

# Oxalate in Crop Plants

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Oxalate is produced and accumulated in many crop plants and pasture weeds. Although oxalate can be a major constituent of plants, important aspects of its biosynthesis, accumulation, and catabolism are unresolved. A major precursor of oxalate is glyoxylate. Oxalate accumulators can also form oxalate by C2/C3 cleavage of ascorbic acid. Plant oxalate content is affected by nitrogen source, inorganic ion availability, and other environmental factors. Ontogenetic stage and genotype are also important factors. Oxalate may play a role in ion balance and osmoregulation. Oxalate oxidase and oxalate decarboxylase are present in some plants, and oxalate can be catabolized and the carbon recycled. Oxalate accumulation by crop and pasture plants affects the nutritional quality of foods and feed negatively. Consumption of plants with high oxalate content has led to poisoning and death of livestock. High-oxalate diets can increase the risk of renal calcium oxalate formation in certain groups of people and may also affect calcium absorption.

## 1. INTRODUCTION

Oxalate is a common constituent of plants, and several species, including some crop plants, accumulate high levels of this C<sub>2</sub> dicarboxylic acid anion. Dependent upon species, oxalate accumulates primarily as soluble oxalate, insoluble calcium oxalate, or a combination of these two forms. From an agricultural point of view, oxalate is of concern because of health-related hazards due to high oxalate levels in food and animal feed. Reviews on oxalate in plants by Oke (1969) (nutritive aspects), Hodgkinson (1977), and Franceschi and Horner (1980) (calcium oxalate in plants) have appeared. This review will survey current knowledge on agricultural and horticultural aspects of oxalate in plants. Emphasis will thus be given to crop plants and certain pasture weeds where oxalate content is considered a potential health risk.

Oxalate is involved in several metabolic processes, possibly even within the same species. Therefore, discussion of its biosynthesis and possible functions is kept general and is restricted to plants that accumulate high levels. Most of the extreme oxalate accumulators (more than 5% oxalate by dry weight) belong to the three families Caryophyllaceae, Chenopodiaceae, and Polygonaceae. The relationship between these families (all within the subclass Caryophyllidae) suggests that similar biosynthetic pathways and functional roles for oxalate exist within this group.

## 2. CHEMISTRY AND METHODS OF ANALYSIS

Oxalic acid is the simplest of the dicarboxylic acids (Figure 1) with pK<sub>a</sub> values of 1.23 and 3.83. Besides being a relatively strong acid it is also a reducing agent. The acid normally crystallizes as a dihydrate. The oxalate ion is a very strong chelating agent, and salts formed with divalent cations are, as a rule, sparingly soluble. An important example is the calcium salt, which is soluble in water to only 6.0 mg/L at 18 °C. The solubility and instability of calcium oxalate increase with the level of hydration, the trihydrate being most soluble and unstable, followed in order by the dihydrate and the monohydrate.

In the analysis of oxalate levels in plants, a distinction is made between procedures for extracting total and water-soluble oxalate. Total oxalate is extracted in strong-acid solution (e.g., hot 1 M HCl) leading to the dissolution of crystalline calcium oxalate. Extraction of

tissues with water removes free oxalic acid as well as its potassium and sodium salts but does not remove calcium oxalate. Classical methods of oxalate analysis involve separation by means of calcium oxalate precipitation at pH 4-5 followed by gravimetric, titrimetric, or spectrophotometric determination (AOAC, 1975). In recent years methods have been developed that utilize gas chromatography (Charransol et al., 1978; Hesse et al., 1980), high-performance liquid chromatography (Libert, 1981; Wilson et al., 1982; Grün and Loewus, 1983), isotachopheresis (Schwendtner et al., 1982), and enzymology (Beutler et al., 1980; Kohlbecker and Butz, 1981), most of which are less time consuming and more precise than the earlier approaches.

## 3. BIOSYNTHESIS, CATABOLISM AND TRANSPORT

### 3.1. Oxidation and Dismutation of Glyoxylate.

Glycolate and glyoxylate are effective precursors of oxalate as shown by labeling experiments with a number of oxalate-accumulating plants (Richardson and Tolbert, 1961; Millerd et al., 1963a; Chang and Beevers, 1968; Osmond and Avadhani, 1968; Seal and Sen, 1970; Piquemal et al., 1980). Richardson and Tolbert (1961) showed that glycolate oxidase from plant tissues catalyzes the oxidation not only of glycolate to glyoxylate but also of glyoxylate to oxalate. The affinity of the enzyme for glycolate was much greater than for glyoxylate ( $K_m = 3.8 \times 10^{-4}$  and  $5.4 \times 10^{-3}$ , respectively). Chang and Huang (1981) concluded that, considering the low glyoxylate concentrations in vivo, glyoxylate oxidation by glycolate oxidase was unlikely. However, compartmentation of the enzyme and substrate could provide conditions favoring the reaction. The characteristics of the enzyme may, furthermore, vary between species. An enzyme oxidizing glycolate and glyoxylate in *Oxalis pes-caprae* (Millerd et al., 1963b) differed from the glycolate oxidase mentioned above by its more efficient use of glyoxylate as the substrate for oxidation. Evidence for multiple enzymes with glycolate and glyoxylate oxidase activity was demonstrated by Havir (1983) in *Nicotiana* while Nishimura et al. (1983) claim the existence of only one glycolate oxidase in spinach. Interestingly, Bornkamm (1969) found there was no consistent difference in capacity to oxidize glycolate to oxalate between oxalate-accumulating species and nonaccumulators.

Oxalate may be produced by plants through the action of lactate dehydrogenase on glyoxylate (Davies and Asker, 1983). Two glyoxylate molecules dismutate to yield glycolate and oxalate. The distribution and activity of this enzyme in oxalate-accumulating species have not been determined, and it is difficult to assess the contribution of this pathway to total oxalate in the plant.

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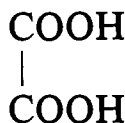


Figure 1. Oxalic acid molecule.

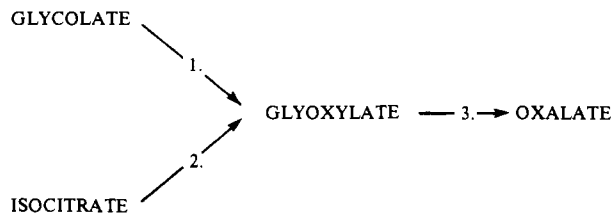


Figure 2. Oxidation and dismutation of glyoxylate in the biosynthesis of oxalate. Key: 1, glycolate oxidase (peroxisomes) or glycolate dehydrogenase (peroxisomes); 2, isocitrate lyase (glyoxysomes); 3, glycolate oxidase (peroxisomes) or lactate dehydrogenase (cytosol).

There are various sources of glyoxylate in higher plants. Glycolate is formed in the chloroplast during photorespiration and is subsequently converted to glyoxylate in the peroxisomes (Tolbert, 1981). Evidence against photorespiratory glyoxylate as a major oxalate precursor in spinach was presented by Raven et al. (1982).  $^{18}\text{O}_2$  incorporation into phosphoglycolate and glycolate did not extend to oxalate. Furthermore,  $\text{C}_4$  photosynthesis, with little or no photorespiration, is frequently found in plants accumulating high concentrations of oxalate (section 6). The high levels of oxalate produced in the dark (Zindler-Frank, 1974; Libert, 1987a) or under low-light conditions (Grütz, 1953) also indicate that photorespiration is not a prerequisite for oxalate synthesis. On the other hand, low oxalate production in *Lemna* plants grown in the dark and supplied with organic nutrients was correlated with lower glycolate oxidase activity (Bornkamm, 1965).

Glycolate dehydrogenase oxidizes glycolate to glyoxylate with a concomitant reduction of  $\text{NAD}^+$  (Roth-Bejerano and Lips, 1973; Kaplan and Lips, 1984). This  $\text{NADH}$  may be utilized for nitrate reduction (Kaplan and Lips, 1984; see section 4.2). In plants examined, glycolate dehydrogenase activity was found to be small compared to the glycolate oxidase activity, suggesting that the contribution of the dehydrogenase to total glyoxylate formation is minimal.

Isocitrate is cleaved by isocitrate lyase to form glyoxylate and succinate. This enzyme is part of the glyoxylate cycle found in glyoxysomes (Tolbert, 1981). The glyoxylate cycle is generally considered to be present only in early stages of seed germination of some oil-rich seeds (Tolbert, 1980). The limited occurrence of this process seems to exclude this pathway of biosynthesis from being a general source of oxalate in plants. However, isocitrate lyase activity in green leaves of some plant species (including spinach) (Osmond and Avadhani, 1968; Godavari et al., 1973) as well as in white shoots of *Oxalis pes-caprae* (Millerd et al., 1963b) has been demonstrated.

The suggested pathways for formation of oxalate via glyoxylate are summarized in Figure 2. It is indeed likely that glyoxylate is included in the biosynthetic pathway of oxalate, but neither isocitrate nor photorespiratory glycolate is an undisputable precursor.

**3.2. Cleavage of Oxalacetate.** Chang and Beever (1968) demonstrated an enzyme activity in beetroot and spinach preparations that cleaves oxalacetate into oxalate and acetate. Whether this is a major or even a common pathway of oxalate formation is unknown. As oxalacetate also is a precursor to malate, this biosynthetic pathway could

account for the negative correlation between malate and oxalate content of rhubarb petioles described by Libert (1987a,b).

**3.3. Cleavage of Ascorbate.** Wagner and Loewus (1973), Yang and Loewus (1975), and Nuss and Loewus (1978) presented convincing evidence that oxalate may be formed by C2/C3 cleavage of ascorbate. Conversion proceeds in the dark as well as the light. In oxalate-accumulating plant species 22–50% of the label from [ $1\text{-}^{14}\text{C}$ ]-ascorbic acid appeared in oxalate, while only 2–19% was recovered in oxalate in some low-oxalate species (Nuss and Loewus, 1978). Label is also incorporated into developing calcium oxalate crystals (Franceschi, 1985). The enzymatic basis of this pathway is yet to be determined. Glyoxylate may be an intermediary substance in the ascorbate to oxalate conversion (Franceschi, 1985).

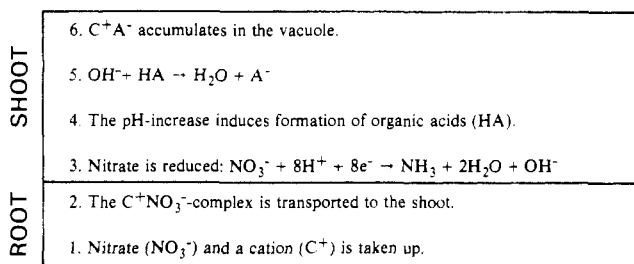
**3.4. Catabolism.** Oxalate is generally regarded as an end product, and few examples of net oxalate breakdown *in vivo* are recorded in the literature (Wyman and Palmer, 1964; Clements, 1964). Diurnal variation of oxalate content was observed by Seal and Sen (1970) in *Oxalis corniculata* and by Jones and Ford (1972) in *Setaria sphacelata*. Roughan and Warrington (1976) failed to find diurnal variation in the latter species. Label from [ $^{14}\text{C}$ ]oxalate applied to plants or plant parts incorporates into other substances including glycolate (Zbinovsky and Burris, 1952; Millerd et al., 1963a; Calmes and Piquemal, 1977), malate, and sugars (Havir, 1984). Plant enzymes capable of oxalate oxidation and decarboxylation have been detected. Oxalate oxidase activity was described by Franke et al. (1943), Pundir and Nath (1984), and others. The oxidase activity is often found in oxalate-accumulating species. Oxalate decarboxylation activity in a pea seed extract was described by Giovanelly and Tobin (1961).

**3.5. Localization of Synthesis and Transport.** Information on localization of oxalate biosynthesis and oxalate transport in plants is scarce. Considering the various pathways possible, synthesis could occur in peroxisomes or the cytoplasm (see Figure 2). Most labeling experiments have been made with cell-free extracts, detached leaves, and germinating seedlings. As for oxalate transport, it can be deduced from the low levels of  $\text{Ca}^{2+}$  in the phloem sap (Raven, 1977) that transport of oxalate in the phloem is possible.

**3.6. Calcium Oxalate Dissolution.** Calmes and Carles (1970) hypothesize that calcium oxalate trihydrates may be dissolved, whereas the monohydrates represent an end product. Dissolution of calcium oxalate crystals in Boston ivy (*Parthenocissus tricuspidata*) was observed by Calmes and Piquemal (1977). There have been occasional reports of seasonal dissolution of calcium oxalate crystals in other plants [see Franceschi and Horner (1980)].

#### 4. POSSIBLE FUNCTIONS

**4.1. Protection.** As early as 1888, Stahl [see Pat-schovsky (1920)] proposed that oxalate provided protection for plants against foraging animals in as much as oxalate-rich plants tended not to be protected by hairs, thorns, etc. Ecological factors behind evolutionary changes of metabolic character are difficult to prove. Besides the observations of Stahl, there is some circumstantial evidence that a high oxalate level may be of some importance for protection of plants against insects and foraging animals. In rice (*Oryza sativa*), genes for resistance to the brown planthopper appear to be associated with an elevated oxalate level of the leaf sheath (Yoshihara et al., 1979). Furthermore, low concentrations of oxalate in test solutions inhibit sucking by the planthopper (Yoshihara et al., 1980). Similar results were obtained by Massonie (1980) in ex-



**Figure 3.** Simplified model for accumulation of organic acid anions in connection with nitrate uptake and reduction.

periments with the aphid *Myzus persicae*.

The background of a possible protective effect of oxalate is clearly not the same for foraging animals, insects, and microbial pathogens. For foraging animals, preference for an oxalate-rich plant may be affected by taste or texture, rather than oxalate toxicity. As for taste, acidity may be influenced by the oxalate level. Further, texture can be strongly affected by calcium oxalate crystals (Franceschi and Horner, 1980) (section 5.6). Microorganisms are more likely to be affected by the presence of soluble oxalate and its effects on calcium availability and metabolism. For insects both preference and toxicity may play a significant role. Some plants may themselves show wilting symptoms when fed with oxalate, and there are examples of plant pathogens where isolates inducing oxalate accumulation are more virulent than other isolates (Noyes and Hancock, 1981; Punja et al., 1985).

**4.2. pH Regulation and Osmoregulation.** Cellular processes are pH sensitive, and it is essential that mechanisms for pH regulation in subcellular compartments are developed. Nitrate reduction and excess cation over anion uptake are examples of important processes that consume  $H^+$  ions, while organic acids can serve as a source of  $H^+$  ions (Raven and Smith, 1974, 1976). A homeostatic relationship between nitrate uptake/reduction and organic anion accumulation has long been recognized (Ergle and Eaton, 1947; Figure 3). Malate is the dominant anion in some plants (Kirkby and Knight, 1977; Naik and Nicholas, 1986). Raven and Smith (1976) proposed that oxalate has the same function with the further advantage that deposition of calcium oxalate in the vacuole will not influence the osmotic potential of the cell. A metabolically plausible pH-stat involving oxalate was suggested by Davies and Asker (1983). Oxalate has also been found to be an important counterion to inorganic cations such as sodium and potassium (Osmond, 1963, 1967; Austenfeld and Leder, 1978; Karimi and Ungar, 1986). Soluble salts of oxalate may play an important role in turgor generation and osmoregulation.

In a number of plant species, nitrate as the source of nitrogen as opposed to ammonium lead to a higher oxalate content of the plant (Clark, 1936; Gilbert et al., 1951; Scharrer and Jung, 1954; Grütz, 1956; Becker, 1964; Ehrendorfer, 1964a; Joy, 1964; Knauer and Simon, 1968; Kick and Massen, 1973; Kpodar et al., 1978). Some representative data are summarized in Table I. Higher oxalate levels with ammonium as the nitrogen source were, however, found by Crombie (1954) and Olsen (1939). It should be kept in mind that the source of nitrogen has a great effect on aspects of plant metabolism other than nitrate reduction. Meeuse and Campbell (1959) identified nitrate as a potent inhibitor of oxalic acid oxidase in *Beta vulgaris*. If photorespiratory glyoxylate would be a precursor of oxalate, the supply of nitrogen could influence whether glyoxylate will accumulate and be further oxidized or be transaminated to glycine (Kpodar et al., 1978). Moreover,

**Table I.** Effect of the Source of Nitrogen on Oxalate Content (as Anhydrous Oxalic Acid) and Fresh Weight Yield of Plant Tissues

tissue	oxalate content, % DM		rel yield $NH_4$ nutr, % of $NO_3$ -N	reference
	$NO_3$ -N	$NH_4$ -N		
tomato leaf + stem <sup>a</sup>	2.7	0.3	34	Clark, 1936
spinach leaf <sup>b</sup>	8.4	6.8	92	Becker, 1964
spinach leaf <sup>c</sup>	7.8	7.7	97	Becker, 1964
spinach leaf <sup>a</sup>	9.0	4.7	104	Ehrendorfer, 1964a

<sup>a</sup> Sand or solution cultures. <sup>b</sup> Field trial, spring. <sup>c</sup> Field trial, autumn.

the source of nitrogen appears to affect the aggregation state of glycolate oxidase, which may have physiological significance (Emes and Erismann, 1982).

It should be emphasized that relatively high levels of oxalate are found in oxalate-accumulating plants even when ammonium is the sole source of nitrogen. Also, Roughan and Warrington (1976) observed that oxalate accumulation continued in older tissue of *Setaria sphacelata* after nitrate reduction activity had ceased. However unclear the picture remains, the role of oxalate accumulation in pH regulation and osmoregulation of the cell appears quite real.

**4.3. Precipitation of Calcium Ions.** Two explanations for high levels of calcium oxalate in plants have been offered:

1. Oxalate synthesis has evolved as a means of neutralizing the negative effects of excessive calcium uptake [de Vries, 1881, see Olsen (1939)].

2. Oxalate, which is a toxic product for the plant, is made harmless by precipitation as the highly insoluble calcium salt [Schimper, 1888, see Olsen (1939)].

Whether either or both explanations is correct is difficult to prove experimentally. Kinzel's (1963) observation that oxalate-accumulating plants tend to occur on noncalcareous soils can be interpreted as an indication that oxalate is not produced for decalcification primarily. Examples can also be found where oxalate production induces calcium deficiency in the plant (Brumagen and Hiatt, 1966; Lötsch and Kinzel, 1971). On the other hand, the existence of species with very high levels of water-soluble oxalate proves that oxalate can be efficiently "detoxified" without calcium oxalate precipitation in some species.

A positive correlation between calcium content of the growth medium and oxalate formation (Olsen, 1939; Gilbert et al., 1951; Scharrer and Jung, 1954; Grütz, 1956; Rasmussen and Smith, 1961; Bornkamm, 1965; Zindler-Frank, 1975) appears to be related to processes involving chemical regulation: Uptake of calcium leads to calcium oxalate precipitation, altering the equilibrium of oxalate synthesis and/or breakdown in the plant. In some investigations on plants with a high level of water-soluble oxalates (Chenopodiaceae and Polygonaceae), little or no correlation was found between calcium in the medium or plant and oxalate in the plant (Wittwer et al., 1946, 1947; Osmond, 1967).

It must be pointed out that calcium oxalate formation is not a simple precipitation phenomenon. Before the calcium oxalate crystals are formed, specialized membranes develop within the cell (Franceschi and Horner, 1980). Often cells that produce crystals are formed in very specific regions of the plant and appear modified in morphology and cytology so that they are specialized for this particular function. The number of crystal-forming cells produced is related to calcium availability (Frank, 1972; Franceschi

and Horner, 1979; Van Balen et al., 1980). It is clear that the plant controls the precipitation process and so exerts some control over soluble and insoluble levels of calcium and oxalate.

## 5. ANTINUTRITIVE EFFECTS AND TOXICITY OF DIETARY OXALATE

### 5.1. Absorption and Metabolism of Dietary Oxalate.

Absorption of oxalate in the gastrointestinal tract is an important factor for assessing the health risk associated with high oxalate levels in food or animal feeds. The reported percentage of dietary oxalate absorbed varies from 1.3 to 42% depending on the form of the oxalate loads, general diet, and the individual [cf. Libert et al. (1987)]. Calcium oxalate is frequently regarded as unavailable for uptake by the human digestive tract. The recent results of Prenen et al. (1984) give, however, a clear indication that oxalate in this form may be absorbed. The level of hydration, purity, and size of crystals are most likely of importance for bioavailability (section 2). In ruminants and horses, a substantial portion of calcium oxalate in the feed is dissolved during digestion (Negi, 1971; Blaney et al., 1982; Blackall and McKenzie in Blaney et al., 1982).

It is not clear whether the process of oxalate uptake is active or passive. Conflicting conclusions are drawn by Binder (1974) and Freel et al. (1980). Reduction of oxalate levels in a specific food matrix did not seem to increase relative absorption (Libert et al., 1987). Earlier studies have concluded that oxalic acid, once taken up in the digestive system, is not further metabolized but normally excreted in the urine [see Hodgkinson (1977)].

Particularly in ruminants, but also in other animals, ingested oxalate is partially degraded in the digestive system by microorganisms (Talapatra et al., 1948a; Brune and Bredehorn, 1961; Justice, 1985). An anaerobic bacterium, *Oxalobacter formigenes*, isolated from human faeces degrades oxalic acid quite efficiently (Allison et al., 1986). Allison and Cook (1981) and others have shown that the rate of degradation increases if oxalate-rich feed is given regularly, probably through adaptation of the intestinal microflora.

**5.2. Chronic Deficiency of Calcium and Other Minerals.** Dietary calcium is less available for absorption when present as calcium oxalate. A low ratio of calcium vs. oxalate in the diet may thus cause chronic calcium deficiency owing to the complexation of calcium by oxalate before or in connection with ingestion [review Kelsay (1985)]. Human diets based on oxalate-rich vegetables and with a low intake of calcium-rich products, like milk and cheese, and vitamin D may lead to calcium deficiency.

Hodgkinson (1977) claims that in a typical English diet (molar ratio calcium vs. oxalate approximately 17) oxalate is not an important factor for the availability of calcium. In contrast, in a specific regional Indian diet Sing et al. (1972) found a seasonal ratio as low as 0.2, which certainly could lead to calcium deficiency.

Mineral nutrition of livestock is similarly disturbed by high oxalate levels in the feed [see Swartzman et al. (1978)]. The situation is less serious for ruminants, as they can recover some calcium from the dietary calcium oxalate (Blaney et al., 1982). Although dietary calcium oxalate is partly dissolved in horses, dissolution takes place in the large intestine whereas the bulk of dietary calcium is absorbed earlier in the upper small intestine (Blaney et al., 1981). Chronic calcium deficiency can be overcome by a calcium supplement, even if the oxalate load is continued.

In addition to calcium, absorption of other minerals such as iron (Gillooly et al., 1983), magnesium (Wittwer et al.,

1947), and copper (Kelsay, 1981) are sometimes claimed to be negatively affected by the presence of dietary oxalate. In a study by Van Campen and Welch (1980) iron absorption in rats was unaffected or possibly positively affected by dietary oxalate.

**5.3. Acute Toxic Effects.** The acute toxic effects of excessive oxalic acid intake can be divided into three overlapping phases (Hodgkinson, 1977): 1. a local corrosive action in the mouth or gastrointestinal tract (corrosive gastroenteritis with vomiting and diarrhea); 2. effects on the nervous system (muscle cramp, tetany), cardiovascular collapse, lowered blood coagulability, and other symptoms caused by low levels of calcium in the body fluids; 3. renal insufficiency caused by a direct toxic action of oxalic acid or deposition of calcium oxalate. The lethal dose of oxalic acid for man varies from 2 to 30 g depending on a variety of factors.

**5.4. Calcium Oxalate Stones.** The majority of the urinary stones formed in man are calcium oxalate stones (Hodgkinson, 1977). Elevated oxalate concentration in the urine (hyperoxaluria) is one of the initiating factors for calcium oxalate stone formation (Robertson and Nordin, 1969). When high-oxalate food is included in the diet, a significant part of the renal oxalate comes from dietary oxalate, especially for specific risk groups (Chadwick et al., 1973; Hodgkinson, 1978). If oxalate-rich food is not included in the diet, endogenously produced oxalate constitutes the greater part of urinary oxalate (Hodgkinson, 1977).

**5.5. Arthritis.** There is a possible connection between symptoms of arthritis and dietary oxalate. Grott (1942) observed an association of high oxalate levels in the blood and arthritis in some cases. Another indication is the observation by James (1970) that cattle grazing on *Halimolobos* acquired locomotor disturbances. Although the evidence is tenuous, it is perhaps worthy of further study.

**5.6. Penetration of Skin and Membranes.** Some plants form calcium oxalate crystals, which are involved in irritation and burning sensations due to mechanical penetration. *Colocasia* and *Dieffenbachia* are two examples (section 6). A highly developed example is seen in *Tragia*, where a large calcium oxalate crystal is part of the mechanism in the stinging trichomes (Thurston, 1976). In other species there appears to be a relationship between size and shape of crystal and degree of irritation (Sakai et al., 1984).

**5.7. Oxalate and Toxic Metals.** Crystalline forms of oxalate commonly occur in many crop and weed species, often in extremely large quantities. Van Balen et al. (1980) and Franceschi and Schueren (1986) have demonstrated that cadmium and strontium incorporate into plant calcium oxalate crystals. Strontium accumulated to concentrations several times in excess of that present in the nutrient solution. This process occurred exclusively in calcium oxalate crystal forming cells (Franceschi and Schueren, 1986). The toxic nature of Cd is well-known and <sup>90</sup>Sr, a common, long-lived isotope formed by industrial nuclear processes and certain types of nuclear explosions, is considered to be a highly toxic radioisotope due to its rapid incorporation into bone with subsequent irradiation of the marrow. The possibility of soil contamination by these elements is considerable, and health risks posed by accumulation of such metal ions into plant oxalate crystals should not be ignored.

## 6. OCCURRENCE IN CROP PLANTS

According to Zindler-Frank (1976) the majority of the species of only 11 out of 93 orders of higher plants do not store oxalate. In three of the orders accumulation of

water-soluble oxalate is a prominent feature: polygonales, caryophyllales (including chenopodiales), and begoniales (Zindler-Frank, 1976). A brief discussion of some important oxalate-accumulating crop plants and weeds is given below, and examples of oxalate levels are presented in Table II. For a more thorough account of oxalate-accumulating weeds in Australia see Mathams and Sutherland (1952).

**Amaranthaceae.** *Alternanthera sessilis* (racaba) is locally cultivated as a leaf vegetable in the tropics. *Amaranthus spp.* (amaranth) are used as leaf vegetables throughout the temperate zone and the tropics. The genus also includes weedy species such as *Amaranthus retroflexus*, which probably has caused lethal poisonings of pigs (Osweiler et al., 1969). Deutsch (1978) recorded a large genotype-environment interaction for oxalate content in several *Amaranthus* species, suggesting a low probability for selection of low-oxalate lines. Both *Alternanthera* and *Amaranthus* include species with  $C_4$  photosynthesis.

**Apiaceae (Umbelliferae).** *Coriandrum sativum* L. (coriander) is mostly cultivated for the seeds, but the young leaves are locally used for soups, e.g. in India.

**Araceae.** *Colocasia esculenta* (L.) Schott (taro, dash-reen) is a perennial herb cultivated throughout the tropics and subtropics. The calcium oxalate content of the edible corm has been associated with irritation in the mouth when eating it raw, but the symptom has also been attributed to other agents (Osisiogu et al., 1974). Genetic variation of oxalate content was reported by Asokan et al. (1980). The leaves of some cultivars are used for food or feed, as are leaves of some closely related *Xanthosoma spp.* *Dieffenbachia spp.* are important as ornamental plants but are toxic when (accidentally) ingested. The toxicity has earlier been attributed to the content of calcium oxalate but may at least partly be caused by other agents (Pohl, 1964). Genetic variation of oxalate content has been recorded (Zettler and Rhodes, 1975).

**Basellaceae.** *Basella rubra* L. (syn. *Basella alba* L.) (basella, Ceylon spinach) is used as a garden green vegetable in the tropics [see Bendana-Brown and Lim (1959)].

**Chenopodiaceae.** *Atriplex* species (salt bush) are sometimes grazed in semiarid regions. Selection of low-oxalate *Atriplex halimus* L. populations has been attempted by Ellern et al. (1974). *Atriplex hortensis* L. (garden orach) is cultivated as a leaf vegetable. Many *Atriplex* species have  $C_4$  photosynthesis. *Beta vulgaris* L. (chard, mangold, sugarbeet, fodderbeet, beetroot, table beet, etc.) is cultivated for the leaves or the root in several forms and is used for food or feed all over the world. Oxalate levels are considerably lower in the beets (roots) than in the leaves. Leaf oxalate increases with the development of the plant (Sing and Saxena, 1972). *Chenopodium spp.* (chenopods) are occasionally used as leaf vegetables but are also widely distributed weeds. The chenopods contain very high levels of water-soluble oxalate. Total oxalate increases with developmental stage (Sing and Saxena, 1972). *Halogeton glomeratus* (M. Bieb.) C. A. Mey (halogeton) is an annual weed with  $C_4$  photosynthesis growing in cold, saline, and arid regions. In the western United States the high oxalate content has caused frequent poisonings resulting in death of sheep (James et al., 1968) and nonlethal injuries of cattle (James, 1970; section 5.5) and seriously reduces the acreage suitable for grazing. Total and water-soluble oxalate of leaves increase over the season (Williams, 1960). *Spinacia oleracea* L. (spinach) is a leaf vegetable widely distributed all over the world. Oxalate levels of spinach have been studied extensively, and various sources of variation are accounted for in sec-

tion 7 (Tables I and III). Genetic variation is low to moderately high (Doesburg and Zweede, 1948; Eheart and Massey, 1962; Yukura and Hongo, 1963; Kitchen et al., 1964; Srivastava et al., 1977; Handke et al., 1982). According to Kitchen et al. (1964) savoy cultivars of spinach are lower in oxalates than smooth and semisavoy.

**Convolvulaceae.** *Ipomoea batatas* (L.) Lam. (sweet potato) is a perennial root crop cultivated in the tropics and in some areas of the temperate zone. Leaves and tender shoots of *Ipomoea* species accumulate oxalate and are used as a green vegetable.

**Fabaceae (Leguminosae).** *Arachis hypogaea* L. (peanut) is cultivated in warm regions for the ripe seeds. *Dolichos uniflorus* Lam. (horse gram) is an important crop in Burma and the south of India. The dry seed is used as a boiled pulse. It is questionable whether its relatively low oxalate content is of any significance. Although *Medicago sativa* L. (lucerne, alfalfa) is one of the world's most important forage crops, its content of oxalate has attracted surprisingly little attention from agricultural scientists.

**Malvaceae.** The young leaves of *Hibiscus sabdariffa* L. (roselle) are sometimes used as a vegetable although the calyces and the fibers are the principal reason for cultivation. Roselle is cultivated in West Africa and India.

**Oxalidaceae.** *Averrhoa carambola* L. (carambola tree) is cultivated in Florida. The fruit is sold in small amounts on the fresh fruit market. There is considerable variation of water-soluble oxalate content between cultivars (Wilson et al., 1982). *Oxalis pes-caprae* L. (syn. *O. cernua*) (sour sob) is a widespread herb, which has caused a large number of deaths among sheep of Australian pastures (Smith, 1951). In the Mediterranean it is sometimes used for cattle feed (Maymone et al., 1962). Poisonings by *O. corniculata* L. have also been recorded (Mathams and Sutherland, 1952).

**Piperaceae.** *Piper betle* L. (betel) is cultivated in the Far East for its leaves, which are chewed together with the betel nut. To neutralize the effect of oxalate, slaked lime is often applied before consumption.

**Poaceae (Graminae).** *Cenchrus ciliaris* L. (buffel grass;  $C_4$  photosynthesis) is used for grazing or hay in Australia. Calcium deficiency in horses has been attributed to extensive feeding on this grass and setaria (see below) (Blaney et al., 1982). *Oryza sativa* L. (rice, paddy straw) is not only a grain crop; the straw is also used for feed. Total and water-soluble oxalate concentration decrease with ontogenetic stage of the plant (Hans et al., 1984). *Panicum maximum* Jacq. (Guinea grass;  $C_4$  photosynthesis) is an important fodder grass as well as a weed in the warm humid tropics. *Paspalum dilatatum* Poir. (Dallis grass,  $C_4$  photosynthesis) is an important fodder grass in the southern parts of the United States and Africa. *Pennisetum americanum* (L.) Leeke (syn. *P. glaucum* and *P. typhoides*) (pearl millet, bulrush millet, bajra) is an annual crop mostly grown for the seed, but frequently also as a fodder grass. It has  $C_4$  photosynthesis and is cultivated in Africa and India. Genetic variation has been indicated (Goswami et al., 1970; Gupta and Batra, 1980). *P. purpureum* Schum. (Napier grass;  $C_4$  photosynthesis) is a perennial fodder grass in tropical regions. Interspecific hybridization with *P. americanum* as a means to decrease oxalate levels was evaluated by Sidhu and Gupta (1974). Varieties of the *Setaria sphacelata* complex (setaria;  $C_4$  photosynthesis) are used as tropical and subtropical forage crops. Lethal poisonings of cattle were reported by Seawright et al. (1970). High levels of urea fertilization increased oxalate accumulation while the oxalate levels decreased with ontogenetic stage (Jones and Ford, 1972).

**Polygonaceae.** *Rheum spp.* (rhubarb) is a perennial crop in temperate regions, mostly cultivated in home gardens of Europe and the United States. The petioles are sour and used in pies, jam, etc. Lethal poisoning after excessive ingestion of rhubarb leaves has, by some authors, been attributed to high oxalate levels (Tallqvist and Väänänen, 1960), but the content of various anthraquinone derivatives may play a significant role (Fassett, 1973). The blades contain less oxalate than the petioles, and the upper part of the petiole less than the lower part (Libert, 1987a). Total oxalate levels increase and water-soluble oxalate decreases with ontogenetic stage of the individual leaf. However, petioles of established plants accumulate less oxalate than seedlings or recently cloned plants (Libert, 1987a). Varietal differences of oxalate content are considerable, and growth vigor is positively related to low oxalate levels and high malate levels (Libert, 1987a,b). Forced rhubarb accumulate less total but more water-soluble oxalate than field-cultivated rhubarb (Kmieciak, 1974a,b; Libert, 1987a). *Rumex acetosa* L. (sorrel) is occasionally used as a leaf vegetable. Its occurrence on pastures has caused poisoning of sheep (Mathams and Sutherland, 1952).

**Portulacaceae.** *Portulaca oleracea* L. (purslane, pigweed;  $C_4$  photosynthesis) is a leaf vegetable cultivated in the northern hemisphere. It is also a widely distributed weed. Purslane contains high levels of water-soluble oxalate and is believed to have caused severe oxalate poisoning of grazing cattle and sheep in Australia (Mathams and Sutherland, 1952). Oxalate levels increase with ontogenetic stage (Sing and Saxena, 1972). *Talinum spp.* are widely used as green vegetables in the tropics.

**Solanaceae.** Pepper leaves (*Capsicum spp.*) are sometimes used as a vegetable and accumulate relatively high oxalate levels [see Bendana-Brown and Lim (1959)].

**Sterculiaceae.** *Theobroma cacao* L. (cacao) is cultivated in the tropics, the beans being the main source of cacao.

**Tetragoniaceae.** *Tetragonia tetragonioides* (Pall.) Kuntze. (New Zealand spinach) is widely cultivated in subtropic climates. The leaves are eaten as a vegetable.

**Theaceae.** *Camellia sinensis* (L.) Kuntze. (tea) is mostly cultivated in Asia. Tea is the main source of dietary oxalate in England (Hodgkinson, 1977). Wight (1959) proposed the use of calcium oxalate crystal frequency in the leaf petioles as a taxonomic character.

**Tiliaceae.** *Corchorus olitorius* L. (Jew's mallow, jute mallow) is cultivated in the tropics as a leaf vegetable. Other *Corchorus spp.* are occasionally used for the same purpose.

## 7. MEASURES TO REDUCE LEVELS OR TOXIC EFFECTS OF OXALATE

**7.1. Plant Nutrients.** Fertilization practice will alter oxalate content of certain crops. Interactions between mineral nutrients, soil, species, etc., can be complex, and interpretations of fertilization experiments must be made with caution. An example of how differential fertilization of nitrogen, phosphorus, and potassium affects oxalate levels in spinach is presented in Table III.

Ammonium vs. nitrate as sources of N has been discussed above (section 4.2, Table I). Although ammonium N is primarily associated with lower oxalate levels in the plant compared to nitrate N, little is gained in practice. First, ammonium fertilization is sometimes associated with depressed yield. Second, even if ammonium N is applied, a proportion of the ammonium is normally oxidized to nitrate before uptake (Table I). The effect of increased nitrogen fertilization has been studied in spinach and beet.

Increasing levels of nitrate fertilization result in an increased oxalate accumulation, while higher levels of ammonium scarcely influence accumulation (Scharrer and Jung, 1954; Becker, 1964; Ehrendorfer, 1964a; Nicolaisen and Kuhlen, 1967). Grütz (1956) found decreased oxalate accumulation in spinach leaves with increased ammonium nitrate fertilization.

The effect of phosphorus is less obvious, but in most studies increased levels of phosphate decreased oxalate accumulation (Grütz, 1956; Ehrendorfer, 1961, 1964b). The influence of potassium levels on oxalate accumulation has been attributed to the interference of potassium with calcium uptake. Increased levels of potassium (and sodium) usually lead to a decreased total oxalate but an increased water-soluble oxalate concentration (Karimi and Ungar, 1986). At very high soil potassium levels or when calcium levels are low and are not interfering with potassium uptake, oxalate accumulation will, however, increase (Scharrer and Jung, 1954; Grütz, 1956; Ehrendorfer, 1964b).

Effects of calcium in the growth media are described in section 4.3. High levels of calcium enhance oxalate accumulation in most cases. Calcareous soils also tend to have a basic soil reaction, promoting nitrification that may further accentuate oxalate accumulation. High calcium levels in the soil tend to decrease the proportion of water-soluble oxalate (Scharrer and Jung, 1954). The positive effect of boron fertilization on calcium uptake is also reflected in higher oxalate accumulation (Scharrer and Jung, 1954).

In conclusion, to avoid extreme levels of oxalate accumulation calcium nitrate fertilization should be avoided, and phosphate nutrition should be kept on a high and potassium on a moderately high level.

**7.2. Harvest Practices.** The time of the season for harvest is not a simple factor to consider. Ontogenetic stage, light conditions, temperature, and nutrient status will all influence oxalate production and accumulation. Metabolism of oxalate in the plant appears to be slow (section 3.4), and oxalate content on a plant basis increases generally with age. The growth rate may influence whether the oxalate concentration of the plant increases or decreases. The relationship of ontogenetic stage to oxalate concentration in the plant tissue is complex and may even differ between studies on the same crop (see section 6 for differences between crops). For example, total and water-soluble oxalate concentration of spinach has been found to decrease (Sengbusch et al., 1965; Bengtsson et al., 1966) as well as increase (Sing and Saxena, 1972) with ontogenetic stage. A plausible generalization might be the following: With a low rate of growth, oxalate concentration of the plant tissues increases, and with a high rate of growth, it decreases. If this is true, then the harvest should take place after a period of vigorous growth in order to minimize total oxalate levels.

The time of the season (at the same ontogenetic stage) appears to be insignificant for oxalate accumulation in rhubarb (Libert, 1987a), but spinach exhibits increased total and water-soluble oxalate levels with increasing day length (Sengbusch et al., 1965). As a rule green vegetables contain less oxalate in the petiole (stalk) than in the leaf blade (Table II). Rhubarb is a notable exception (Libert, 1987a).

**7.3. Plant Breeding.** Some effort has gone into recording genetic variation of oxalate content between cultivars, breeding lines, etc. (section 6). From Table II it can be concluded that genetic variation seems to be of potential importance for crops like spinach, rhubarb, and the car-

Table II. Oxalate Content of Some Crop Plants and Important Weeds

species, plant part	oxalate content <sup>a</sup>		source of variation <sup>b</sup>	reference
	water-sol	total		
Amaranthaceae				
<i>Alternanthera sessilis</i> , leaf		6.8		Hoover and Karunairatnam, 1945
<i>Amaranthus</i> spp., leaf		6		Deutsch, 1978
<i>A. gangeticus</i> , leaf	4.4	12.8		Srivastava and Krishnan, 1959
<i>A. gangeticus</i> , leaf	3.3-5.7	8.6-11.9	O	Sing and Saxena, 1972
<i>A. gangeticus</i> , leaf	0.7 <sup>c</sup>	1.9 <sup>c</sup>		Anantha Samy et al., 1960
<i>A. gangeticus</i> , leaf		6.0		Hoover and Karunairatnam, 1945
<i>A. polygonoides</i> , leaf		11.2		Hoover and Karunairatnam, 1945
Apiaceae (Umbelliferae)				
<i>Coriandrum sativum</i> , leaf	0.8 <sup>c</sup>	1.3 <sup>c</sup>		Anantha Samy et al., 1960
Araceae				
<i>Colocasia antiquorum</i>				
mature leaf	1.0	7.0		Srivastava and Krishnan, 1959
main tuber	0.2	2.2		Srivastava and Krishnan, 1959
<i>C. violacea</i>				
mature leaf	0.9	1.4		
tuber	0.7	2.1		
<i>Dieffenbachia picta</i> Schott., leaf		3.9-6.4	G	Zettler and Rhodes, 1975
Chenopodiaceae				
<i>Atriplex halimus</i> , leaf	1.0-3.0	2.5-6.4		Ellern et al., 1974
<i>A. hortensis</i> , leaf	0.66-0.75 <sup>c</sup>	1.2-1.6 <sup>c</sup>	M	Halen, 1985
<i>A. muelleri</i> , aerial part	4.0-4.5	6.2-7.0		Mathams and Sutherland, 1952
<i>A. semibaccata</i> , aerial part	0.6-7.4	1.9-10.2		Mathams and Sutherland, 1952
<i>Beta vulgaris</i> , tops		5.9-8.1		Eriksson, 1955
<i>B. vulgaris</i> , tops		4.0-5.8	G, M	Wittwer et al., 1947
<i>B. vulgaris</i> , leaf	3.5-7.2	7.8-14.1	O	Sing and Saxena, 1972
<i>B. vulgaris</i> , leaf	0.6-1.5	2.3-4.6	G	Baker and Eden, 1954
<i>B. vulgaris</i> , leaf	0.9-3.6	2.3-12.3	S, G	Baker and Eden, 1954
<i>B. vulgaris</i> , leaf	10.2	12.9		Srivastava and Krishnan, 1959 <sup>d</sup>
<i>B. vulgaris</i> , beet	0.4	0.7		Srivastava and Krishnan, 1959 <sup>d</sup>
<i>B. vulgaris</i> , beetroot	0.03-0.09 <sup>c</sup>	0.06-0.12 <sup>c</sup>		Herrmann, 1972
<i>B. vulgaris</i> , beetroot		0.68 <sup>c</sup>		Kasidas and Rose, 1980
<i>Chenopodium album</i> , leaf	0.9 <sup>c</sup>	1.4 <sup>c</sup>		Anantha Samy et al., 1960
<i>C. album</i> , leaf	8.1-10.5	9.3-18.5	O	Sing and Saxena, 1972
<i>C. album</i> , leaf	0.5 <sup>c</sup>	2.6 <sup>c</sup>		Halen, 1985
<i>C. murale</i> , leaf	8.2-14.5	9.9-21.0	O	Sing and Saxena, 1972
<i>Halogeton glomeratus</i> , leaf	17.2-34.6	21.8-38.7	S	Williams, 1960
<i>Spinacia oleracea</i> , leaf		8.7-10.5	G	Eheart and Massey, 1962
<i>S. oleracea</i> , leaf		6.5-15.7	G	Yukura and Hongo, 1963
<i>S. oleracea</i> , leaf		5.4-9.8	G	Kitchen et al., 1964
<i>S. oleracea</i> , leaf	1.7-4.4	4.4-6.5	O	Sengbusch et al., 1965
<i>S. oleracea</i> , leaf blade	3.6-5.4	8.7-10.0		Sengbusch, et al., 1965 <sup>d</sup>
<i>S. oleracea</i> , petiole	1.2-2.8	1.5-4.1		Sengbusch et al., 1965 <sup>d</sup>
<i>S. oleracea</i> , leaf	3.4-8.0	6.5-12.3	O	Sing and Saxena, 1972
Convolvulaceae				
<i>Ipomoea aquatica</i> , leaf		2.2		Hoover and Karunairatnam, 1945
Fabaceae (Leguminosae)				
<i>Arachis hypogaea</i> , roasted peanut		0.19 <sup>c</sup>		Kasidas and Rose, 1980
<i>A. hypogaea</i> , raw peanut		0.22 <sup>c</sup>		Andrews and Viser, 1951
<i>Dolichos uniflorus</i> , seed		0.2-0.4		Pore, 1979
<i>Medicago sativa</i> , hay		0.96-1.10		Ward et al., 1982
<i>M. sativa</i> , hay		0.35		Talapatra et al., 1948b
Malvaceae				
<i>Hibiscus sabdariffa</i> , leaf	1.1 <sup>c</sup>	1.9 <sup>c</sup>		Anantha Samy et al., 1960
Oxalidaceae				
<i>Averrhoa carambola</i> , fruit	0.08-0.73 <sup>c</sup>		G	Wilson et al., 1982
<i>Oxalis cernua</i> , whole plant	3.7-14.9	5.9-16.6	O	Maymone et al., 1962
<i>O. corniculata</i> , aerial part	4.1	7.0		Mathams and Sutherland, 1952
Piperaceae				
<i>Piper betle</i> , leaf	1.2 <sup>c</sup>	1.4 <sup>c</sup>		Anantha Samy et al., 1960
Poaceae (Graminae)				
<i>Cenchrus ciliaris</i> , hay	1.1	1.8		Blaney et al., 1982
<i>C. ciliaris</i> , hay	3.5-4.3		G	Silcock and Smith, 1983
<i>Oryza sativa</i> , paddy straw	1.0-2.5	1.6-4.0	O	Hans et al., 1984
<i>O. sativa</i> , paddy straw		1.7-2.4		Talapatra et al., 1948b
<i>O. sativa</i> , paddy straw	1.3	1.4		Negi, 1971
<i>Panicum maximum</i> , hay		2.2		Talapatra et al., 1948b
<i>P. maximum</i> , hay		1.0-2.3	G	Garcia-Rivera and Morris, 1955
<i>Paspalum dilatatum</i> , hay		2.8		Talapatra et al., 1948b

Table II (Continued)

species, plant part	oxalate content <sup>a</sup>		source of variation <sup>b</sup>	reference
	water-sol	total		
<i>Pennisetum americanum</i>		1.2-2.2	G	Goswami et al., 1970
<i>P. americanum</i> , hay		1.0-2.8	G	Sing et al., 1980
<i>P. americanum</i> , hay	0.3-1.4	1.5-2.3		Lal et al., 1966 <sup>d</sup>
<i>P. americanum</i> × <i>purp</i> , hay	0.2-2.3	1.3-3.0		Lal et al., 1966 <sup>d</sup>
<i>P. purpureum</i> , hay	2.4-2.6	3.3-3.6		Lal et al., 1966 <sup>d</sup>
<i>P. purpureum</i> , hay		3.3		Talapatra et al., 1948b
<i>P. purpureum</i> , hay		2.5-2.6	G	Garcia-Rivera and Morris, 1955
<i>Setaria sphacelata</i> , hay		3.4-9.1		Roughan and Warrington, 1976
<i>S. sphacelata</i> , hay	1.0	1.3		Blaney et al., 1982
		Polygonaceae		
<i>Rheum</i> spp., petiole	1.8-5.7	4.7-9.6	G	Allison, 1966
<i>Rh. spp.</i> , petiole forced	4.2-8.4	5.0-9.4	G	Kmiecek, 1974a <sup>d</sup>
field cultivated	3.4-5.6	6.1-8.5	G	Kmiecek, 1974b <sup>d</sup>
<i>Rh. spp.</i> , petiole		3.4-9.5	G	Libert and Creed, 1985
<i>Rh. spp.</i> , petiole		1.8-21.6		Libert, 1987b
<i>Rumet acetosa</i> , leaf		5.8-12.9		Shivrina, 1961
<i>R. acetosa</i> , leaf	0.55 <sup>c</sup>	1.0 <sup>c</sup>		Halen, 1985
		Portulacaceae		
<i>Portulaca oleracea</i> , aerial part	4.3-9.4	3.5-13.3		Mathams and Sutherland, 1952
<i>P. oleracea</i> , leaf	7.2-10.3	9.9-17.0	O	Sing and Saxena, 1972
<i>P. oleracea</i> , leaf		9.7		Tabekhia, 1980
<i>P. oleracea</i> , young leaf	10.3			Silcock and Smith, 1983
<i>Talinum speciosa</i> , leaf		12.7		Hoover and Karunairatnam, 1945
<i>T. triangulare</i> , tops		24.8		Bendana-Brown and Lim, 1959
		Sterculiaceae		
<i>Theobroma cacao</i> , cocoa powder	0.6			Zaremski and Hodgkinson, 1962
<i>Th. cacao</i> , cocoa powder	0.3-0.4			Lagemann et al., 1985
<i>Th. cacao</i> , chocolate	0.06-0.12 <sup>c</sup>			Hodgkinson, 1977
<i>Th. cacao</i> , chocolate	0.12 <sup>c</sup>			Kasidas and Rose, 1980
		Tetragoniaceae		
<i>Tetragonia tetragonioides</i> , tops		6.7-7.6	M	Wittwer et al., 1947
<i>T. tetragonioides</i> , leaf		11.7		Kohman, 1939
<i>T. tetragonioides</i> , stalks		7.9		Kohman, 1939
		Theaceae		
<i>Camellia sinensis</i> , beverage	0.005-0.013 <sup>c</sup>			Zaremski and Hodgkinson, 1962
<i>C. sinensis</i> , beverage	0.005-0.026 <sup>c</sup>			Ohkawa, 1985
		Tiliaceae		
<i>Corchorus olitorius</i> , leaf	0.6	4.5		Oke, 1964
<i>C. olitorius</i> , leaf		2.9		Tabekhia, 1980

<sup>a</sup> Content usually as percent anhydrous oxalic acid per dry weight. <sup>b</sup> Sources of variation: O, ontogeny; S, time of the season; G, genotype, cultivar; M, mineral nutrition. <sup>c</sup> Percent anhydrous oxalic acid per fresh weight. <sup>d</sup> The same plants/genotypes are used; comparisons can be made.

Table III. Effects of Fertilization Levels on Relative Yield and Total Oxalate Content (as Anhydrous Oxalic Acid) of Spinach Leaves<sup>a</sup>

	P <sub>1</sub>		P <sub>2</sub>	
	oxalate, % DM	rel yield	oxalate, % DM	rel yield
N <sub>1</sub> K <sub>1</sub>	7.2	100	6.9	91
N <sub>1</sub> K <sub>2</sub>	6.8	124	4.9	110
N <sub>1</sub> K <sub>3</sub>	7.9	122	5.9	171
N <sub>2</sub> K <sub>1</sub>	9.7	214	6.0	238
N <sub>2</sub> K <sub>2</sub>	8.3	235	5.2	260
N <sub>2</sub> K <sub>3</sub>	8.9	263	5.1	320
N <sub>3</sub> K <sub>1</sub>	9.1	145	4.3	250
N <sub>3</sub> K <sub>2</sub>	9.2	180	5.3	365
N <sub>3</sub> K <sub>3</sub>	9.8	193	7.2	544

<sup>a</sup> Data from Ehrendorfer (1964b), sand cultures. Key: K<sub>1-3</sub> = 181, 461, 1208 mg of K (as K<sub>2</sub>SO<sub>4</sub>)/container; N<sub>1-3</sub> = 113, 450, 1800 mg of N (as NH<sub>4</sub>NO<sub>3</sub>)/container; P<sub>1-2</sub> = 35, 393 mg of P (as Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O)/container.

ambola fruit. However, to our knowledge a low oxalate content has in no case been an argument for using a cultivar in practice. Total oxalate levels in low-oxalate gen-

otypes or populations of high-oxalate species are not consistently lower than approximately 50% of normal levels, and the inheritance is quantitative with low *h*<sup>2</sup> estimates (Yukura and Hongo, 1963; Kitchen et al., 1964; Chawla and Gupta, 1982; Libert, 1987b). Major genes blocking oxalate accumulation have not been found.

Several examples of successful breeding to eliminate toxic constituents in the harvested parts of crop plants are recorded (glucosinolates and erucic acid in Brassicas, alkaloids in lupin, gossypol in cotton). Total elimination of oxalate in high-oxalate tissues is probably difficult to achieve. Oxalate is likely to be a substance produced in the primary metabolism of oxalate-accumulating plants and can be important for vital processes of the plant (section 4). Also, from the viewpoint of food or feed, oxalate has to be eliminated in vegetative parts of the plant, not in a highly restricted part such as the seed.

**7.4. Postharvest Treatment.** The most common approach to reduce the risk of high oxalate levels in food or feed is to add calcium under conditions favorable for calcium oxalate precipitation. Blanching (scalding in steam or water) is commonly used for spinach and other green vegetables. It results in leaching of water-soluble



oxalate and, to a lesser extent, calcium oxalate precipitation (Richter and Handke, 1973). Freeze-drying leads to sublimation of free oxalic acid (Kallistratos and Sengbusch, 1964). However, as the undissociated form rarely is found in plants, the effect is low in practice (Richter and Handke, 1973). In silage (e.g., from beet tops) oxalate is degraded to a considerable extent. Gorb and Maksakov (1959) tested the effect of additives such as hydrochloric acid and ammonium sulfate to promote microbial breakdown of oxalate. This led to metabolism of up to 56% of total oxalates when applied after harvest.

**7.5. Prevention of Oxalate-Induced Health Problems.** The obvious approach to decrease the risk of chronic calcium deficiency associated with consumption of oxalate-rich foods is to eat more calcium-rich food or feed. In cases of acute oxalate poisoning, ingestion or injection of calcium gluconate has been recommended as an antidote (Tallqvist and Väänänen, 1960). Ingestion of milk is an alternative. As has been mentioned (section 5.1) some animals, especially ruminants, adapt to increasing oxalate loads, probably through changes in their intestinal microflora. Finally, high water intake will lower calcium and oxalate concentration in the urine, decreasing the risk of renal calcium oxalate stone formation.

## 8. CONCLUSIONS

Our knowledge of the physiology of oxalate in plants is scant but improving. Biosynthetic pathways, regulation of accumulation, and function of oxalate in the overall metabolism of the plant are not established. It is unlikely that one general pattern of oxalate biochemistry exists in higher plants. Bornkamm (1969) and Zindler-Frank (1976) attempted to group plants as to synthesis, accumulation, and metabolism of oxalate, but there still are no unifying criteria. Results of Raven and coworkers (1982) indicate differences even between the related species *Spinacia oleracea* and *Chenopodium album*. Possibly, the study of genotypes differing in oxalate accumulation would be a worthwhile approach to gain further insight into the physiology of plant oxalates.

Oxalate content of crop plants and weeds constitutes in some cases a risk to the health of man and livestock. As to the toxicity of plant oxalates, an important but unsolved question concerns the absorption of oxalate from plant-derived calcium oxalate. Further efforts to decrease oxalate levels in harvested products would be of value. Both breeding programs and postharvest treatments, as well as controlled plant nutrition regimes, may prove effective in some crop species and should be selectively pursued. Agroecological effects of oxalate, such as effects on parasite resistance and plant growth, are largely unknown. Some information in this area is essential for the development of crops that are healthy and pest resistant and are of lower oxalate content for improved edibility and decreased health risk.

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## Effect of Maillard Reaction Products on Protein Digestion. Studies on Pure Compounds

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The effects of pure, synthetic compounds, similar to or identical with those formed in the Maillard reaction, on the activity of carboxypeptidase A (EC 3.4.12.2) and purified, intestinal brush border enzyme aminopeptidase N (EC 3.4.11.2) were studied. A number of furans and pyrroles inhibited carboxypeptidase A. The strongest inhibitors had a carboxylic acid as a substituent on the furan ring, and their effects were characterized as an induced substrate inhibition. A few competitive inhibitors of aminopeptidase N were found, the most effective being DL-2-formyl-5-(hydroxymethyl)pyrrole-1-norleucine. This compound also inhibited carboxypeptidase A. When fed to rats (3 mg in a 1-g portion), it reduced the plasma level of lysine coming from the diet.

There are a number of possible physiological implications of the Maillard reaction in food. The reaction may consume the essential amino acid lysine and lower the protein quality. This effect has been extensively studied and is probably the most important of the mechanisms impairing the quality of food proteins during processing (Mauron, 1981; Adrian, 1982). Besides the loss of protein quality, the Maillard reaction may produce compounds that possess other effects on biological systems. These include the production of antibacterial compounds (Einarsson et al., 1983; Anderson et al., 1984; Mester de Parajd et al., 1986), enzyme inhibitors (Öste et al., 1986; Gomyo and Miura, 1986), mutagenic compounds (Shibamoto, 1983; Jägerstad et al., 1986), and nephrotoxic compounds (von Wangenheim et al., 1984; Finot and Furniss, 1986).

In addition to the loss of the lysine, severe heating may reduce protein utilization further, obviously by mechanisms other than the loss of biologically available lysine (Valle-Riestra and Barnes, 1970; Ford and Shorrock, 1971; Pronczuk et al., 1973; Percival and Schneeman, 1979). In two preceding publications we examined the effect of a glucose-lysine reaction mixture per se on the utilization of dietary protein by rats and on the activities of gastrointestinal proteolytic enzymes (Öste and Sjödin, 1984; Öste et al., 1986). We observed that low molecular weight reaction compounds may affect the utilization of dietary protein, possibly due to enzyme inhibition.

In the present paper we report studies on the effect of some pure compounds on the activities on two enzymes, aminopeptidase N (EC 3.4.11.2) and carboxypeptidase A (EC 3.4.12.2). These enzymes were inhibited *in vitro* by the low molecular weight glucose-lysine reaction mixture (Öste et al., 1986). Aminopeptidase N is located in the

membrane of the epithelial cells of the small intestine in swine, rats, and humans (Sjöström et al., 1978; Kenny and Maroux, 1982; Tobey et al., 1985), constitutes a considerable portion of the membrane protein (Sjöström et al., 1978), and may play a central role in the final digestion of peptides escaping the action of pancreatic enzymes, as observed with rat small intestine specimens (Friedrich et al., 1980a,b).

The experiments were performed essentially with various substituted pyrroles and furans that are structurally related to compounds formed in the Maillard reaction. A few inhibitors of aminopeptidase N were found as well as a number of inhibitors of carboxypeptidase A. We also observed that a competitive inhibitor of aminopeptidase N affected the dietary protein utilization, when fed to rats.

### EXPERIMENTAL SECTION

**Materials.** Compounds (structures and names given in Table I) 1 (Miller et al., 1984), 2 (Olsson et al., 1978), 4 (Miller and Olsson, 1981), 5 (Silverstein et al., 1963), 8 (Clemon and Ramage, 1931), 13 (Olsson et al., 1977), 14 (Rapoport and Volcheck, 1956), and 20 (Finot and Mauron, 1969) were prepared according to the literature. Compound 7 was obtained by saponification of the ester ethyl 2,5-dimethylpyrrole-1-acetate prepared as described (Dann and Dimmling, 1953). Compound 12 was prepared from commercial 5-methylfurfural by the Cannizzaro reaction. The other compounds shown in Table I as well as all other chemicals used were commercial samples of analytical grade.

Each compound was dissolved in a small quantity of methanol prior to assaying its enzyme inhibitory effects. This addition of methanol did not affect the enzymes at the conditions of the assays.

**Enzyme Assay.** Sources of the enzymes and the assay procedures for carboxypeptidase A and aminopeptidase N were as described earlier (Öste et al., 1986). The effects of the compounds on enzyme activities were tested with 0.1-0.8 mM solutions of compound in the assay mixtures, to which the enzymes were added and immediately assayed.

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