sensitivity to sanguinarine, i.e. they are not drawn from the same parental population. Student's t-tests were also performed taking any 2 biotypes at a time and the results demonstrating the degrees of significance are presented in table 2. Table 2 thus reveals that the difference between any 2 vibrio biotype is also statistically significant.

The present study has shown that *V. cholerae* (classical), *V. cholerae* (El Tor), NAG vibrios and *V. parahaemolyticus* may be differentiated from each other on the basis of their sensitivity to sanguinarine and may thus be of importance for the taxonomy of vibrios.

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Phytotoxic effects of cadmium in leaf segments of *Avena sativa* L., and the protective role of calcium

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Summary. It is shown that ethylene production can be stimulated in peeled oat leaf segments in the dark by cadmium at concentrations below 0.01 mM, and that cadmium-stimulated stress ethylene accelerates senescence processes. Higher cadmium concentrations cause membrane deterioration and inhibit ethylene production and senescence. These effects of cadmium are antagonized by calcium. This indicates a possible protection of plant cells against cadmium toxicity by calcium.

Cadmium is an environmental contaminant of increasing importance because of industrial processes and refuse incineration. It is readily taken up by plants from soils[2] and nutrient solutions[3], and accumulates in various tissues during the growing season. This accumulation is toxic to plants. Reduced rates of photosynthesis[4], transpiration[5] and respiration[6] have been observed, as well as stimulated or inhibited enzyme activities[6,7]. Among the early effects of excess cadmium uptake in beans, short-term stimulation of ethylene biosynthesis was reported in a recent paper[3]. Such stimulation is limited to a relatively short incubation period. Changes in peroxidase activity and phenolic materials accompany the subsequent decline. Cadmium is known to bind to macromolecular cell constituents[8]. In bean leaf discs, membrane damage seems to be the cause of the inhibition of a membrane-involving step in ethylene biosynthesis[9]. The interaction with membranes and soluble proteins may be responsible for the impact of cadmium on cellular metabolism. Membranes function as important regulators of specific pathways, such as ethylene biosynthesis, and developmental processes, such as senescence. Stress-induced senescence in oat leaves includes a rapid increase in protease activity leading to an enhanced content of α-amino nitrogen, ethylene biosynthesis and chlorophyll loss[10]. In the present study, the oat leaf-system has been used to show that membrane damage induced by cadmium is associated with inhibited ethylene production and an altered senescence rate, and that calcium protects the cells against cadmium damage.

Materials and methods. The 1st leaf of 14-day-old seedlings of *Avena sativa* L. (var. Victory) was used for all experiments. The seeds (Swedish Seed Company, Ltd, Svalöv, Sweden) were grown in vermiculite in controlled growth chambers at 25 °C with a 16-h photoperiod (about 12,000 lux). Leaf sections were excised and sterilized as described before[11]. The abaxial epidermis was peeled off with fine forceps. Five segments of 50 mm length each were rinsed in sterile distilled water and floated in glass bottles, stripped side down, on 5 ml of Na-phosphate buffer (1 mM, pH 5.7). Cadmium was added as $CdCl_2$ at concentrations in the range 0–1 mM. Where indicated, $CaCl_2$ (1 mM) was included. The bottles were sealed with serum rubber caps and incubated for 48 h in the dark at 25 °C. At the end of the incubation period, air samples were withdrawn with a 5 ml syringe and injected into a gas chromatograph (Perkin Elmer Model F-11) equipped with an activated alumina column and a flame ionization detector. Ethylene was identified and quantified by comparison with the retention time and peak height of ethylene standards. After taking the air samples, the bottles were opened, samples of the incubation solution were removed and their UV-absorbance at 280 nm was determined spectrophotometrically (Aminco DW-2a). The presence of UV absorbing materials in the incubation solution was used as measure for leakage due to membrane damage[9]. For the determination of chlorophyll and α-amino nitrogen, leaves were extracted with hot 80% ethanol and extracts were analyzed as described before[12].

Results and discussion. Peeling off the epidermal cells of oat

Effect of cadmium (0.01 mM) and aminoethoxyvinylglycine (AVG, 0.1 mM) on dark-induced chlorophyll loss during 48 h of incubation

	Chlorophyll loss (in % of initial value)
H_2O	60 ± 4
Cadmium	71 ± 5
Cadmium + AVG	52 ± 4
AVG	55 ± 5

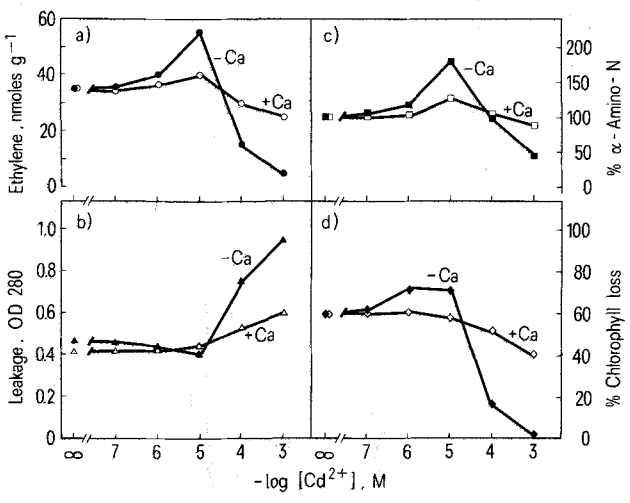
Values are means (N = 4) + 1 SD.

leaf segments prior to incubation in darkness led to the production of wound ethylene (fig., a). It was shown in an earlier report that most of the ethylene formed under these conditions is due to the mechanical wounding and not the dark-stress, and that the biosynthetic pathway of ethylene is regulated at the step of 1-aminocyclopropane-1-carboxylate (ACC) synthesis[13]. Ethylene induced by peeling was enhanced in the presence of 0.1, 1.0 and 10 µM cadmium. The later was found to be an inducer of ethylene biosynthesis in several systems[3,14,15]. It stimulates ACC synthesis at low tissue concentrations similarly to mechanical wounding[9]. It is therefore not surprising that the chemical and physical treatments applied in this study act synergistically on ethylene production. At concentrations above 10 µM, cadmium was inhibitory to wound ethylene production. Cadmium is known to inhibit ACC synthase in vivo and in vitro, and ACC conversion, depending on the tissue concentration[9]. Conversion of ACC to ethylene presumably involves an intact membrane system. As membranes become impaired by physical or chemical treatments, cells lose their ability to convert ACC[9,13].

The figure a also shows a strong antagonism between cadmium and calcium. In the presence of calcium, cadmium-stimulation of ethylene production at low, as well as inhibition at high concentrations were strongly antagonized.

The figure b shows that cadmium concentrations above 10 µM induced leakage of UV absorbing material. This absorbance change of the incubation solution was due to amino acids and proteins (data not shown). Cadmium-induced leakage was reduced in the presence of calcium, indicating the protective effect of calcium against cadmium-induced membrane damage.

The apparent link between membrane damage and inhibition of ethylene production, as observed in this study, is similar to what has been found with bean leaf discs[9]. Cadmium thus inhibits ethylene production when tissue concentrations are high enough and acts antagonistically to the wound-stimulus from peeling. This may be important in cases where ethylene is necessary for the induction of secondary responses to mechanical wounding, such as lignification[16].



Effect of various cadmium concentrations on *a* wound ethylene production, *b* leakage, *c* α-amino nitrogen content (value of control = 100%), and *d* chlorophyll loss (expressed in percent of initial chlorophyll content) of peeled oat leaves incubated for 48 h in the dark in the presence (open symbols) or absence (closed symbols) of 1 mM $CaCl_2$.

It was shown that during senescence of oat leaves the content of α-amino nitrogen and of chlorophyll changes rapidly[12]. The effect of cadmium on these senescence processes is shown in the figures c and d, respectively. The 2 processes responded in very similar ways to the Cd treatment. There was an acceleration of protein degradation (indicated by an increased content of α-amino nitrogen) and chlorophyll loss at low concentrations. The question arises at this point to what extent cadmium-stimulated ethylene could be responsible for this effect. It was suggested earlier that ethylene plays a minor role in senescence of oat leaf segments in darkness[13]. The results in the table show that the increase in chlorophyll loss due to 10 µM cadmium was abolished in the presence of aminoethoxyvinylglycine, a specific inhibitor of ethylene biosynthesis. Stress-induced chlorophyll loss in the absence of cadmium, on the other hand, was relatively insensitive to this inhibitor. This suggests that cadmium-induced acceleration of senescence is mediated by ethylene.

Chlorophyll and protein degradation were inhibited in leaves floating on cadmium concentrations higher than 10 µM. Such an inhibition of senescence may be due to the inactivation of chlorophyll and protein degrading enzymes. Enzyme inhibition by cadmium in vitro and in vivo has been reported[7,9]. Another possibility is that membranes lose functions which are important for the regulation of senescence processes, as observed in experiments with non-toxic, membrane-active substances[13].

The cadmium-calcium antagonism seen in the case of ethylene production and leakage was also found for chlorophyll loss and increase in α-amino nitrogen (see the figure, c and d). Calcium protection of plant tissues against heavy metal toxicity was reported before[7,18], and was observed with wounded storage tissue and *Lemna minor* L. (unpublished observation). The mechanism of such calcium protection is not known. The results of this study suggest an interaction at the membranes.

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