buds is not yet clear although many changes in growth regulators have been reported during its onset and release (9). It has been shown that several nitrogenous compounds, such as nitrate, nitrite, hydroxylamine, and thiourea, which can break dormancy in seeds, also decrease catalase activity (5). Low temperature was reported to decrease catalase activity in cucumber seedlings (12) and in peach flower buds, in which it also increased bud break. In peach flower buds, the activity of catalase was lowest at the end of the dormant period (7).

Calcium cyanamide has been widely described as an efficient dormancy breaking agent for grapevines (6, 8, 16). H_2CN_2 was found to be the active form (16). Dormant buds on grapevine cuttings treated with 0.125 to 0.250 M cyanamide sprouted 9 to 12 d after treatment when exposed to a sufficiently high temperature (23°C), while untreated buds sprouted 10 d later.

Thus, it was of interest to study the mechanism of cyanamide's action on dormant grapevine buds, and in particular we tried to determine the involvement of catalase in the development of and release from dormancy in grapevine buds, in the field and in an *in vitro* system.

MATERIALS AND METHODS

Canes of the grapevine cultivar 'Perlette' were collected from autumn until early spring. The canes were cut into single node cuttings for bud break studies or were left intact for bud analysis. In some cases, we used buds collected from intact canes in the vineyard. The canes or buds were taken from two climatically different regions: the coastal plain, with Mediterranean subtropical climate, and the southern Jordan Valley, a hot region characterized by a warm autumn and winters having many clear days with relatively high temperatures.

The intensity of dormancy was determined by placing into the light 5 replicates of 5 to 10 single node cuttings, with their bases immersed in water at 23°C, and recording the time-lag to bud opening.

For determining the effect of temperature on the activity of catalase, detached canes were placed for 17 d at 5°C, or were left in the laboratory at room temperature (23–28°C). On the 13th d, some of the canes were removed from 5°C to room temperature and the activity of catalase was measured in their buds 24, 48, and 96 h thereafter.

Enzyme Extraction. Ten buds weighing about 1 g were removed and immediately homogenized with an Ultraturax in 10 ml of 0.05 M phosphate buffer (pH 6.9) on ice. Another 10 ml of phosphate buffer was added and the homogenate was transferred to a mechanical pulverizer (Fritsche, Pulverisette) for 10 min at 4°C. The homogenate was centrifuged for 10 min at 1080*g*, and the supernatant was used as crude enzyme solution for assays. Two to four such replicates were used for each treatment.

Enzyme Assays. Catalase activity was determined by the floating disc method (4). Discs with a diameter of 6 mm were cut from a Whatman 3MM chromatographic paper. The discs were dipped for 1 s in the crude extract and put in a vial containing 5 ml of 30 mM H_2O_2 at 20°C. The time until the discs floated was determined with a stopwatch. Ten to 20 replicates of individual discs were used for each crude extract. Catalase activity results were verified using the Clark type O_2 monitor (YSI Co.) (14). Results obtained by the two methods were almost identical. The activity of catalase in the different plant extracts was calculated according to the activity of bovine-liver catalase (σ = 21,000 units/mg protein).

Peroxidase activity was measured in the same extracts using 4 mM of H_2O_2 and 5 mM of guaiacol (Merck) at pH 5.8 as substrates and expressed as changes in the O.D. at 470 nm (10). When the enzymic assays were more than duplicated the standard errors of the mean were given.

Protein was determined by the method of Bradford (3) using BSA (Sigma) as standard.

The dormancy-breaking agents used were 0.125 to 0.25 M pure analytical cyanamide (Sigma), 50 g/L technical cyanamide containing 50% active ingredient (SKW CO., Trostberg, W. Germany), 0.2 M thiourea (BDH Co., London, England), and 0.05 M dinitro-*ortho*-cresol (Taphazol, Israel).

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RESULTS AND DISCUSSION

Catalase activity in buds of cv 'Perlette' grapevines was followed from autumn to spring. The activity in buds from the Jordan Valley increased markedly in autumn, reaching a maximum at the end of October (Fig. 1) and then started to decrease, reaching the minimum in January. The gradient of catalase increase was steep in the winter of 1982-1983, which was exceptionally cold. The increase in catalase activity in autumn and its decrease with the approach of spring were negatively correlated to bud opening on cuttings from the same plant material. The winter of 1983-1984 was very mild and warm, and there were many fluctuations in both the courses of the decrease of catalase activity and bud opening.

At the coastal plain in 1983-1984 the peak of catalase activity in the buds also occurred at the end of October (Fig. 2), the decrease however, was more rapid than in the Jordan Valley. The catalase activity and the intensity of dormancy began to decrease parallel to the drop in temperatures. These results may explain the inhibiting and delaying effects of high temperatures during winter on bud break.

Artificial chilling (5°C) applied to detached canes also caused a significant decrease in the catalase activity in the buds, while the activity of catalase in buds held at room temperature (23–28°C) slightly and gradually increased. Canes removed from the cold room and placed at room temperature maintained low catalase activity in their buds for almost 4 d after the transfer (Fig. 3). Thus the decrease in catalase activity due to low temperature is probably not due to the general effect of low temperature on the rate of reactions. Similar results were obtained with artificially chilled cucumber seedlings (12). Chilling peach shoots to 5°C resulted in a decrease in catalase activity (7). Buds moved to 25°C after an initial 2 weeks at 5°C showed a reversal of the inhibitive effect of chilling and also resulted in inhibition of bud break. Furthermore, it was recently reported that the chilling of sensitive species induced the formation of a catalase inhibitor which could be eliminated by returning the chilled tissues to 20°C, or by passing a tissue extract through a Sephadex column (13).

Cyanamide, which is a very potent dormancy breaking agent

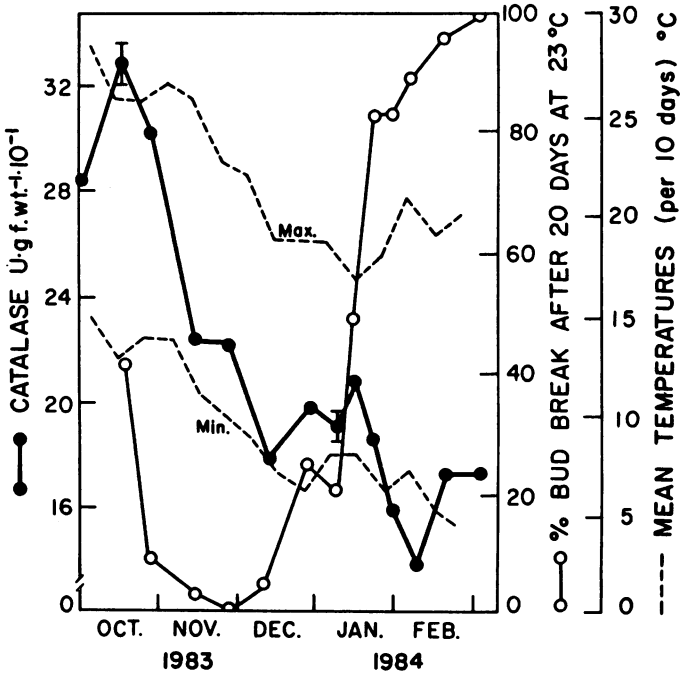

FIG. 2. Temperature changes during winter, catalase activity, and bud break of grapevine, cv 'Perlette,' in the coastal plain. (Mean minimum and maximum temperatures are for 10-d periods. Bud break results represent means of 5 replicates of 5–10 single node cuttings having SE not greater than 10%.)

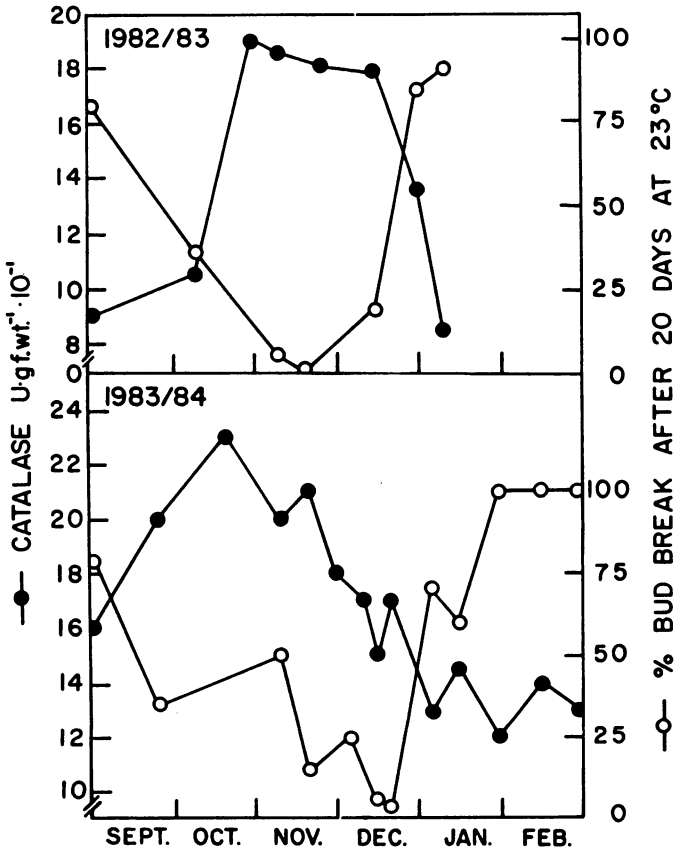

FIG. 1. Catalase activity and bud break of grapevine, cv 'Perlette,' in the Jordan Valley during the winters of 1982-1983 and 1983-1984. (Bud break results represent means of 5 replicates of 5 to 10 single node cuttings having SE not greater than 10%.)

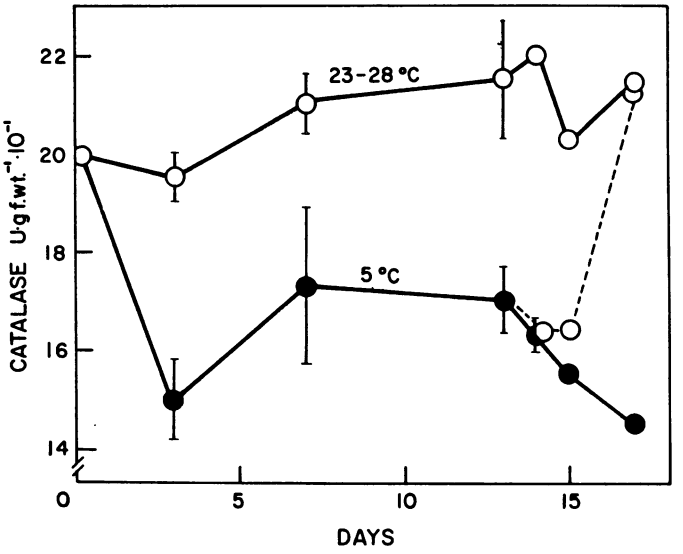

FIG. 3. The effect of chilling on catalase activity in buds of detached grapevine canes, cv 'Perlette.' (On day 13 some canes were transferred from the cold chamber to room temperature. The bars represent SE of 5 replicates.)

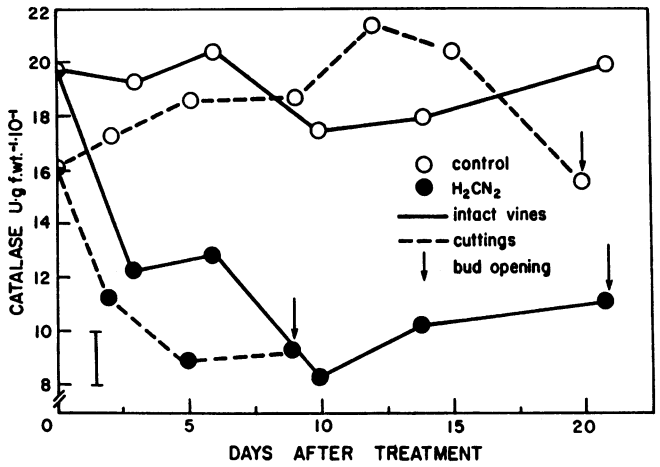

FIG. 4. The effect of cyanamide on catalase activity in buds taken in November 1983 from cuttings and intact shoots of grapevine, cv 'Perlette.' (Cuttings treated with 0.25 M cyanamide (H_2CN_2) and placed at 23°C, shoots sprayed in the field with 50 g/L technical cyanamide. The bar represents twice the pooled SE.)

Table I. *Effects of Dormancy-Breaking Agents on Catalase Activity and Bud Opening in Grapevine Cuttings of cv 'Perlette'*

Treatment	Catalase Activity	Bud Opening
	% of control	
Cyanamide 0.25 M	50	150
Thiourea 0.25 M	64	140
Dinitro-*ortho*-cresol 0.05 M	100	114

for grapevines (6, 8, 16) and other species (11, 18), has been shown to inhibit specifically catalase activity both in fungi and in higher plants (1, 2).

Catalase activity was followed in buds of cyanamide-treated intact vines and in cuttings. Cyanamide caused a very significant decrease in the activity of catalase (Fig. 4). The effect of cyanamide on the reduction of catalase activity was similar in buds of cuttings having a pronounced wound effect, and in buds of intact vines which were not subjected to wounding or to winter chilling. Buds on cyanamide treated cuttings opened 9 d after treatment while buds on untreated cuttings opened only after 20 d. Buds on intact vines treated in November with cyanamide opened 21 d after treatment, and the control ones did not open until natural bud opening occurred in the next spring 3 months later.

The effects of chilling and of cyanamide on catalase and peroxidase activities in buds were also tested. Chilling and 0.25 M cyanamide reduced catalase activity by 45 and 50%, respectively. Peroxidase was reduced only 10% by chilling and was even increased 12% by cyanamide. An increase in peroxidase activity during chilling was also shown in cucumber seedlings (12).

Other dormancy-breaking agents were tested for their influence on catalase activity and on bud opening. Thiourea, which can substitute for light and low temperature requirements in dormant seeds, also decreased their catalase activity (5). This chemical is an efficient dormancy-breaking agent in deciduous fruit trees (20). In this study on grapevines, we observed with thiourea a 40% increase in bud opening and a 64% reduction in catalase activity (Table I).

A less efficient dormancy-breaking agent for grapevine buds, dinitro-*ortho*-cresol which acts as an uncoupler (15), increased bud opening by only 14% and did not inhibit catalase activity. Thus the intensity of dormancy of grapevine buds is positively related to the activity of catalase. The activity of catalase was decreased by cool winter temperatures and dormancy-breaking chemicals such as cyanamide and thiourea. It is possible, as previously suggested for seeds (17, 19, 20), and found by us in grapevine buds (G. Nir, S Lavee, unpublished data), that the decrease in catalase activity causes an increase in the level of H_2O_2 in bud tissue. The increased level of H_2O_2 might cause the activation of the pentose-phosphate pathway, and thus lead to dormancy termination, bud burst and rapid growth (17).

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