Table 2 *p*H and concentrations of di- and monovalent malate (mol m^{-3}) in the various cells of the stomatal complex of *C. communis* when the stomata are closed and also about to open

Stomata closed	GC	ILS	OLS	EPID
*p*H	5.1	5.5	5.7	5.8
Monovalent malate	32	50	45	60
Divalent malate	9	25	53	120
Pre-opening	(*p*H 5.8)			
$Malate^-$	10	50	45	60
$Malate^{2-}$	20	25	53	120

GC, Guard cell; ILS, inner lateral subsidiary; OLS, outer lateral subsidiary; EPID, epidermal cell.

closing would be expended in bringing about the change in *p*H of the guard cell from the *p*K value of malic acid of 5.1 to 5.8 and back again. It is assumed that all the environmental factors which cause stomatal opening and closure work by changing the *p*H of the guard cell and so operating the malate switch. This would explain why a number of factors can influence stomata by acting independently and also by seeming to act together. For example, before dawn endogenous rhythm could cause a partial rise in guard-cell *p*H which would be completed by a reduction in ambient CO_2 at the onset of photosynthesis at dawn. This would explain how stomata anticipate dawn by partially opening before the light period. It is not the purpose of this paper to suggest how environmental factors cause the change in guard cell *p*H.

Table 3 *p*H and concentrations of di- and monovalent malate (mol m^{-3}) in the various cells of the stomatal complex of *C. communis* with the stomata open and also about to close

Stomata open	GC	ILS	OLS	EPID
*p*H	5.8	5.7	5.5	5.1
$Malate^-$	60	60	50	32
$Malate^{2-}$	120	70	25	9
Pre-closing	(*p*H 5.1)			
$Malate^-$	192	60	50	32
$Malate^{2-}$	53	73	25	9

It follows from this hypothesis that: (1) Opening and closing of stomata should always be preceded by a *p*H change in the guard cells. (2) The important ion is not K^+ but malate and this would explain why other monovalent cations are effective in bringing about stomatal opening[9,10].

Although malate is considered to be the important ion in *C. communis* because of its ability to switch its valence, it is possible that other organic acids may provide the switch anion in some other species. There are, however, few organic acids which have *p*K values at the *p*H normally found in plant cells.

The mechanism of transport of the ions between the cells of the stomatal complex is not crucial to the validity of the hypothesis but the gradients envisaged suggest that diffusion could be the most important factor. In this case the active transport found between the cells[4,6] would be secondarily active. Primary active transport between the cells cannot, however, be ruled out at the present time.

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Plant uptake and leaching of dimethylnitrosamine

The occurrence of carcinogenic nitrosamines is of considerable interest because they have been found in foods consumed by humans[1] and they can be produced, at least *in vitro*, from agricultural chemicals[2,3]. Furthermore, the widespread occurrence of potential precursors of nitrosamines—secondary or tertiary amines and inorganic nitrogen compounds that are readily converted to nitrite by microorganisms—led to studies demonstrating that the carcinogens can be formed in soil samples and aquatic environments[4,5]. A hazardous chemical in soil is not of toxicological significance, however, unless it is volatilised or enters the human food chain by being assimilated by food crops or leached through soil into ground waters ultimately used for drinking. We report here that dimethylnitrosamine (DMNA) can be assimilated by the roots of lettuce and spinach, is translocated to the tops and that it also moves readily through soil.

Lettuce was grown from seed in quartz sand or Williamson silt loam contained in clay pots and incubated at 16 °C in a growth chamber in which the daylength was 16 h. The plants were watered daily with Hoagland's inorganic nutrient solution[6] at one-quarter strength; the leaves were not wetted during the watering. Spinach was grown from seed in the same nutrient solution, the liquid being aerated constantly and replaced weekly. The spinach plants were maintained at 20 °C under Growlux fluorescent lights timed to provide a 16-h day. After two months, the various pots received either 0.57 μCi of ^{14}C-DMNA plus the unlabelled nitroso compound to a final concentration of 100 $\mu g\ ml^{-1}$, or 0.057 μCi of ^{14}C-DMNA plus unlabelled nitrosamine to a final concentration of 10 $\mu g\ ml^{-1}$. The total amounts of DMNA added were 2.50 and 0.25 mg for the high and low levels of exposure, respectively. After 2 d, the aerial portions of these plants, as well as of plants not exposed to the nitrosamine, were harvested and washed, and 100-g portions homogenised in 150 ml of 6.0 N NaOH. The homogenised slurry was refluxed for 30 min, filtered through glasswool, and steam-distilled, half of the volume being collected as distillate. The distillate was extracted three times with volumes of methylene chloride one-third that of the distillate, and the extract was dried with Na_2SO_4 and concentrated to 2 ml by evaporation in a Kuderma–Danish evaporative concentrator operating at 60 °C. Half of the extract was added to 10 ml of a scintillation cocktail containing 5 g PPO, 0.1 g POPOP, and 1 l of toluene, and the radioactivity was counted on a Beckman model LC-100C liquid scintillation system. A second experiment with lettuce growing in sand was conducted similarly, but the plants were harvested at 4, 9 and 15 d.

The results show that both spinach and lettuce can absorb radioactivity from the growth medium (Table 1), the amount assimilated being proportional to the amount of ^{14}C-DMNA added to the pots. The absorbed ^{14}C disappeared with time, in agreement with results on nitrosamine uptake by *Lepidium sativum*[7]. The percentage of radioactivity taken up by lettuce grown in soil was slightly higher than that of plants grown in sand. The quantity of DMNA taken up per unit weight of plant material was, however, severalfold greater for the soil-grown plants, possibly because of the lower yield obtained when the lettuce was grown in the silt loam. It has been reported that cereals can absorb nitrosamines[8], and the present findings not only extend previous observations, but pertain to two major vegetable crops.

To confirm the identity of the labelled product, 2-month-old lettuce plants were exposed for 2 d to 5.7 μCi of ^{14}C-DMNA in 25 ml of nutrient solution made to 500 $\mu g\ ml^{-1}$

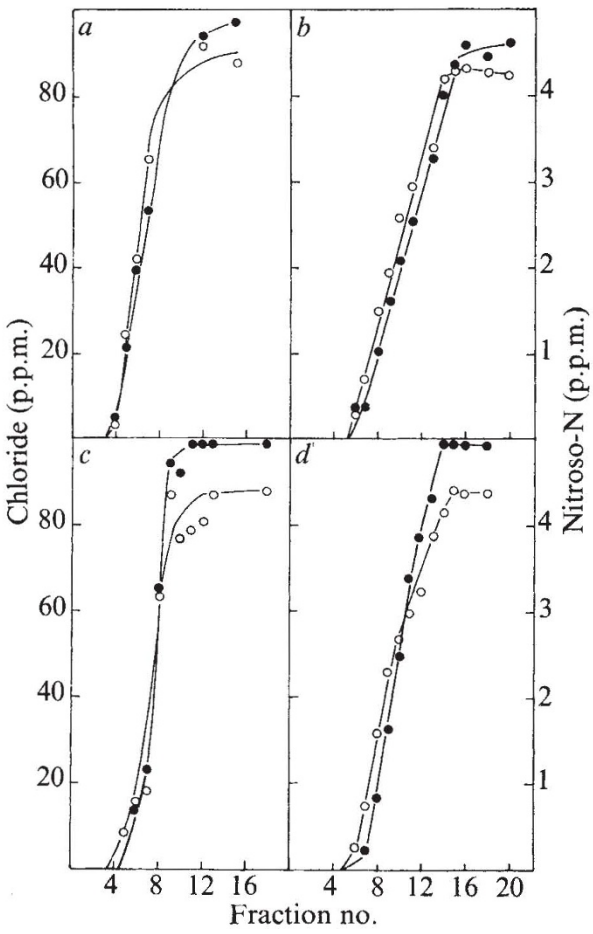
Table 1 Uptake of dimethylnitrosamine by spinach and lettuce

Plant	Growth medium	^{14}C-DMNA supplied (μCi)	Length of exposure (d)	DMNA taken up* (μg per g dry wt)	DMNA taken up by plant* (%)
Lettuce	Sand	0.057	2	1.38	3.20
		0.57	2	14.38	3.25
Lettuce	Soil	0.57	2	106.0	5.06
Spinach	Water	0.057	2	0.54	0.38
		0.57	2	5.60	0.27
Lettuce	Sand	0.57	4	7.04	1.56
			9	1.40	0.21
			15	0.07	0.02

*Each figure represents the average of four replicates.

with the unlabelled nitrosamine. Compounds containing the radioactivity were extracted and concentrated, and then 0.5 ml of the methylene chloride extract was placed on each of two silica gel thin-layer plates (Eastman chromatogram sheets), only one of which was exposed to ultraviolet radiation (240 nm) for 15 min before development. After development in hexane–diethyl ether–methylene chloride (4 : 3 : 2), the chromatograms were counted on a strip chart recorder (Actigraph III, Nuclear Chicago), and the location of the radioactivity was compared with that on chromatograms of authentic ^{14}C-DMNA. The radioactivity on the non-irradiated chromatogram had the same R_f value (0.34) as ^{14}C-DMNA, and the radioactivity on the ultraviolet-irradiated chromatogram had the same R_f value (0.03) as ^{14}C-dimethylamine, which is formed by the photolytic cleavage of DMNA. The yield of dimethylamine was essentially stoichiometric. As a further confirmation of identity, the extract was analysed by gas chromatography using a Varian Aerograph series 1700 gas chromatograph equipped with a flame ionisation detector and a 1.84 m × 3.2 mm stainless steel column packed with 10% Carbowax 20M on Chromosorb W-HMDS. The column was maintained at 80 °C for 5 min and then programmed to 110 °C at a rate of 2 °C min^{-1}. The retention times of the unknown and authentic DMNA (512 s) were identical.

Fig. 1 Leaching of chloride (●) and dimethylnitrosamine (○) through columns of four soils. *a*, Deerfield; *b*, Scarboro; *c*, Lima; *d*, Williamson.



To determine whether DMNA can leach through soil, columns of Williamson silt loam (*p*H 5.8, 1.9% organic matter), Lima loam (*p*H 7.8, 3.8% organic matter), Deerfield sand (*p*H 5.1, 2.5% organic matter), and Scarboro sand (*p*H 5.6, 14.8% organic matter) were prepared using 100–200 g of air-dry soil that had been passed through a 2-mm sieve. The columns were moistened with distilled water to bring the soil to field capacity, and a solution containing 100 μg ml^{-1} of chloride and DMNA (5 μg nitroso nitrogen ml^{-1}) was allowed to pass through the column. Equal fractions (10 ml) were collected, and the fractions analysed for chloride and DMNA content using the methods of Bergmann and Sanik[9] and Daiber and Preussmann[10], respectively. Nitrite, which gives a positive reaction in the method of DMNA analysis, was determined in all samples by the procedure of Montgomery and Dymock[11], and corrections were made for the small amounts present in the first few fractions. The results in Fig. 1 demonstrate that DMNA is not retained by the soils but moves as readily as chloride. Because the soils were open to the atmosphere, some of the added DMNA may have been lost through volatilisation.

The earlier findings that nitrosamines can be formed and are persistent in samples of soil[4,5,12] and our observation that DMNA can potentially move from soil into food crops and downwards into groundwater suggest that a hazard may exist from a previously unrecognised source of exposure. Investigations on nitrosamine uptake by edible crops and movement through soil are also important in light of the report that the *N*-nitroso derivative of at least one pesticide has been found in a municipal water supply (D. H. Fine, personal communication). Further research is required to determine whether such a possible hazard is in fact a reality.

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Non-enzymatic photochemical reduction of nitrate in rice seedlings

LIGHT plays an important role in nitrate assimilation in leaves by providing the reductants and by stimulating the synthesis of nitrate and nitrite reductases[1–3]. Activity of nitrate reductase is considered to be the rate-limiting step in nitrate assimilation and in the production of grains and grain proteins in crop plants[4,5]. It has been known for some time, however, that the ultraviolet radiation in sunlight photochemically reduces nitrate in aqueous solution to nitrite[6,7]. Stoy[8,9] reported that ultraviolet light had a much stronger effect on nitrate assimilation in wheat leaves than light of longer wavelengths. We have observed, in experimental conditions, significant accumulation of nitrite in rice leaves exposed to sunlight, even when nitrate reductase activity was negligible, so we have investigated non-enzymatic photochemical reduction of nitrate in leaves.

The absorption spectrum of 0.2 M KNO_3 measured in a Gilford spectrophotometer showed a trough at 260 nm ($E = 0.3$) and a sharp peak at 302 nm ($E = 1.04$). Negligible absorption was observed at wavelengths 340 nm and above. On the other hand, 0.2 M $NaNO_2$ revealed a broad absorption band ($E = 1.25$–1.75) in the whole range of wavelengths from 260 to 325 nm, after which it showed a decline, with negligible absorption at 400 nm and above.

When 10 mM KNO_3 solution was exposed to bright sunlight of intensity from 7,000 to 10,000 foot candles (70–108 klx), about 10 to 20 nmol NO_2^- ml^{-1} h^{-1} was produced. This could be compared with the amount of nitrite produced *in vitro* by an active preparation of nitrate reductase from rice leaves, incubated with 0.34 mM NADH and 10 mM KNO_3, which was found to be 20–30 nmol NO_2^- ml^{-1} h^{-1}. When 10 mM $NaNO_2$ was irradiated with sunlight, negligible loss of nitrite was observed.

That the ultraviolet component of solar radiation was responsible for the reduction of nitrate was easily confirmed by using filters, which absorbed that part. Negligible quantities of nitrite were formed when nitrate solution was treated with such filtered sunlight. Exposure of nitrate to ultraviolet light from a mercury lamp was as effective for reduction as direct sunlight.

To detect non-enzymatic accumulation of nitrite in leaves exposed to sunlight, it was necessary to eliminate, or diminish considerably, the activities of both nitrate and nitrite reductases. In order to achieve this, rice seedlings were grown in complete darkness. It is known that synthesis of the two enzymes is retarded in etiolated plants[2,3]. When these leaves are exposed to sunlight, however, the enzymes are likely to be synthesised as the chloroplasts develop and to prevent this, nitrate was added in the presence of sodium tungstate and chloramphenicol. Tungstate blocks the *in vivo* synthesis of an active nitrate reductase, by forming the enzymatically inactive tungsten analogue by replacement of molybdenum in the enzyme molecule[10]. Chloramphenicol, an inhibitor of protein synthesis on 70S ribosomes, inhibits the formation of nitrite reductase, which requires protein synthesis on chloroplastic ribosomes, without affecting the synthesis of nitrate reductase, which takes place on 80S ribosomes in the cytoplasm[3,11]. Thus, by a combination of tungstate and chloramphenicol treatments, we hoped to diminish the activities of nitrate reductase and nitrite reductase considerably.

Results show that in normal seedlings grown in sunlight with nitrate (Table 1; treatment 1), the activities of the two enzymes were quite high. Free nitrite was not detected in the leaves because of its rapid reduction to ammonia in healthy seedlings. The seedlings in which the activities of the two enzymes were blocked (treatment 3) progressively accumulated nitrite for up to 4 h in leaves exposed to sunlight. The same result was brought about by ultraviolet light from a mercury lamp; but detached leaves kept in the dark did not accumulate nitrite. When the seedlings were treated with chloramphenicol (treat-

Table 1 Effect of sunlight on the accumulation of nitrite in leaves of rice seedlings

Conditions of growth of seedlings			Period of exposure of detached leaves to sunlight (h)	Nitrate reductase (μmol NO_2^- per 20 min per g tissue)		Nitrite reductase (μmol NO_2^- reduced per 20 min per g tissue)	Nitrate (μmol per g tissue)	Nitrite
Treatment number	Light regime	Additions to nutrient solution		*In vitro*	*In vivo*			
1	Sunlight	KNO_3	4	1.92	0.182	13.4	12.1	Nil
2	Sunlight	KNO_3 + chloramphenicol	4	1.08	0.096	1.2	11.4	0.220
3	Dark	KNO_3 + tungstate + chloramphenicol	0					0.012
			1					0.027
			2					0.044
			3					0.062
			4	0.04	Nil	1.0	12.2	0.083
		(Detached leaves kept in the dark)	4					0.012
		(Detached leaves exposed to ultraviolet light from a mercury lamp)	1					0.041

Seedlings of rice (*Oryza sativa*), variety Taichung Native-1 were raised in well washed quartz sand with Hoagland solution lacking nitrate as described earlier[3]. Seedlings grown in sunlight in treatments 1 and 2 were exposed during the day (10 h) and kept in dark during the night. In treatment 1, 15 mM KNO_3 was added on the 14th day. In treatment 2, 15 mM KNO_3 and 3 mg ml^{-1} chloramphenicol were added on day 14 and seedlings kept in the dark for 2 d. In treatment 3, on day 14, 15 mM KNO_3 with 5 mM sodium tungstate and 3 mg ml^{-1} chloramphenicol were added to the nutrient solution and also sprayed on the etiolated plants and seedlings kept in the dark for 2 more days. Leaves from 15-d-old seedlings from the three treatments were detached from plants and immersed in 15 mM KNO_3 solution so that the bottom 1 cm dipped into the solution and the remaining portion was exposed to sunlight. The nitrate solution was kept in flasks covered with a black paper, so it was not exposed to sunlight and photochemical reduction of nitrate in the flasks was avoided. The protruding leaves alone were exposed to light for different intervals as indicated in the table. At the end of this period, leaf extracts were prepared and *in vitro* activities of nitrate reductase and nitrite reductase were assayed as described previously, using NADH and reduced methyl viologen as electron donors, respectively[3]. *In vivo* nitrate reductase activity in the leaf disks was assayed by the method of Jaworski[12]. Nitrite content in the leaf extracts as well as in the enzyme assays, was determined by the standard Greiss–Ilosvay colorimetric method. Nitrate content in suitably diluted leaf extracts was determined after its quantitative enzymic reduction to nitrite by an active dialysed nitrate reductase obtained from rice leaves.