Limestone and calcium in plants

Jean-Georges Barth[1]

Introduction

The term 'limestone' refers to sedimentary rocks comprising mostly calcium carbonate (at least 50% $CaCO_3$), a mineral compound of carbon. Apart from rocks, limestone is ubiquitous: it is found in low concentrations in water[2] and in the air in the form of aerosols (*Perakis & Sinkhorn* 2006, *Perakis et al.* 2013) where it is in trace amounts (*Benesch & Wilde* 1983, *Steiner* 10-6-1924 GA 327). Calcium also comes to the Earth in the form of meteoric dust. Amounts from this source are negligible. It occurs in the form of achondrites whose calcium content is variable, low or zero, but can exceed 5% (*Gounelle* 2017).

Calcium rocks include calcium carbonate, magnesium carbonate (dolomite) and magnesium carbonate (magnesite) (Fig. 1) (*Benesch & Wilde* 1983, *Bouillard* 2016). In his conception of chalk substance, Steiner included the alkaline sodium and potassium substances (*Steiner* 7-6-1924 GA 327), as well as phosphates (apatites) (*Steiner & Wegman* GA 27) and sulphates (gypsum) (*Steiner* 5-7-1924 GA 317). In the agriculture course (*Steiner* 13-6-1924 GA 327), Steiner implicitly broadens his conception of limestone to organic derivatives such as calcium oxalate (whewellite = $CaOx.H_2O$) ; this interpretation is confirmed by Benesch and Wilde (1983). Calcium oxalate is a rare form in the mineral world, but abundant in plants[3] (*Bouillard* 2016) (Boxes 1 and 2).

Figure 1. From left to right: examples of crystals of calcite, aragonite and dolomite (with siderite).

Limestone results principally from the activity of living organisms (biogenic rock) and constitutes an insoluble[4] and inert deposit that accumulates after their death. Its attack by acids releases free, mobile, water-soluble calcium into the medium which can be taken up by living systems and become active again. These two aspects alternate and present themselves as limits of an oscillation between life and death (*Bouillard* 2016, *Steiner* 17-2-1923 GA 349).

This work aims to highlight certain aspects of the essential nature of limestone based on the indications of both Steiner and university knowledge. It is mainly concerned with verifying the existence in plants of this oscillation between inert deposit and biological activity, with clarifying the role of the plant in the genesis of calcareous rocks, with researching the correspondence between the plant and edaphic factors of its biotope, and with giving a survey of the biological roles of limestone and calcium, especially in sexual reproduction, as well as in the management of abiotic and biotic stress.

---

[1] email : jean-georges.barth@gmail.com

[2] Potable water may contain up to 900 mg calcium per litre.

[3] In the 5th lecture of the agriculture course, Steiner recommended using the calcium that is found in oak bark. Calcium is found there only in the form of particularly insoluble calcium oxalate (solubility in water at 18°C = 6 mg/l), a property shared with calcium carbonate (limestone). As regards biological effects, in that lecture Steiner attributed the same properties to calcium and to limestone.

[4] The solubility of calcium carbonate in water is 14 mg/l at 20°C and a pressure of 1013 hPa.

Box 1– Limestone and calcium in the inorganic realm

Calcium is the 5th most abundant element in the Earth's crust after oxygen (46%), silicon (28%), aluminium (8%) and iron (5%). It represents about 3.5% of its constituents. It never occurs in the native form. It is sequestered in magmatic, plutonic or volcanic rocks and in sedimentary rocks (*Goguel* 1959, *Gehlig* 1994). Calcium becomes available only after it has been released into the medium by erosion.

Among the sedimentary rocks we find so-called biochemical rocks such as carbonates of calcium (limestone), phosphates of calcium (fluoro-, chloro- and hydroxapatite) and evaporites such as sulphate of calcium (gypsum). They represent 1.6% of the Earth's crust. Limestone ($CaCO_3$), in the form of calcite, alone represents about 1.5%. The distribution between 'silica' and 'limestone' seems to be balanced; in reality, the sedimentary rocks only occur on the surface, whereas deeper down, the Earth's crust (up to about 35 km thick at the continental level) is largely composed of silica and silicates (*Goguel* 1959, *Benesch & Wilde* 1983, *Gargaud et al.* 2009, *Bouillard* 2016, *Johnsen* 2016).

The crystal structures of limestone ($CaCO_3$) are very varied. A great number of forms are known which belong to three groups, namely calcite, aragonite and dolomite (Fig. 1). Calcite and aragonite are the main ones. The diversity of calcite's forms is explained by the proportions in greater or lesser amounts of impurities comprising the other alkaline earth elements (Mg, Sr, Ba) and metals of all kinds (Mn, Fe, Zn, Co, Pb, Cu, Al, Ni, V, Cr, Mo) (*Bouillard* 2016, *Johnsen* 2016).

From this brief description of 'limestone' we learn of its great chemical and morphological variability (*Benesch & Wilde* 1983).

Box 2– Basic chemistry of calcium and limestone (Huheey et al. 1997)

Calcium can be produced by electrolysis of calcium fluoride, but more recently by vacuum reduction of lime (CaO) with powdered aluminium.

Calcium is a powerful reducing agent, which is why it is used as a deoxidant in metallothermic reduction with calcium in the metallurgy of chrome, thorium, uranium and zirconium. In the presence of oxygen it burns with a yellow-orange flame forming lime (CaO) which, together with water, becomes slaked lime (calcium hydroxide, $Ca(OH)_2$). Its violent reaction with water immediately produces slaked lime.

Calcium hydroxide ($Ca(OH)_2$) is a base of intermediate strength which reacts with acids. With carbonic acid (H_2CO_3), a weak acid, it forms soluble calcium bicarbonate ($Ca(HCO_3)_2$) or insoluble carbonate ($CaCO_3$). At low temperatures, water in contact with air takes up CO_2 and the carbonic acid formed reacts with the carbonate and produces calcium bicarbonate according to the reactions:

$$\mathbf{CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-} \quad (1) \qquad \mathbf{CaCO_3 + H_2CO_3 \rightarrow Ca(HCO_3)_2} \quad (2)$$

The reverse reaction occurs if the air or water temperature increases:

$$\mathbf{Ca(HCO_3)_2 \rightarrow CO_2 + H_2O + CaCO_3} \quad (3)$$

Generally the carbonate and bicarbonate of calcium react with very many acids whose pKa is < 5.1 (HA), such as nitric acid (HNO_3) and sulphuric acid (H_2SO_4).

$$\mathbf{Ca(HCO_3)_2 + HA \rightarrow CaA + CO_2 + H_2O} \quad (4a) \qquad \mathbf{CaCO_3 + HA \rightarrow CaA + CO_2 + H_2O} \quad (4b)$$

The intense reactivity with oxygen, water and acids constitute aspects that Steiner called the craving nature of calcium and limestone (*Steiner* 11-6-1924 GA 327).

Flora on calcareous soils

Calcicole and calcifuge plants

The elements of the environment create very diverse habitats that plants can occupy according to their ecological amplitude, so that it is often difficult to observe a clear dividing line between calcicolous and calciferous or basophilic and acidophilic species. The geography of habitats, edaphic factors (parent rock, acidity, base richness, type of humus, porosity, aeration, etc.), the biological activity of soils (bacteria, fungi, microfauna, very diverse vertebrates and invertebrates), climatic conditions (water, rainfall, drought, heat, altitude), the presence of other plant species (competition) and the consequences of human activities (livestock farming, chemical fertilizers, acid rain, pollution) are all parameters influencing plant habitats.

However, some plants only inhabit soils of siliceous and acidic substrates or have an exclusive affinity for soils of calcareous substrates. We note that towards the limits of the geographical or altitudinal limits of the area of distribution of the species, their preference for a given type of soil is often clearer[5] (*Salisbury* 1920). It is the same at the initial stages of the cycle of the plant (germination, seedling) where the chemical conditions are decisive in relation to the other influences. Thus, the seeds of acidophilic plants show a poor rate of germination on calcareous soil and reciprocally for the seeds of calcicole plants (*Simpson* 1938, *Rorison* 1960, *Jefferies & Willis* 1964, *Jefferies & Willis* 1964a, *Wenk & Dawson* 2007).

Strictly calcifuge plants can grow in soil in which the calcium content is dominant compared with that of other essential minerals provided that the total concentration of mineral nutrients is low. These plants cannot regulate the absorption of the calcium they take from the soil in preference to other cations. On the other hand, strictly calcicole plants (fig. 3; for example, *Origanum vulgare*, Lamiaceae) prefer soils poor in nutrients where they can find calcium available in large amounts and are able to control calcium absorption. It follows that their tissue content of calcium is of the same order of magnitude as that of calcifuge plants. Consequently, what is attributed to a silicicole character is in fact an expression of a preference for soils poor in calcium, as are acidic soils on a siliceous substrate (*Jefferies & Willis* 1964, *Jefferies & Willis* 1964a, *Clarkson* 1965, *Bahr et al.* 2012, *Labidi et al.* 2012, *McCormick & Gibble* 2014).

Finally, in the natural environment, very similar species or subspecies are distinguished by their marked preference for limestone or silica: these species are termed ecological vicariants. Thus, *Gentiana acaulis* (Trumpet Gentian), an acidophilic species, is the vicarious of *G. clusii*, a calcicole. It is the same with *Achillea erba-rotta* subsp. *moschata* and *Achillea atrata* or *Rhododendron ferruginum* and *R. hirsutum*, the first of each pair being acidophilic, the second calcicole.

A few examples described below illustrate the diversity of edaphic characteristics of plant habitats (Box 3) (Salisbury 1920 ; Simpson 1938, Rorison 1960, Jefferies & Willis 1964, Jefferies & Willis 1964a ; Clarkson 1965 ; Rameau 1993 ; Wenk & Dawson 2007 ; Rameau et al. 2008, Ducerf 2008 ; Bahr et al. 2012 ; Labidi et al. 2012 ; McCormick & Gibble 2014 ; Ducerf 2015 et 2017 ; Bothe 2015).

Limestone substrates and soils : generalities

Usually nothing grows on limestone rocks. This is in contrast to siliceous rocks on which can be found algae, mosses and lichens. Limestone rock is hostile to life (Fig. 2A and 2D).

Carbonic acid contained in snow or rainwater slowly erodes the rock, dissolving the limestone (Box 2, reactions 1 and 2) generating characteristic reliefs, limestone pavements, karst, causses (Fig. 2D), sink holes, grottos, caverns (Fig. 2C), subterranean rivers (Fig. 2B), stalagmites and stalactites. Limestone rocks are fissured. Rainwater infiltrates them rapidly and disappears into their depths.

---

[5] The beech, found on slopes and calcareous meadows, also thrives on soils that are well drained, siliceous, sandy and gravelly, but it does not tolerate waterlogging. The requirements of the beech are all the more pronounced as it approaches the limit of its range: in the south of France (Cévennes) the beech prefers soils devoid of limestone, while in the north of France and in Denmark its requirement for a calcareous soil is more marked; it is then almost exclusively calcicole (*Salisbury* 1920).

The calcium (Ca++) liberated by erosion is involved in the constitution of clay-humus complexes producing lumpy aggregates whose size and solidity are influenced by the amount of available calcium. These aggregates stabilize the soil structure, favouring the enlargement of its pores, its permeability to water and air as well as its rewarming in spring. They encourage root system development.

Figure 2. Stacking of strata (A), underground rivers (B), grottos (C) and crevices (D) are characteristic of limestone massifs.

Figure 3. From left to right, *Laserpitium halleri* (Apiaceae), *Jasione montana* (Campanulaceae) and *Erica cinerea* (Ericaceae) are plants of siliceous substrates.

Figure 4. Arnica (*Arnica montana*, Asteraceae) can also grow on shales containing limestone provided that they have a high silica content; ferruginous rhododendron (*Rhododendron ferrugineum*, Ericaceae) growth on acidic crude humus, whatever the substrate, siliceous or calcareous.

Figure 5. From left to right : *Lomelosia graminifolia* (Caprifoliaceae), *Teucrium pyrenaicum* (Lamiaceae), *Daphne alpina* (Thymeleaceae), *Rosa spinosissima* (Rosceae), typical calcicole plants.

Box 3. Edaphic diversity of plant habitats: some examples

The Haller's laser (*Laserpitium halleri*, Apiaceae) and the star hare’s ear (*Bupleurum stellatum*, Apiaceae) are heliophilic and indicator species of acidic soils (acidophilic) in the subalpine and alpine levels, which are common on siliceous substrates (Reduron 2008) (Fig. 3).

The sheep’s-bit scabious (*Jasione montana*, Campanulaceae; Fig. 3) and the round-readed rampion (*Phyteuma hemisphaericum*, Campanulaceae) are typical plants of acidic (siliceous) soils, the former from the hill to the subalpine zone and the latter from the subalpine to the nival zone. Bell heather (*Erica cinerea*, Ericaceae; Fig. 3) and common heather (*Calluna vulgaris*, Ericaceae) are typical acidophilic (silica) plants from the plains to the mountains (Rameau et al. 1993; Aeschimann et al 2004).

Ear willow (*Salix aurita*, Salicaceae) is a subboreal acidophilic and hygrophilic species of peaty and alluvial soils (Rameau 1993).

Arnica (*Arnica montana*, Asteraceae; Fig. 4) is a heliophilic and calcifuge orophyte of acid humus soils of siliceous substrates (broad-spectrum acidophilic). However, it is also found on schistose substrates containing limestone, but rich in silica. The explanation for this paradox could be found in the experimental demonstration that silica, when added to the culture medium, reduces calcium absorption and tissue content, thus favouring plant growth. This antagonistic effect of silica and limestone (Steiner GA 312-29-3-1920 and GA 327-10 and 11-6-1924) has been demonstrated for many field crops and could also explain why plants such as *Arnica montana* can be found on silica-rich limestone shales (Ma and Takashi, cited by Epstein, 1994; Mehrabanjoubani et al., 2015; Diederich & Riggers 2003; Barth 2019).

Alpine rose (*Rhododendron ferruginum*, Ericaceae; fig. 4) is an acidophilic species of subalpine and alpine shady sides that grows on acidic crude humus, the result of the blocking of decomposition of organic matter due to altitude, accumulated on calcareous or siliceous substrates. On the other hand, hirsute alpine rose (*Rh. hirsutum*) is only found in subalpine shady sides with calcareous substrates (Rameau et al. 1993).

The grass-leaved scabious (*Lomelosia graminifolia*, Caprifoliaceae; fig. 5) is typical of limestone mountains (Aeschimann et al. 2004); the same applies to the Pyrenean germander (*Teucrium pyrenaicum*, Lamiaceae; fig. 5), alpine mezereon (*Daphne alpina*, Thymeleaceae; fig. 5) or garland flower (*D. cneorum*) calcicole plants commonly found up to the subalpine zone of the Pyrenees or southern Alps. The spanish broom (*Genista hispanica*, Fabaceae) is a calcicole plant of the Franco-Iberian hills and mountains up to 1400 m, while the burnet rose (*Rosa spinosissima*, Rosaceae; Fig. 5) occurs from the hills to the calcareous subalpine zone (Rameau 1993; Dumé et al. 2018).

The glaucous trinny (*Trinia glauca*, Apiaceae) is indicative of very dry, base-rich soils; it is often calcicole, but also occurs on basaltic or crystalline substrates; it has been described as thermal calcicole (Reduron 2008).

Boxwood (*Buxus sempervirens* Buxaceae) is a thermophilic and xerophilic supramediterranean species, usually found on calcareous substrates, also found on clays and siliceous rocks, up to 1600 m (Rameau et al 1993).

The yellow gentian (*Gentiana lutea*, Gentianaceae) behaves differently depending on the region and altitude: in the mountains it is characterized by a very wide ecological amplitude, ranging from acidic (Vosges) to basic (Jura) soils.

On the other hand, many generalist species are indifferent to the nature of the soil, such as the common juniper (*Juniperus communis* L. subsp. communis, Cupressaceae), a pioneer species with a very wide range, found on soils of all kinds, from very acidic to basic (limestone). Other plants, such as marigold (*Calendula officinalis*, Asteraceae), evening primerose (*Oenothera biennis*, Onagraceae), mat grass (*Nardus stricta*, Poaceae) and many others, herbaceous or woody, are indifferent to the nature of the substrate (Rameau et al. 1993, Rameau et al. 2008; Dumé et al. 2018).

Soils on limestone substrates are thin, only 10 to 20 cm deep, and poor in nutrients (humus). They are largely composed of calcium carbonate ($CaCO_3$), sometimes in association with magnesium carbonate (dolomitic soils). They are characterized by a stronger proportion of soluble salts and by their neutrality or even their alkalinity (up to pH = 8.5). In strongly basic soils (pH 8 and above), calcium carbonate precipitates round organic particles, inhibiting the activity of micro-organisms, impeding the formation and mineralization of humus, reducing availability of nitrogen, iron and phosphorus.

These soils strongly reflect sunlight. The fraction of incident solar radiation reflected (albedo) depends on the nature of the limestone. White limestone reflects incident radiation almost completely. On such soils, the plant is therefore subject to both incident and reflected radiation to the extent that in high summer at about 15 cm above soil level the temperature can reach 50 to 60°C (Fig. 5).

Plants on these calcareous substrates therefore have to cope with drought, heat and nutrient poverty. These factors reduce plant development and limit its biomass production (*Bothe* 2015). They encourage a diverse flora frequently composed of annual plants, geophytes and suffructescent champhytes* or shrubs. This explains not only, compared with other geological substrates, the flora of a limestone massif is schematically characterized by the precocity of spring, but also that, in our latitudes, most plant cycles are completed by late June (Benesch & Wilde 1983, Chytry et al. 2010, Collin-Bellier et al. 2010, Bahr et al. 2012, Atkinson 2014, Bothe 2015).

Figure 6. Limestone rocks reflect the light. Plants found there grow in the soils formed in holes in the rock. In the foreground of this photo is *Allium schoenoprasum* (Amaryllidaceae) a generalist plant* and in the background *Achillea atrata* (Asteraceae) a calcicole plant (Col du Sanetsch limestone pavement, Switzerland).

Plant species that require substrates rich in calcium carbonate are called calcaricole. The hoary plantain (*Plantago media*, Plantaginaceae) is an example; but paradoxically these plants do not need the element calcium and can grow on other substrates provided they are basic.

Species frequently present on calcium-rich soils (calcicole species) can, in mediterranean regions, also inhabit soils of any kind, provided that they are warm and dry; these species are called thermal calcicole. Other plants called ‘chemical calcicoles’ plants, such as dog’s mercury (*Mercurialis perennis*, Euphorbiaceae), require soil rich in minerals often containing calcium in abundance. However, the calcium is not necessary for them and they can grow on other substrates than limestone (Collin-Bellier et al. 2010).

Hard limestone parent rock (lutetian limestone, Paris stone) is so resistant to acids produced by soil organisms that the soil contains less or very little calcium, allowing the acidophilic species to establish there. Snow woodrush (*Luzula nivea*, Juncaceae) is often cited as an example. The same thing is observed in regions of high rainfall where calcium (Ca^{++}) liberated from the parent rock can be driven into the depths of the soil medium, or be eliminated by leaching of rainwater.

This very brief overview indicates the great diversity of soils in limestone localities and the special conditions they offer to plants. This overview is further enriched by the variety of parent rocks, such as chalk, tufa, dolomite, clay-limestone shale, marls, gypsum, by association with other rocks (clay) and by the influence of general or local climatic conditions. All these data show that the term 'calcicole plant' should be clarified by the diversity of trophic and water conditions of a particular biotope (*Leurquin* 2010, *Escudero et al.* 2015). (*Benesch & Wilde* 1983, *Chytry et al.* 2010, *Collin-Bellier et al.* 2010, *Bahr et al.* 2012).

Limestone supplementation

Positive effects: the buffering power of limestone

Limestone parent rock or other substrates rich in carbonates act as a buffer vis-à-vis acids carried by rain or produced by plant root exudates, litter degradation and respiratory processes of soil organisms, namely bacteria, fungi, algae and invertebrates (Box 2, reactions 1 and 2). The reaction of these acids with the carbonate anion keeps the soil medium neutral or alkaline, thus protecting the plant from their harmful effects.

The aim of limestone amendment is to reduce soil acidity and achieve pH values between 6 and 6.5 (Box 2, reaction 4a and 4b); this improves growth and yield of many cultivated plants. Plant water uptake and nitrogen content are improved. Neutralization is less favourable to fungi than to soil bacteria, especially nitrogen-fixing rhizobiums in Fabaceae. In addition, the neutrality obtained stimulates the development of soil fauna, especially earthworms; the latter help to reduce the acidity of the soil that passes through their bodies. Acidic soils represent a considerable proportion (around 36%) of cultivated land, which shows the importance that limestone amendment can have in reducing soil acidity (Box 3) (*Salisbury* 1920, *Atkinson* 2014, *Rothwell & Dodd* 2014, *Bothe* 2015).

Box 4 – Calcium supplementation of acid soils and haricot bean cultivation

The haricot bean (*Phaseolus vulgaris*, Fabaceae) prefers calcareous soils (*Aeschimann et al.* 2004). It is sensitive to calcium deficiency and this is manifested by decreased growth and necrosis of root apices and caulines. When the soil is acid, limestone supplementation is useful for ensuring satisfactory nourishment of the plant with calcium and to neutralize soil acidity as well as the toxic effects of aluminium (Al, see below). In bringing up soil pH from 5 to 6, yield doubles. The number of seeds and pods per plant, as well as above and below ground biomass, are increased. Calcium accumulates in the leaves and seeds in proportion to the concentration of calcium in the growth medium. These positive effects are observed up to growth medium calcium concentration of 5mM (*Rothwell & Dodd* 2014, *Domingues et al.* 20).

Negative effects: alkalinization of the medium and excess calcium availability.

Many abiotic stressors (salinity, fungicides, pollution) have a negative effect on the formation of mycorrhizae*. The enrichment of the growth medium in calcium carbonate causes its alkalinization which constitutes the most important factor in the disruption of the fungal life cycle. This disruption relates to the pre-symbiotic stages (spore germination, lengthening of the hyphae) and symbiotic stages (speed of colonization of the roots, non-root development of hyphae and spores). Although the fungal cycle is not completely stopped and a certain level of symbiosis is possible, the resulting biomass is reduced[6] (*Salisbury* 1920, *Labidi et al.* 2012).

Normally the calcium concentration of the rhizosphere is higher than is necessary for assuring a satisfactory plant development. That is why the way limestone supplementation is used needs to be specified according to the nature of the soil and the species of plant cultivated, calcicole, neutrocline* or acidicline*[7], mindful that Ca^{++} is involved in stomatic* conductance and consequently in overall gas exchange and the assimilation that depends on it. Increasing the availability of calcium in the rhizosphere can reduce CO_2 absorption, thus reducing biomass and growth (*Atkinson* 2014).

Calcium biomineralizations in plants and the carbonatation process

Biominerals and mineralisation in the living world

Many mineral deposits can be found in living organisms, among which are mainly silicic and calcic deposits. For their formation, these biominerals need a more or less complex matrix of mucopolysaccharides* or protein to which the minerals can attach themselves. Products formed under these

---

[6] These findings have been demonstrated experimentally in a fungus (*Rhizofagus irregularis)* that is responsible for the arbuscular mycorrhizae of chicory (*Cichorium intybus*, Asteraceae) (*Labidi et al.* 2012).

[7] In peat bog plants, the enrichment of their growing medium with calcium carbonate leads to significant physiological disturbances, as these acidophilic plants are unable to regulate the intracellular concentration of calcium and bicarbonate. These disturbances affect the osmotic balance and enzyme activity and are reflected in reduced root and shoot growth and nutrient uptake. The significant browning of the leaves is a sign of calcium-induced stress. In addition, seed germination is reduced, seedling mortality increases (McCormick & Gibble 2014).

conditions have physical properties beyond those of the pure minerals of the inorganic world or obtained in the laboratory. For example, an oyster pearl (aragonite) is about 300 times harder than mineral aragonite (*Mann* 2001).

Calcium biominerals are found in all groups of eukaryotes, unicellular, multicellular, protists (Foraminifera), algae[8], plants, invertebrate and vertebrate animals, as well as in bacteria (Prokaryotes)[9] (*Bauer et al.* 2011). They constitute the components of the skeletons of calcareous sponges and the exoskeletons of lower animals (Cnidaria[10], Molluscs, Crustaceans[11]) in the form of carbonate (calcite and aragonite). They also constitute the endoskeletons of vertebrates, largely in the form of apatites. This is why these calcium compounds occupy a special place among biominerals.

Calcium mineralisation in plants

Generally, all marine or terrestrial photosynthetic organisms from bacteria to plants, including unicellular algae, produce calcium biominerals in bulk. These include calcium carbonate ($CaCO_3$) and whewellite (calcium oxalate monohydrate, CaOx, $1H_2O$; fig. 7), which are among the most important minerals produced on Earth (*Stephens* 2012)[12].

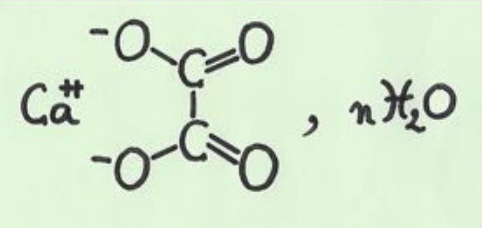

Figure 7. Extended formula for calcium oxalate; n = 1 (whewellite); n = 2 (weddelite).

In plants, the most common calcic mineralization comprise deposits of amorphous or crystalline calcium oxalate or calcium carbonate, the latter primarily in the form of calcite, aragonite or even vaterite. The calcic deposits occur most often in scattered specialized cells in the tissues. These are the idioblasts where CaOx is deposited, and lithocysts, in which $CaCO_3$ forms cystoliths (fig. 8).

---

[8] **Coccolithophores**, of which mainly of *Emiliana heuxlei*, are essential constituents of marine phytoplankton. The coccoliths that they produce are calcite skeletons which, at cell death, settle to the bottom of the sea where the CO_2 is thereby sequestered. For this reason, the algae play a major part in climatic equilibrium by reducing the mass of atmospheric carbon dioxide (*Gehlig* 1994, *Mann* 2001).

[9] **Stromatolites** comprise the oldest known limestone deposits. The earliest of them date from 2.15 to 2.7 million years ago, but they are still forming today. They are layered sedimentary structures comprising primarily photosynthetic cyanobacteria. The calcium bicarbonate dissolved in the surrounding water is fixed in a mucilaginous matrix secreted by the bacteria, then transformed into $CaCO_3$ (cf. Box 2 reaction 3). The trapped particles form a solid crust, called cyanobacterial laminate. Over time, a stack of mineralized crusts is formed in which CO_2 is sequestered in large quantities (*Amaudric du Chaffaut* 2008, *Gargaud et al.* 2009) (Fig. 2).

[10] **Corals and madrepores** (Anthozoa) are colonies of polyps living in symbiosis with photosynthetic algae (Dinoflagellates) in shallow waters of tropical regions. Colony development leads to the massive accumulation of limestone. The amount produced is such that underlying geological structures subside and atolls or barrier reefs form. The limestone skeletons produced represent a form of CO_2 sequestration and for this reason it contributes to climatic control (*Grassé & Doumene* 1993, *Campbell & Rice* 2004, *Birkeland* 2015, *Hubbard* 2015, *Muller-Parker et al.* 2015).

[11] **European crayfish** (*Astacus astacus*) is a freshwater crustacean whose chitinous carapace, impregnated with calcite, is renewed at each moult. Before the moult, the crayfish dissolves the limestone of its carapace and accumulates it in the form of gastroliths in the stomach wall. In less than 4 weeks, these limestone deposits are completely consumed to consolidate the new carapace. The 'stones' of the crayfish are used, combined with flint, in the prophylaxis of renal calculi (Lapis cancri) (*Cukerzis* 1984, *IAAP* 2017).

[12] **Whewellite** is also present in many animals and the human being. In the latter it is found in pathological conditions in the form of extracellular deposits such as renal calculi. Fungi and lichens also produce whewellite, most of the time towards outside along the hyphae* of the mycelium. Whewellite produced by lichens can damage building materials, but in some cases it has a protective effect, for example, when a whewellite-rich patina has formed on prehistoric wall paintings (*Stephens* 2012).

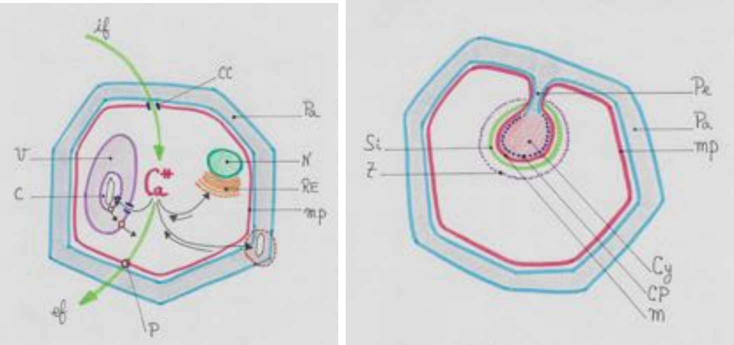

Figure 8. Schematic representation of an idioblast (A) and a lithocyst (B).
C: intravacuolar chamber; CC: calcium channel; CP: cellulose, pectin; Cy: cystolith; ef: calcium efflux; if: calcium influx; m: membrane; mp: plasma membrane; N: nucleus; P: calcium pump or transporter; Pa wall (apoplasm*); Pe: peduncule; RE: endoplasmic reticulum*; Si: silica; Z: future extension of cystolith.

Carbonates are found in Bryophytes, Ferns, and Angiosperms among which they have been observed in Moraceae, Acanthaceae, Cannabaceae and Urticaceae. Other carbonates (of magnesium or strontium) as well as sulphates and phosphates (of calcium, strontium and barium) have been found in several plant groups, notably of the genus *Acacia* (*Franceschi & Nakata* 2005, *Faheed et al.* 2012, *Meyer et al.* 2013, *He et al.* 2014).

Whewellite is generally the dominant mineral in plants, although some do not produce it. It is frequently associated with less stable weddelite (CaOx dihydrate). CaOx deposits can represent up to 80% of the plant's dry weight and up to 90% of the total calcium [8]. Depending on the family, CaOx production is constitutive or inducible. Still other crystals of organic calcium compounds are known, such as citrate, tartrate and malate. These organic biominerals are found in all groups of plants, but they are especially abundant in Angiosperms and Gymnosperms (box 5) (*Epstein* 1994, *Mann* 2001, *Hopkins* 2003, *Franceschi & Nakata* 2005, *Meyer et al.* 2013, *He et al.* 2014, *Raman et al.* 20014).

Box 5 – Some examples of plants that produce calcium oxalate

Iroko, a tree of the Ivory Coast (*Milicia excelsa*, Moraceae) flourishes on acid soils and stores CO_2 as large amounts of calcium oxalate crystals in its trunk and tissues. This accumulation can be so great that the trunk is almost petrified to the extent that sawing it is very difficult. Decomposition of the tree's dead organs enriches the soil in calcium carbonate and contributes to increasing soil pH. The subsequently formed carbonates are stabilized. The phenomenon is significant quantitatively (*Stephens* 2012, *He et al.* 2014).

During their growth, the Cactaceae such as **prickly pear** (*Opuntia ficus-indica*) and saguaro (*Carnegiea gigantea*) produce calcium oxalate in significant amounts which changes to calcite during decomposition of the plant or of its dead organs (*Stephens* 2012, *Bothe* 2015).

Whewellite is present in the foliage of many species (4% dry weight in tomato, 3.3% in apple, 4.3% in peach, 3.7% in spinach, etc.). Most of the leaves mentioned also contain weddelite. Moreover, spinach contains magnesium oxalate (glushinskite). The idioblasts of leaf mesophyll* of water lettuce (*Pistia stratiotes*, Araceae, Alismatale) accumulate CaOx in large amounts (*Stephens* 2012).

Calcium oxalate in oak. In the oaks (*Quercus macranthera, Q. aucheri, Q. cerris, Q. petrae*, Fagaceae), calcium oxalate crystals are localised in the uniseriate* cells of the parenchyma* stems forming long chains and containing very compact crystals of variable shape and size, prismatic, tetragonal or rhomboidal. Likewise are found acicular crystals, isolated or in raphides in parts of the conduction vessels of the summer wood (final wood) where they adhere to the wall. Sometimes it is sand, made up of small.

compact crystals (*Q. aucheri*). In sessile oak (*Q. petraea*) we also find in the same locations as CaOx amorphous granules containing 66% iron as well as sulphur (0.65%), calcium (0.59%) and silica (0.57%) (*Serdar & Demiray* 2012).

The morphological diversity of CaOx crystals is very great. Five principal types have been found: prisms or tetrahedra which can aggregate into a druse, acicular crystals that are isolated (styloid; from Greek stylos, column) or grouped (raphides; from Greek raphis, needle), as well as small angular crystals constituting sand. Currently six different types of raphides are known (*Franceschi & Nakata* 2005, *Faheed et al.* 2012, *Raman et al.* 2014). Raphides are the most common forms among Monocotyledons, while druses are more common in Dicotyledons.

It has never been possible to produce raphides *in vitro*, which demonstrates the influence of cellular constituents and processes on crystal morphology. In idioblasts, the crystals are deposited in the 'chambers', intervacuolar spaces surrounded by membranes. Nucleation, growth, morphology and crystal dimensions are controlled by the crystallization chamber's chemical characteristics, such as the ratio of concentrations of calcium and oxalic acid, the composition of its membranes as well as the structure and chemical functions of the macromolecules and proteins it contains. The idioblasts synthesize oxalic acid[13], while their Golgi* apparatus is involved in the transport to the vacuole of a protein constituting the crystal matrix (*Mann* 2001, *Bauer et al.* 2011, *Franceschi & Nakata* 2005, *Le et al*. 2010, *Serdar & Demiray* 2012, *Raman et al.* 2014). These mineralizations are controlled by influx and efflux of calcium. The influx occurs through more or less specific calcium channels*. They are more numerous or more active in cells accumulating these minerals than in ordinary cells. These phenomena are genetically controlled. Mutations can go in the direction of exaggerated or insufficient accumulation (*Franceschi & Nakata* 2005, *Stephens* 2012, *Pinto & Ferreira* 2015).

CaOx crystals can be found in all tissues, vegetative, reproductive, storage organs, photosynthetic and non-photosynthetic tissues, developing organs as well as in seeds and their integuments (*Domingues et al.* 2016). The distribution of crystals in tissues and organs as well as the crystal morphological type are species specific (genetically determined) (*Franceschi & Nakata* 2005, *Serdar & Demiray* 2012). The plant's living conditions, such as aridity or soil calcium content, influence the abundance of the deposits, but not their morphology[14] (*Brown et al.* 2013 ; *Dauer & Perakis* 2014).

Extracellular deposits (apoplasm deposits) are commonplace, especially in Gymnosperms where they are produced in any layer of the wall of most cell types. In Angiosperms, these deposits occur in the apoplasm of specialised cells called asterosclereids. Such deposits are strictly genetically regulated.

Calcium also occurs outside these locations, associated with proteins, in other cellular compartments such as the endoplasmic reticulum*, mitochondria* and chloroplasts* (*Dayod et al.* 2010, *Franceschi & Nakata* 2005).

Degradation of calcium oxalate and the carbonatation process

In temperate forests, calcium fixed during the plant's life in the form of compounds with cell wall pectin and in the form of calcium oxalate is released during bacterial and fungal decomposition of the litter. Calcium oxalate, which is almost insoluble in the dead tissues of the plant, is transformed into a soluble form that allows calcium to be reintroduced into a new life cycle. Calcium oxalate acts as a long-term calcium reservoir in ecosystems subject to stress induced by acidification of sites (see below) or by repeated harvesting of wood (*Dauer & Perakis* 2014).

In arid environments, at the death of the plant whewellite accumulated in its organs can be mineralized to calcium bicarbonate thence to calcium carbonate (calcite) under the influence of the bacterial and fungal decomposers. The process accompanies the release of the oxalate's carbon moiety in the form of CO_2. The other moiety is sequestered in the form of carbonate, a mineral form of carbon which has the advantage of remaining in the soil for a longer time than the organic matter[15]. Many factors influence the

---

[13] According to the authors, oxalic acid is formed from ascorbic acid (vitamin C) (*Franceschi & Nakata* 2005) or from the glycolate / glyoxylate system. The formation of the latter precursors is independent of photorespiration (*Le et al*. 2010).

[14] These observations were made with *Acacia* sp. and with Douglas fir.

[15] The breakdown of whewellite after the death of the plant or of its organs explains why it is rare in documents of geological history. It can be found in low-temperature spas in contact with graphitic schists, carbonaceous rocks of plant origin. Calcium

carbonatation process among which are nutrients, salinity, temperature and the deposit conditions. Limestone accumulation can even be observed on granitic substrates where it would not be expected. CaOx carbonatation influences the carbon and calcium cycles and has significant climatic consequences (*Stephens* 2012, *He et al.* 2014, *Bothe* 2015) (box 6). The phenomenon can be summarized by the following diagram:

Air CO_2 (photosynthesis) → oxalic acid and soil calcium → calcium oxalate → death and decomposition of the plant → CO_2 release and accumulation of carbonate in the soil

Box 6 – Calcium and climate

The sequestration of CO_2 in limestone rocks is more longer lasting than that in vegetable matter produced by photosynthesis. Thus, after a plant's death, the CO_2 is returned more or less quickly to the atmosphere according to the speed of breakdown of the organic matter by the decomposers (bacteria, fungi, human activity). Therefore, limestone plays a leading part in climatic regulation.

But environmental acidification disturbs the normal development of the plant and the normal formation of exoskeletons and calcareous coccoliths (*Birkeland* 2015, *Muller-Parker et al.* 2015). This threatens the survival of many living organisms and endangers climatic stability. Environmental degradation constitutes a threat to the plant, to its physiology, especially for its reproductive function and, correspondingly, for the quality and yields of cultivation.

Active calcium in plants

Calcium is an essential macronutrient* involved in membrane stabilization and cell wall consolidation. It is also a ubiquitous element acting as a second messenger* in numerous signal phenomena (calcium signature) as well even though its tissue and cell storage is heterogeneous (*Gilliham et al.* 2011).

Calcium homoeostasis: regulation of cell calcium concentration

Water and calcium supply to the plant

The transport of water and solutes, including calcium, to tissues occurs primarily via apoplasmic and symplasmic* routes. The relative share of the two circulation systems depends on the species, the developmental state of the plant, the nature of the tissues involved and on environmental factors. However, the current view is that the main long-distance transport of calcium and water between plant tissues is via the apoplasmic route. This means that the supply of calcium to tissues closely depends on transpiration* and that its accumulation takes place in organs with high transpiration. The flux of water through the plant is itself regulated by Ca^{++}, partly in the apoplasm by its effects on the cell wall structure and on the stomatic conductance (open / closed), and partly in the symplasm where cytosol calcium concentration ($[Ca^{++}]_{cyto}$) regulates calcium and water flow and influences aquaporin* activity (AQPs) and membrane calcium channels (*Dayod et al.* 2010, *Gilliham et al.* 2011, *Atkinson* 2014, *Bothe* 2015).

Several mechanisms restrict the movement of calcium, such as the Caspary band at the level of the root endodermis* and the anionic apparatus of the cellular apoplasm, thus allowing the fixation of calcium (see above) (*Dayod et al*. 2010, *Gilliham et al*.2011, *Atkinson* 2014, *Bothe* 2015). Furthermore, certain plants on calcareous soils can secrete excess absorbed calcium in the form of oxalate (*Dayod et al.* 2010) or calcium carbonate on their leaf margins, on the outgrowths or trichomes* of their leaves (*Saxifraga paniculata, Saxifraga caesia*, Saxifragaceae; fig. 9; *Bothe* 2015, *Thomas et al.* 2016).

---

carbonate and oxalate are markers for the previous presence of life and for this reason are included among the biochemical rocks (*Bouillard* 2016).

Figure 9. White calcium carbonate deposits on the leaf margins of *Saxifraga paniculata* (Saxifragaceae).

Cytosol calcium concentration

Generally, the concentration of free calcium (Ca^{++}) in the resting cell cytosol* is about 0.1 µM (micromole/L). This concentration depends on a number of factors including the characteristics of the environment (soil pH, calcium availability) and the genetically determined features of the species. Calcium released in excess into the cellular cytoplasm interferes with energy transport by precipitating phosphates. It also disturbs phosphorylated protein metabolism as well as calcium signalling processes. Excess calcium competes with magnesium at active* sites on enzymes, which can change their corresponding functions. Furthermore, during calcium signalling events (see below), calcium concentration can briefly exceed 1 µM. This is why its concentration is narrowly controlled in order to avoid its negative effects on growth and its toxic effects at the cellular level (*Franceschi & Nakata* 2005, *Dayod et al.* 2010, *Bauer et al.* 2011, *Gilliham et al.* 2011, *Stephens* 2012, *Atkinson* 2014, *He et al.* 2014).

Elements of the regulation of the calcium concentration of the cytosol

At the level of the cytoplasm, excess calcium is actively secreted from the cytoplasm in the form of insoluble calcium compounds, most often crystallized and osmotically and physiologically inactive, of which calcium oxalate is the main compound. Carbonate and other insoluble calcium salts (sulphate in *Acacia* sp.) also contributes to this regulation (see above biomineralisations and fig. 8). Each idioblast's regulatory capacity is limited. The density of these specialized cells increases with that of the calcium content of the growth medium. Calcium is likewise stored in the apoplasm and in the cell organelles (mitochondria, endoplasmic reticulum) where it is strongly bound to high affinity proteins. In the apoplasm and vacuole, calcium is present at millimolar concentrations, i.e. at levels several orders of magnitude higher than those of resting cytoplasm (*Tuteja* & *Mahajan* 2007, *Bauer et al*. 2011, *Gilliham et al.*2011, *Franceschi & Nakata* 2005, *He et al.* 2014, *Pinto & Ferreira* 2015).

Water flux and calcium oscillation

When the calcium concentration in the cytosol falls sufficiently, the aquaporins (AQPs) and calcium channels are activated, thus permitting the entry of water and calcium into the cytoplasm. But the flows of water and calcium are separate. Water passes via the AQPs and spreads from cell to cell by the symplasmic route (hydraulic conductivity*), whereas calcium moves via the calcium channels. The increase in its concentration in the cytosol is also fed from the cellular calcium stores, such as those in the vacuole.

Gradually, the calcium content of the cytosol that results from these flows becomes such that it causes the closing of aquaporins (AQPs) and inactivates calcium channels. At the same time as the transporters (antiports* Ca^{++}/H^{+}) and calcium pumps (Ca-ATPases) of the cytoplasmic membranes and the vacuole (tonoplast) facilitate its outflow to the apoplasm and the vacuole in which it is stored in the form of oxalate (CaOx).At the same time as the carriers (antiporters* Ca^{++}/H^{+}) and cytoplasmic membrane and vacuolar (tonoplast) calcium pumps (Ca-ATPases) the apoplasm, storing the calcium in the vacuole as calcium oxalate (CaOx). Not being able to return to the cell, the water and calcium accumulate in the apoplasm. This results in a further decrease in the calcium concentration of the cytosol and the cycle starts again. Therefore, the calcium concentration of the cytosol **oscillates** (*Tuteja & Mahajan* 2007, *Gilliham et al.* 2011, *Bothe* 2015).

Conclusion

A plant's calcium content is correlated with the size of ionic exchanges, the capacity of the roots to mobilise calcium from the soil, the ascending sap flow, i.e. through transpiration, and the capacity to sequester calcium in particular compartments. The movements of water are linked to those of calcium, not only at the cellular level, but also at the whole plant level by calcium's influence on stomatal conductance and the transpiration* which depends on it.

Calcium deficiency

When limestone is barely present in the soil, plants tend to produce thin or sinuous stems like those of climbing plants. Their flowers open but remain sterile, and do not provide much nutrient (seeds). On the other hand, when 'limestone' is active in the soil, flowering is abundant and diverse (*Steiner* 10-6-1924 GA 327).

When calcium deficiency occurs, it reduces seed germination rates, slows seedling growth and reduces the productivity of herbaceous plants and trees, leaf chlorophyll content, photosynthesis and antioxidant activities. Calcium deficiency disrupts the development and resistance of the cell wall, making the membrane and cellular integrity fragile. Apices of roots, leaves and flowers necrose, fruit softens and rots (apical rot). Calcium deficiency induces wasting. The cold resistance of conifer needles decreases. It disrupts calcium signalling which governs many essential cellular phenomena. Calcium deficiency weakens plant tolerance of biotic* and abiotic* stress and diminishes the quality and the yields of cultivars (*Driscoll et al.* 2001, *Ricles & Miller* 2005, *Tuteja & Nahajan* 2007, *Dayod et al.* 2010, *Liu et al.* 2011, *Atkinson* 2014, *He et al.* 2014, *Du et al.* 2017).

In case of calcium deficiency or increased demand for it, calcium can be released preferentially from apoplasm, but also from the vacuole or cell organelles where it is stored. In the vacuole, crystals already formed reduce in size or disappear, releasing the necessary calcium through the action of enzymes. This does not occur in the vacuole when the calcium supply is satisfactory (*Franceschi & Nakata* 2005, *Tuteja & Nahajan* 2007, *Bauer et al.* 2011).

Box 7. Physiological causes of calcium deficiency

- Tissues such as leaves are nourished by ascending sap (xylemic apoplasmic route) characterized by a significant transpirational flow. These organs are richer in calcium than those having weak transpirational flow supplied by refined sap (circulating in phloem*). It is thus not surprising that tissues most sensitive to calcium deficiency are those with low transpirational flows, such as fruit. The severity of deficiency is correlated with the weakness of transpiration in the corresponding organs. In this case, it is not so much the soil content of calcium as its transport that is responsible for the symptoms of deficiency.
- Calcium transporters are more or less specific. This makes possible the root absorption of physiological or toxic elements having characteristics similar to those of calcium. Competitions can result that are unfavourable to calcium at the profit of other elements, possibly generating deficiencies or toxic effects.
- Increasing atmospheric CO_2 content, drought as well as increasing salinity reduce stomatic conductance which results in the reduction of transpiration and of the concentration of calcium in tissues. This effect depends on the species, the cultivars and the plant organs. For example, in leaves and seeds of wheat, a reduction in the calcium content of 7.5% and 15% respectively has been observed when the atmospheric CO_2 concentration is doubled. Consequently, climatic changes tending to increase atmospheric CO_2 concentration could have a negative impact on transpiration phenomena and on plant calcium nutrition, as well as, correspondingly, on the health and quality of harvests (*Tuteja & Nahajan* 2007, *Dayod et al.* 2010, *He et al.* 2014).

The origins and mechanisms of calcium deficiency are very diverse, and can be related schematically to physiological or environmental causes (box 7 & 8; *Hopkins* 2003, *Dayod et al*. 2010, *Bauer et al.* 2011, *Bothe* 2015, *Pinto & Ferreira* 2015). It should be noted that the excessive tissue CaOx content of plants can also lead to slower growth, reduced productivity or even leaf necrosis in some cases (*Bothe* 2015).

Box 8. Environmental causes of calcium deficiency

When acidity (H^+) produced by strong acids is high, it displaces basic cations* (Ca, Mg and K) from their combination with the micelles of clay-humus complexes (Box 2 and reactions 4a and 4b). Water leaching removes these elements from the soil. Therefore, these cations are lost from the ecosystem, plants and trees. Calcium is the element most sensitive to acidity and the first element to be lost. The biological effects of this loss are observable as soon as the soil buffer capacities and soil inputs (erosion of bedrock, atmospheric inputs) are exceeded (box 9).

Furthermore, when neutralization by bases is reduced, organic acids tend to mobilize aluminium and form complexes deposited in the lower horizons of the soil, in which aluminium is barely soluble. When acidification by strong acids is severe, aluminium is mobilized from these organic complexes which leads to poisoning of the plant and to interference with the absorption of basic cations, notably calcium. It is the same with manganese.

Soil acidification causes the decline in calcicole species favouring generalist or calcifuge (acidophiles) species which may be unaffected by it and become highly competitive. The negative effects of acid rains on sensitive plants can be corrected by adding limestone (*Driscoll et al.* 2001, *Ricles & Miller* 2005, *Liu et al.* 2011, *Perakis & Sinkhorn* 2011, *Bahr et al.* 2012, *Perakis et al.* 2013, *Bothe* 2015, *Du et al.* 2017).

Box 9 – Soil acidification

Soil acidity is caused by soil biological activity, by respiration of subterranean plant organs (cf. above) as well as by acid rain and the acid products resulting from the degradation of mineral nitrogen compounds that are in excess of the plant's needs (see below).

Acid rain brings nitric and sulphuric acids (strong acids), formed by contact of volatile oxides with water vapour. These oxides are of natural origin (thunderstorms, fires) but above all are produced by human activity, agricultural and industrial (volatile oxides of nitrogen from reduction of nitrogen fertilizers, oxides of nitrogen and sulphur produced by all sorts of combustion and by certain electric installations). The acids are responsible for the acidification of rainwater, rivers, lakes and soil.

The supply of nitrogen to cultivation is provided by biological fixation (see below), by fertilizers, including chemical fertilizers, and by pollution (NO_x). A moderate increase in soil nitrogen content stimulates plant growth and calcium absorption.

When this content exceeds the needs of plants, i.e. when nitrogen accumulates in the soil, growth is also stimulated, but correspondingly the calcium demand increases. This accumulation of nitrogen favours the storing of N and P in the soil organic matter, but at the same time promotes the weakening of the reserves in alkaline cations (Ca, Mg, K). The main loss of calcium (but also of Mg and K) is due to the magnitude of the processes of nitrification and consequent acidification (until the pH of soil water is about 4), which causes calcium's loss by leaching (rainwater). These losses are helped by exposure of sites to acid rain and by forestry practices such as timber harvesting. Calcium deficiency results, despite the recycling of calcium oxalates from litter or from those produced by roots and soil fungi. While P and K are easily resorbed from senescent foliage and redistributed in the plant to meet its demands, the recycling of Mg is weaker and that of Ca is minimal. The result is that calcium and not phosphorus becomes the element limiting growth (*Perakis et al.* 2006, *Perakis & Sinkhorn* 2011, *Bahr et al.* 2012, *Perakis et al.* 2013).

Biological roles of 'limestone'

Resistance to biotic stress

Repellent effects on herbivores or on bark boring insects results from physical properties of CaOx crystals accumulated in the mature cells. Large crystals (raphids and styloids) deter large herbivores; insects or their larvae (caterpillars) are deterred by the abrasive effect on the mandibles of small prismatic crystals (cf. Sporoptera exigua; Nakata 2015).The accumulation of CaOx is constitutive (conifers, Negev lilies, etc.) or inducible by grazing (*Sida rhombifolia*, tropical Malvaceae) (*He et al.* 2014).

A proper supply of calcium reinforces plant resistance to parasitic diseases, probably by reinforcing the cell wall and other defence systems still unknown. This beneficial effect is not observed in acidophile plants (calcifuges) (*Dayod et al.* 2010). This is why transgenic modification of field crops (maize, canola) is being considered so that they accumulate more calcium oxalate (CaOx) and thus become more resistant to herbivores. CaOx enrichment could be obtained in the laboratory with different plants, including lady's thumb (*Arabidopsis thaliana*, Brassicaeae) (see below "Calcium strengthens the cell wall"; *Nakata* 2015).

Some plants accumulate toxic substances in the same cells as those containing calcium oxalate raphids or styloids; these facilitate the flow of toxins when in contact with the herbivore. An example is a Mexican Euphorbiaceae, *Tragia ramosa*, which has many urticating hairs containing styloids of CaOx; on contact with the animal the tip of the hair breaks, the crystals can pierce the epithelia of grazers and facilitate the flow, along a groove in the crystal, of a urticating toxin from the base of the hair to the tissues of the herbivore (*Stephens* 2012; *Nakata* 2015; box 10).

Resistance to abiotic stress

As for silica (SiO_2), calcium oxalate (CaOx) and calcium carbonate ($CaCO_3$) are able to reduce aluminium and heavy metal toxicity by co-precipitation or co-crystallization (*He et al.* 2014). These have been observed in many species of important cultivars. This phenomenon depends on both the species and the metal under consideration[16] (*Faheed et al*. 2012,).

Vacuolar accumulation of calcium oxalate or co-precipitation of calcium oxalate and toxic elements occur in tissues distant from those performing photosynthesis protects the photosynthetic organs from their toxic influence. Apart from idioblasts, the main tolerant cells involved in these deposits are those of the epidermis and vascular sheaths. When the level of toxicity is high and the possibilities for isolation in the tolerant organs are exhausted, photosynthesis is threatened (*Pinto & Ferreira* 2015).

Light utilization

Calcium biomineralisations in the leaves of various species of radiator plant (*Peperomia* sp., Piperaceae), tropical plants of shady environments, play a special part in light capture. Curiously, the palisade* tissue, of their leaves is richer in CaOx than the idioblasts of the epidermis, where it normally accumulates. In each cell of the palisade tissue there is a druse of CaOx (fig. 10).

In *Peperomia glabella* it has been found that the size and position of the druse in the palisade cells is influenced by the light intensity.

---

[16] The detoxification of heavy metals and of aluminium has been observed for example in beet (*Beta vulgaris*, Amaranthaceae), soy bean (*Glycine cannescens*, Fabaceae), haricot bean (*Phaseolus vulgaris*, Fabaceae) and tomato (*Solanum lycopersicon*, Solanaceae) (He et al. 2014). On the other hand, Malvaceae, (jute mallow, *Corchorus olitorius* and small-flowered mallow, *Malva parviflora*), grown in a hydroponic medium supplemented with heavy metals, do not incorporate them in their CaOx crystals (*Faheed et al.* 2012).

- When light is intense the druses are situated in the upper part of the palisade cells and their size decreases. In this position, they dissipate and attenuate the excess light by reflection. In doing so CaOx crystals protect chloroplasts adapted to shade from damage caused by excess of light (inhibition of photosynthesis).
- When light intensity is low, the druses are positioned in the lower part of the palisade cell, enhancing the optimal use of the small amount of incident light on the leaves.

In other species of plants such as the curtain fig (*Ficus microcarpa*, Moraceae) which contains mineralized calcium carbonate and oxalate, the refractive index of the cystoliths ($CaCO_3$) and druses (CaOx) of cells in their leaves is significantly higher than those of neighbouring cells. The cystoliths and druses are thus able to disperse light and in doing so allow a satisfactory use of the incident light.

These few observations show that calcium biominerals, notably CaOx, can be involved in photosynthetic processes of land plants. It is not known whether this property is general and functionally significant (*He et al.* 2014).

Box 10 – 'Limestone' and resistance to biotic stress

A reduction in free calcium (Ca^{++}) and oxalic acid concentrations is observed in a lucerne species (*Medicago truncatula*, Fabaceae) which forms few or no crystals, or crystals that are abnormal in shape and size. These plants are subject to grazing insects and their larvae.

It has been found, for example, that the leaves of the Negev lily (*Pancratium sickenbergeri*, Amaryllidaceae) are all the more grazed by the dorcas gazelle when their CaOx content is low, which is the case of young shoots (*He et al.* 2014).

Certain conifers accumulate calcium oxalate in their secondary phloem, which protects them against bark piercing insects. On the other hand, larch canker (*Larix decidua*, Pinaceae, acidophile) is aggravated on chalky soils (*Salsbury* 1920).

Supplementation of acid soils with limestone hinders or reduces the severity of diseases, such as cabbage clubroot or iris heterosporiosis (*Salsbury* 1920, *Dayod* 2010).

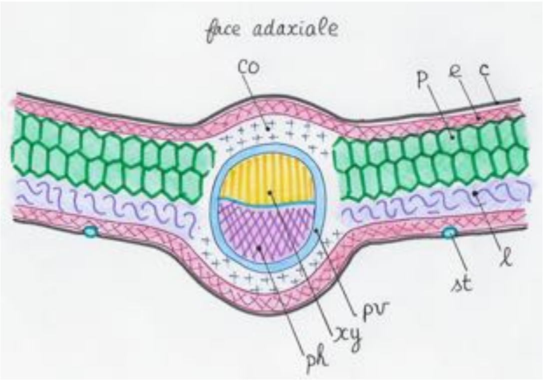

Figure 10. Schematic representation of the tissues of a leaf limb (cross-section).
c: cuticle; co: collenchyma; e: epidermis; l: lacunar tissue; p: palisade parenchyma; ph: phloem; pv: perivascular sheath; st: stoma; xy: xylem

Biological roles of active calcium

Calcium hardens the cell wall

The extracellular matrix (MEC) is the main constituent of the parietal apoplasm. It is a porous structure whose components are secreted by the adjacent cell. Its structural composition changes according to the plant's site and age. It influences liquid and gaseous exchange with the cell as well as the mobility of solutes and their fixation. The primary cell wall matrix is made of proteins (peroxidases, pectin-esterase, extensins and hydroxyproline-rich glycoproteins[17]) and 3 classes of a wide range of interconnected glycans*. In it are found different hydrophilic pectins, arabinans, homogalacturonan (HGA), rhamnogalacturonan (RG)[18]. These pectins form a gel in which float cellulose microfibrils (ß (1,4)-glucan) and 'strings' of hemicelluloses (xyloglucan, arabinoxylan, mannans).

The homogalacturonans are produced by the cell in the form of non-polar methyl and acetyl esters. In the cell wall, they undergo a progressive de-esterification by a pectin methyl-esterase or a pectin acetyl-esterase. The result of this is the uncovering of carboxylic functions of uronic residues* which can fix calcium in the apoplasmic space. Calcium behaves as a cross-linking agent between adjacent groups of the homogalacturonan chains. The matrix becomes a rigid gel (fig.11).

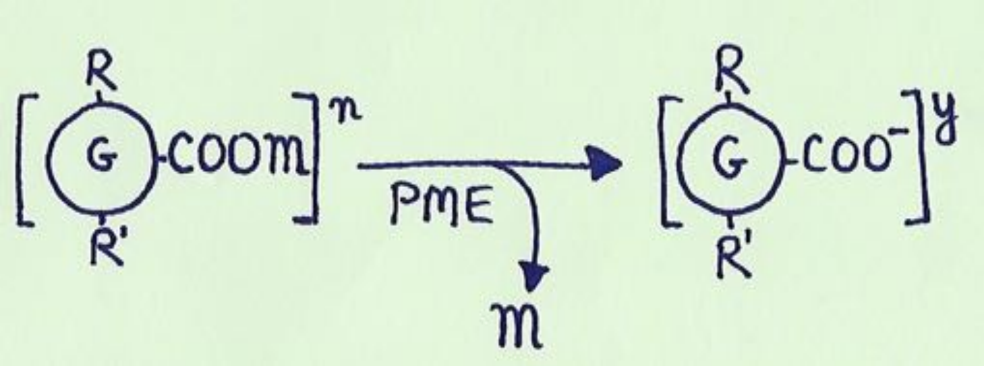

Figure 11. Schematic representation of the de-esterification reaction of homogalacturonan.
G: galacturonic acid residue; PME: pectin methyl-esterase; Ra : methyl or acetyl group; y may be different from n depending on the number of hydrolysed ester functions.

Young cell walls are thin, low in calcium and contain proportionally low amounts of de-esterified pectins. This explains why they are more or less soluble, porous, elastic and subject to extension, allowing growth phenomena to progress in good conditions[19]. On the other hand, with ageing, the extracellular matrix accumulates calcium on its de-esterified pectins that have become more abundant. The cell wall thickens, hardens and rigidifies, losing its elasticity, its ability to extend[20] and its porosity. It forms a kind of skeleton round the cell (*Dayod et al.* 2010, *Gilliham et al.* 2011, *Meyer et al.* 2013, *Zhu* 2016).

---

[17] Comment: the abundance of proline and hydroxyproline (21%) in human collagen is a characteristic of its structural role (*Lehninger et al.* 1994).

[18] The pectin content of cereals is lower than in many dicotyledons. The capacity for cationic exchange (CEC) and the tissue calcium content is weaker, but the Gramineae are rich in silica (*Gilliham et al.* 2011).

[19] It is noteworthy that apoplasmic deposits of calcium impair the elongation of cells and, as a result, hinder growth. It is observed that during periods of growth, the deposits preferentially form in the idioblasts and not in the apoplasm, thus allowing normal development (*Franceschi & Nakata* 2005).

[20] Experimentally increasing the calcium concentration in the apoplasm increases the activity of pectin methyl-esterase, raises the content of calcium in the cell wall, thickening it and decreases its extensibility. Conversely, experimentally reducing the concentration of calcium in the apoplasm reduces the activity of pectin methyl-esterase and the proportion of non-methylated HGA, and reduces the cell wall thickness but increases its extensibility (*Dayod et al.* 2010).

The extracellular matrix structural changes caused by Ca^{++} affect water and calcium transport as well as calcium storage in plant tissues. These changes occur during growth or in response to stress (dehydration, salinity, cold) (*Gilliham et al.* 2011). The effects of these stresses are more obvious in the case of calcium deficiency. Sodium can bind to pectin but it cannot produce cross-linking. Thus, calcium available in sufficient quantities allows a certain tolerance to salt (NaCl) : calcium slows the entry of sodium (Na^+) and competes with it for the acid residues of cell wall pectin. This explains why adding calcium can attenuate weakening of the cell wall due to excess salt (NaCl) (*Dayod et al.* 2010).

Calcium and fruit

Primarily at the maturation phase, the developing fruit is supplied with calcium through flow of the refined sap which is correlated with that of the rising sap (see above). The softening of the fruit at maturity is explained by the reduction of the turgor of the mesocarp* cells and by the weakening of the cell walls induced by local calcium deficiency resulting from decreased flow of rising sap. This last explanation seems to be reinforced by the fact that 1) the external application of calcium increases the shelf life of some crops by delaying the ripening and softening of fruits, 2) the modification by transgenesis of different cultivated plants increases the calcium content of their tissues, roots, tubers or fruits. The shelf life of the latter can be significantly extended. These phenomena can be explained by the reinforcement of the primary wall and the median lamella*, by limiting transpiration, respiratory phenomena and ethylene production in the fruit. These transgenic methods have the disadvantage of reducing growth, slowing down fruiting, reducing fertility and in some cases producing leaf necrosis (*Dayod et al.* 2010).

Calcium signalling

Calcium is one of the second messengers very frequently involved in cell signalling phenomena. We talk about calcium signalling or calcium signature, which is observed throughout the living world. It consists of the transitory increase in the calcium concentration of the cytosol, which occurs in the form of **peaks, waves or oscillations**, characterized by their amplitude, frequency and **propagation kinetics**: these oscillations determine the specificity of the response to stimuli of all kinds that bind to membrane receptors. The calcium signature varies according to the type of cell or tissue; its characteristic is that it is furtive and ephemeral, which makes it difficult to study the mechanisms at work.

In plants is known a similar apparatus to that of mammalian G protein (box 11). It consists of specific transmembrane receptors and proteins soluble in the cytosol belonging to the large family called, because of their small size, small GTPases. The **ROPs (*Rho-like Of Plants*)** belong to that family (*Craddock et al.* 2012, *Himschoot et al.* 2015).

These ROPs are found in the cytosol in inactive form; they become active when they are bound to the plasma membrane and then initiate the corresponding cascade of biochemical events. Each stage in the genesis of events initiated by an elicitor, via the receptor and the active GTPAses, is governed by promoters or inhibitors or by feedback control systems. Calcium plays the role of event coordinator.

A wide variety of biochemical and cellular effectors are involved in calcium signalling phenomena, such as membrane tensions, dynamic reorganization of the cytoskeleton (actin and microtubules), reactive oxygen species, nitrogen oxides and the regulation of inputs of calcium (inflow) and its outputs (efflux) (box 12; figs. 12 and 17) (*Himschoot et al.* 2015, *Zhu* 2016).

Calcium does not directly transmit structural information. The calcium signal has to be detected, decoded and communicated to effectors of the cellular response. This is done by a complex assembly of calcium-binding proteins, some having enzymatic activity (calcium-dependant protein kinase: CDPK), others being without enzymatic activity, such as calmodulin and its related proteins. They are involved, alone or combined with each other, in cascades of biochemical events and produce their effects on their targets, either directly or via the nucleus through activation or inhibition of genes. Signal transduction requires the temporal and spatial co-ordination of all actors in the signalling process (*Tuteja & Mahajan* 2007, *McAinsh &Pittman* 2009, *Bauer et al.* 2011, *Mazars et al.* 2011, *Himschoot et al.* 2015, *Zhu* 2016).

Within a certain period of time (sometimes several hours) after an initial stimulation, a second exposure of the plant to an elicitor does not modify the cytosol calcium concentration. The calcium signature is modified by its first experience: by its effects, a first elicitor antagonistic to a second, renders the latter ineffective. This implies a kind of dialogue between the signalling cascades and a capacity for memory in the plant (*Tuteja & Mahajan* 2007, *Suty* 2015a, *Suty* 2015b).

Box 11. The receptor-protein G system

One of the biochemical devices used for cell signalling in animals and humans is that of transmembrane receptors coupled to the G protein system attached to it (G as guanosine triphosphatase = GTPases), whose activation leads to the sequence "perception, transduction, response". These receptors constitute the largest family of transmembrane receptors in mammals. In the human being it has been possible to identify several hundred specific receptors of exogenous (olfactory and gustatory) and endogenous ligands* (hormones and neurotransmitters). In 1994, the American biochemists Alfred Gilman and Martin Rodbell received the Nobel prize for physiology and medicine for their discovery[21].

The GTPases, when they are active, provide two functions:

that of initiating signal transduction through a cascade of biochemical events, possibly very localized in a region of the cell, where calcium appears as a crucial element coordinating phenomena and

that of degrading the GTP which they had to bind beforehand to become active.

Thus GTPases work like biochemical switches. Their activation is rapidly followed by their inactivation. **This makes the signalling cascades oscillatory phenomena**.

Box 12 – Coordinated calcium inflow and outflow currents

Calcium signalling phenomena result from contrary but co-ordinated currents of somewhat passive influx of Ca^{++} through calcium channels, and efflux due to pumps and transporters. The influx of calcium into the cytosol occurs from the apoplasm, through the plasma membrane or from intracellular organelles (vacuole, mitochondria, chloroplasts, endoplasmic reticulum). The elimination of calcium from the cytosol (efflux) is produced in reverse by two groups of essential proteins, calcium ATPases (energy expenditure) and antiporters of the type H^+/Ca^{++}. The Ca-ATPases have characteristically high affinity and specificity for calcium. The antiporters have a weak affinity for calcium. They are able to quickly rid the medium of Ca^{++} and also transport other metals. The control of influx and efflux mechanisms is essential for forming and modulating the calcium signature and for maintaining Ca^{++} concentrations within limits compatible with cell life, i.e. between a **low calcium concentration and a toxic concentration that is lethal for the cell**.

---

[21] According to Steiner (*Steiner* 4-10-1905 GA 93a, *Steiner* 13-12-1914 GA 156, *Simonis* 1975), in every organ of the body the human being has organs for the perception of taste. The malfunction or alteration of them in a particular organ explains its succumbing to corresponding pathologies. An appropriate diet, and the use of specific medicinal plants, allow the reestablishment of this taste faculty and, as a result, the normal functioning of the organ. Today this applies not only to taste receptors, but also to those of olfaction dispersed in the organs (*Augereau* 2008).

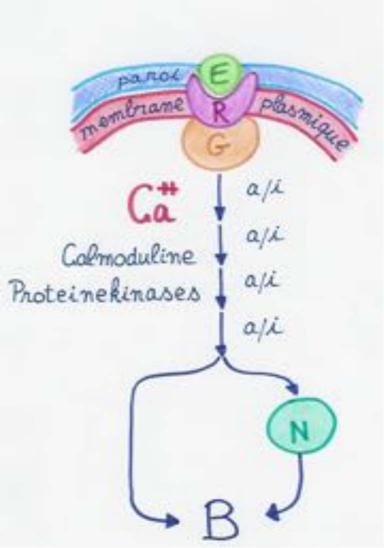

Figure 12. Diagram of receptor (R) and the transduction of a signal (E) leading to a biological effect (B) directly or mediated by the nucleus where calcium plays an important part as modulator. The events of the sequence of transduction are regulated by activators (a) and inhibitors (i).

In the plant, calcium signalling is involved in many aspects of its development and physiology, such as cell division and morphogenesis, polarized growth (cf. pollen tube, absorbent hairs) and stomatal movements. Calcium signalling processes are triggered in response to physiological stimuli (light, CO_2 concentration, phytohormones, contact), and to pathogenic elicitors* as well as to abiotic stresses (salinity, heat, cold, dryness).

This calcium signalling also involves biochemical communication between the plant and certain bacteria involved in biological fixation of atmospheric nitrogen.

In this paper we only consider few examples of calcium signalling such as stomatal movements, pollen tube growth and nodulation phenomena in Fabaceae (*Franceschi & Nakata* 2005, *Tuteja & Mahajan* 2007 *Dayod et al.* 2010, *Bauer et al.* 2011, *Craddock et al.* 2012, *Charpentier & Oldroyd* 2013, *Himschoot et al.* 2015, *Pinto & Ferreira* 2015, *Study* 2015a, *Zhu* 2015).

Stomatic movements

The stomata are places of exchange of carbon dioxide gas[22], water vapour (transpiration) and oxygen produced by the photolysis* of water (ig. 13). Water vapour and oxygen use the same diffusion route as CO_2, but in the opposite direction, which means that the plant cannot absorb CO_2 without losing water. It is estimated that 90% of CO_2 or water exchanged passes through the stomata and that he absorbed water is mostly evaporated (95%)[23]. The outflow of the raw sap through the leaves is the most important route for groundwater flow to the atmosphere. This shows the importance of regulation for limiting these exchanges.

Transpiration is primarily controlled by the water potential gradient (difference in humidity between the inside and outside of the leaf) and by the opening or closing of the stomata. The latter work like hydraulic valves. These movements are influenced by many external factors such as the air concentration of CO_2, the concentration of calcium in the rhizosphere, temperature, light, dark, humidity and by internal factors such as the hydration of the leaf, the CO_2 content of the sub-stomatic chamber, and abscicic acid* (ABA) (*Hopkins* 2003, *Mansfield et al.* 1990, *Meyer et al.* 2013).

---

[22] Toxic gases can enter plants via the stomata.

[23] A tree loses in summer 200 to 400 litres of water per day (or 2500 m^3 of water / km^2 of forest).

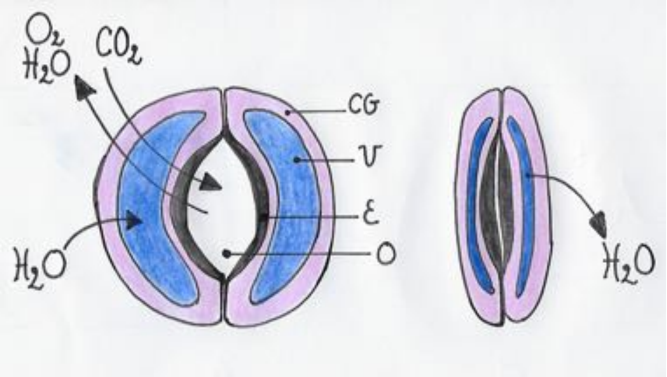

Figure 13. Diagrams of a stoma open (left) and closed (right). The ostiole results from the entry of water into the vacuoles of the guard cells. It is promoted by blue light and by the low CO_2 content of the lacunar tissues of the leaf. Closing of the ostiole results from the exit of water from the vacuoles of the guard cells. It is induced by darkness and water stress (from *Meyer et al.*2013). CG: guard cells; E: thickening of the wall of the guard cell; O: ostiole.

In plants with C_3 metabolism, blue light signals received by the cryptochrome* and a low content of CO_2 in the stomatal crypts promote the opening of stomata in the morning. At the end of the afternoon, the circadian clock and darkness induce their closure. The opening of stomata in the light allows the absorption of CO_2, while their closure at night helps conserve water. Depending on the environmental conditions (CO_2 content of lacunar tissue, humidity or drought, wind), the size of the ostiole is constantly changing in order to optimize the balance between CO_2 capture and water loss (*Mansfield et al.* 1990, *Hopkins* 2003, *Meyer et al.* 2013).

The **opening of stomata** results from the asymmetric deformation of the **guard cells** caused by an increase in their turgor. Turgor changes are initiated by plasma and vacuolar membrane proton pumps, that lead to an increase in the vacuolar content of potassium, chloride, malate, and sucrose. The movement of these electrolytes and organic substances is accompanied by water flows.

The **closure of stomata** results from general efflux of ions, of sucrose and of vacuolar water in the guard cell. This process can be initiated by the binding of abscisic acid (ABA) to its membrane receptor during water stress (drought). Transduction of the signal involves the entry of **calcium (second messenger)** into the guard cell and leads to the outflow of water[24]

The cytosol's content of calcium has to be low to allow stomatal opening. Thus, calcium alone, when its cytoplasmic concentration is raised, can directly cause closure of the stomata, activating calcium dependent protein kinases (*Mansfield* 1990, *Hopkins* 2003, *Dayod et al.* 2010, *Meyer et al.* 2013). The medium calcium concentration is directly correlated, on the one hand, with the profile of the oscillations (period, frequency and amplitude) of the cytoplasmic calcium concentration in the guard cells, and, on the other hand, with the extent of the opening (closing) of the stomata (*Mansfield* 1990, *McAinsh & Pittmann* 2009, *Dayod et al.* 2010, *Webb & Robertson* 2011, *Meyer et al.* 2013).

The foregoing explains that calcium supplementation induces the reduction in stomatic conductance when the concentration of calcium in the xylem reaches a certain threshold that varies greatly according to the species. This is observed in haricot bean (*Phaseolus vulgaris*, Fabaceae) in which the calcium concentration of the xylem sap increases when the availability of exchangeable calcium in the culture medium is doubled. But the reduction in stomatic conductance is equally observed in pea (*Lathyrus oleraceus*, Fabaceae) whose xylemic calcium concentration remains unchanged despite a doubling of the availability of exchangeable calcium in the growth medium. This indicates that a substance other than calcium, as yet unidentified, is responsible for the fall in stomatic conductance in pea and could also be in

---

[24] Similar phenomena of calcium signalling govern other movements such as those of waking and sleeping leaflets of Fabaceae and Oxalidaceae (nastic movements) (*Hopkins* 2003).

other plants even if the calcium concentration of their xylem sap is increased[25] (*Atkinson* 2014, *Rothwell & Dodd* 2014).

Calcium and biological fixation of nitrogen

According to Steiner, there is a close relationship between nitrogen in the air and 'limestone' in the soil resulting from the Sun, at the moment of its separation from the Earth, bequeathing its 'limestone' and nitrogen to the earth and the air respectively. This explains why soil 'limestone' represents a need for nitrogen, just as the lung needs oxygen. It is dependent on some kind of inhalation of nitrogen. This inhalation is brought about by the Fabaceae, a phenomenon linked to the annual solar cycle (*Steiner* 11-6-1924 GA 327).

It is now known that symbiotic* associations in root nodules between certain soil bacteria (rhizobium) and roots of most of the Fabaceae and a small number of plants of other families enable the biological fixation of nitrogen. The formation of these nodules results from a veritable biochemical dialogue between the plant and rhizobium, requiring the contribution of calcium signalling mechanisms in the bacteria and in the absorbent hairs of the plant root (Box 13; fig. 14) (*Charpentier & Oldroyd* 2013, *Meyer et al.* 2013, *Suty* 2015b).

Box 13 – Calcium signaling in the formation of root nodules in Fabaceae

The process of nodule formation is not yet completely elucidated. It starts with the emission by the plant of chemical signals (among which flavonoids) which exert a positive chemotactic effect on soil rhizobium and bind it to specific bacterial receptors. This is followed by the activation of genes on bacterial plasmids* and the release into the medium of nodule-forming factors, compounds closely related to chitin (lipochito-oligosaccharide). These are recognized by the specific cellular membrane receptors on the absorbent root hairs of the plant (lucerne for example). This binding triggers a cascade of calcium signalling, its regulation co-ordinated by many genes leading to cytological, histological and morphological changes in the root enabling the organization of atmospheric nitrogen fixing infectious nodules. Oscillations occur in the region close to the nucleus. The same process is at work in the formation of arbuscular mycohrriza. The same apparatus is used, but the calcium oscillations in arbuscular mycohrriza have a characteristically higher frequency and lower amplitude than the oscillations induced by the nodule factor (*McAinsh & Pittman* 2009, *Charpentier & Oldroyd* 2013, *Singh et al.* 2014, *Suty* 2015b).

These nodule bacteria promote the conversion of atmospheric nitrogen into the ammonium ion, the most reduced form of nitrogen, which, together with glutamate α-ketoglutarate produce glutamate, a metabolic pivot necessary for the syntheses of nitrogen compounds essential for plant life (nucleic acids, proteins). Their nitrogenase, the enzyme that catalyses this reduction, is active only in oxygen-poor conditions and uses a lot of energy (ATP) as well as reducing substances, namely hydrogenated derivatives. The reaction is shown schematically as follows:

Nitrogen (N_2 air) + reducers (NADPH) + energy (ATP) → ammonium (NH_4^+) + hydrogen (H_2) → Glutamine
→ nucleic acids and proteins

or, from a phenomenological point of view:

Nitrogen (depriver of life) → ammonium ion (toxic to life) → glutamine
→ nucleic acids proteins (instruments of life)

---

[25] Limestone supplementation reduces biomass production by different plants that have a preference for calcareous soils, such as haricot bean (*Phaseolus vulgaris*), crown vetch (*Securigera varia*), or by plants indifferent to the nature of the soil such as peas (*Lathyrus oleraceus*), alfalfa (*Medicago sativa*) or sorghum (*Sorghum sudanense*, Poaceae). The biomass reduction results from the significant reduction of stomatic conductance and of processes of assimilation (*Atkinson* 2014).

It is interesting to note that the reaction also generates hydrogen in significant amounts. The hydrogen can be reutilized in a new nitrogen reducing reaction (*Meyer et al.* 2013). The biological fixation of atmospheric nitrogen can be summarized in a pictorial way as the inhalation of atmospheric nitrogen and the exhalation of hydrogen. The process is very large scale: it is estimated that at the planetary level it supplies at least as much assimilable nitrogen as all the nitrogenous chemical fertilizers used annually. This illustrates the importance of calcium in facilitating this phenomenon (*Suty* 2015b).

Figure 14. Some examples of root nodules in Fabaceae. From left to right: *Lupinus angustifolius* (annual lupin), *Phaseolus vulgaris* (haricot bean), *Lathyrus sylvestris* (narrow-leaved everlasting pea), *Trifolium arvense* (haresfoot clover) and *Onobrychis viciifolia* (sainfoin).

Limestone, calcium and reproduction

In spermaphytes, sexual reproduction leads to the formation of a seed, a remarkable living form of plants. Formation of the seed requires the co-ordination of different specialized parts of the flower including pollen formation. When pollen lands on the flower's stigma, it germinates to form the pollen tube which penetrates the stigma tissues and carries male gametes to the embryonic* sac where they fuse with the oosphere and the central nuclei. The seed itself is not evidence of the plant's fertility. The latter is expressed only during the seed's germination (*Steiner* 10-6-1924 GA 327). The pollination process and the germination of the seed are influenced by 'limestone' and calcium. The pollination process has two main aspects: pollen dispersion followed by its germination and pollen tube growth.

Calcium oxalate (CaOx) and pollen dispersion

When pollen of pepper or chili (*Capsicum annuum*, Solanaceae) is ripe, the calcium content of the cytoplasm and cell walls of pollen sacs and the zone of dehiscence (stomium*) is systematically eliminated into the extracellular environment and inactivated as insoluble CaOx. This leads to the weakening of tissues and to their degradation, followed by the release and dispersion of pollen (fig. 15).

Calcium is indispensable for the germination and growth of the pollen tube. The calcium is provided from the calcium oxalate transported by the pollen or produced by the stigma. This is observed in petunia (*Petunia hybrida,* Solanaceae) whose oxalate also comes from the degradation of the stomium cell walls. The phenomenon has also been demonstrated in certain tobacco species (*Nicotiana* sp. Solanaceae) and in plants of other families. It is not known whether the function of CaOx observed in some species holds generally for flowering plants (*Steiner* 14-6-192 GA 327, *Tuteja & Mahajan* 2007, *Bauer et al.* 2011, *He et al.* 2014).

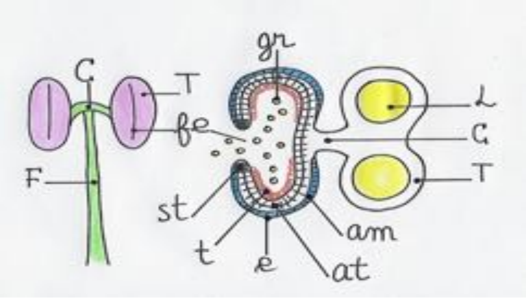

Figure 15. Schematic representation of a stamen and a cross-section of an anther.
am: mechanical layer; at: transitory layer; C: connective; e: epidermis; fe: zone of dehiscence; F: stamen filament; gr: pollen grain; L: locule (microsporangia); st: stomium; t: tapetum; T: theca.

Calcium and the polarized growth of the pollen tube

A basic property of cells is their polarity. In plants, the formation of the zygote* and embryo (fig. 16), growth of absorbent root hairs or that of the **pollen tube** (Tp)[26] involve cellular polarization and apical growth, also called polarized or unidirectional growth. The origin and maintenance of apical growth are governed by complex cellular processes and signalling networks, where Ca^{++} appears as an essential element in their regulatory systems[27]. It is an oscillating phenomenon (*Jamin & Jang* 2011, *Craddock* 2012, *Himschoot et al.* 2015). Depending on the species, the speed of growth is between 1 µm/min and ≤ 1 mm/min and the period of oscillation of growth is between 20 and 80 s.

The growth of the Tp is made possible by a reduction in both the tensions in the cytoplasmic membrane and in the robustness of the cell wall. A phase of progression is quickly followed by a phase of limiting and consolidation. It brings into play many indispensable factors, among which small GTPases (ROP1), the effectors they generate (proteins RIC3 and RIC4), the apical calcium concentration gradient (spatiotemporal signal), the dynamics of actin-F of the cytoskeleton, the signalling routes requiring lipids, the vesicular traffic and the modification of the cell wall (box 14).

The germination of pollen and the growth of Tp are only possible if the culture medium calcium concentration is optimal (2 to 5 mM). Too much calcium (≥ 10 mM) bursts the pollen tube, not enough (< 0.5 mM) slows or even prevents its growth (*Jamin & Jang* 2011). In the first case, calcium influx is disturbed (too much) and the cell wall is rigidified through the activation of PME (pectin methyl esterase) by the excess calcium (see above), which slows or inhibits growth. In the second case, the insufficient supply of calcium is responsible for the weakness of the apical gradient.

---

[26] The purpose of the pollen tube is to transport the two male gametes to the embryonic sac. The fusion of one with the oosphere leads to the formation of the embryo, and that of the second with the central nucleus generates a triploid nutrient tissue, the albumen* (fig. 16). It has been shown that the growth of absorbent hairs and of the Tp are governed by the action of a group of genes called 'TIP' genes which, with other reasons, allow the establishment of an analogy between Tp and root phenomena (absorbent hair) (*Schilperoord* 2011).

[27] The growth of the pollen tube has been the subject of many studies because of its functional importance, but also because it is made of a single cell, robust and easily cultured *in vitro*, which lends itself to all kinds of research techniques without obvious damage to it. It is the preferred study system for scientists to analyse the function and regulation of the calcium signal and its spatiotemporal characteristics.

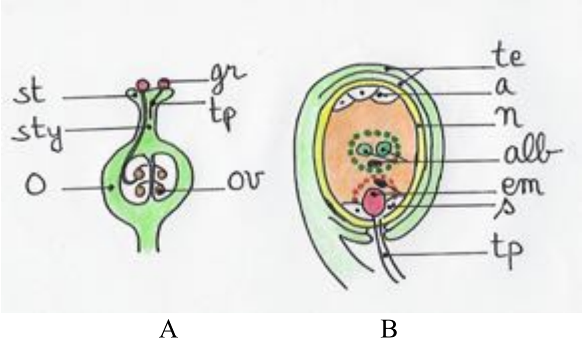

Figure 16. Schematic representation of the growth of Tp and double fertilization. The fusion of the first male gamete with the female gives rise to the embryo (em) and the fusion of the second with the two central nuclei gives rise to the endosperm (alb), the food tissue of the young plant after germination (from Meyer et al., 2013).
A – ovary (O) containing ovules (ov), with style (sty) and stigma (st) above; pollen grains (gr) have germinated and the pollen tubes (tp) are developing in the direction of the ovules. **B** – the Tp has released its gametes into the embryonic sac of the ovule (a: antipodals; n: nucellus; s: synergids; te: teguments).

As with many phenomena in the living world, those of the polarized growth of the Tp are oscillatory. A period of expansion of Tp driven by ROP1 is rapidly followed by **antagonistic effects of calcium** which results in a **slowing** of growth and the **consolidation of the wall**. These oscillations occur according to a certain chronology. ROP1 oscillates in phase with that of growth but precedes it; calcium focuses towards the apex, and the influx of calcium oscillates in phase, but offset compared with the growth of the Tp. The sequence of events can be summarised schematically as follows:

ROP1 oscillations → growth oscillation → [Ca^{++}] oscillation → Ca^{++} influx oscillation)

Box 14 – Regulators of growth of the pollen tube

While the apical calcium concentration of the resting cell is between 0.1 and 0.3 µM, that of the apical calcium gradient is between 1µM and 1mM (10 to 10000 x more). Maintaining an appropriate calcium (Ca^{++}) concentration is essential for normal growth of the Tp and involves the operation of calcium channels (cyclic nucleotide-gated channel) and pumps (Ca ATPases auto-inhibited).

The necessary supply of calcium is secured equally by cellular sources such as the endoplasmic reticulum, and other vesicles. The corresponding increase in the calcium gradient promotes exocytosis* and elongation of Tp, which explains the shift between oscillations of the apical calcium gradient and its (delayed) influx.

When the pollen tube extends, its wall in the apical region is constantly modified by PME (pectin methyl esterase). The uronic residues are exposed, their reaction with calcium reduces the elasticity of the pectins, rigidifies the cell wall and slows Tp growth (*Choi et al*. 2011, *Jamin & Jang* 2011).

Among the proteins of the small GTPases group (cf. Box 9) ROP1 is a GTPase specific to pollen. It is indispensable for normal growth of Tp. ROP1 controls apical growth by modulating calcium movements and generates two downstream effectors proteins called RIC3 and RIC4, which initiate feedback controls loops whose modes of action are antagonistic (Fig. 17).

- RIC4, by promoting the stabilization of actin-F (cytoskeleton) through the assembly of its filaments, increases the activity of ROP1 and growth oscillation. It tends to suppress apical accumulation of calcium by favouring calcium's exocytosis. In addition, the effects of the **guanine nucleotide exchange factor** (Rop-GEF) catalyses the formation of GTP and consequently activates ROP1.
- RIC3 contributes to apical accumulation of calcium by favouring the disassembly of actin-F filaments via profilin (protein); in doing so RIC3 counters the positive effects of RIC4 on the activity of ROP1. Calcium via a protein kinase activates the **Rop-Enhancer (REN1)**, a protein from the GAP group, deactivates ROP1 and may possibly lead to a decrease in Tp growth.
- The role of calcium is that of a negative regulator of the activity of ROP1 and when its apical concentration is excessive, the activity of ROP1 can be suppressed (*Choi et al.* 2011, *Jamin & Jang* 2011, *Craddock* 2012).

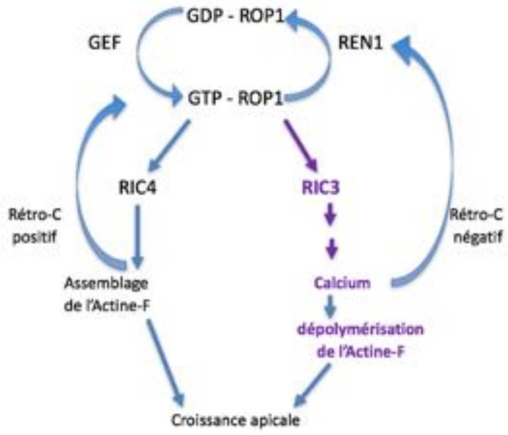

Figure 17. Schematic representation of routes of transduction during pollen tube growth.

Limestone, calcium and seed germination

Depending on whether a plant is calcicole or calcifuge, the influence of soil limestone and calcium is favourable or otherwise to germination and growth during the seedling period (see above; *Liu et al.* 2011). At optimal concentration, calcium can inhibit Na^+ absorption and reduce its negative influence on germination. It is the same with magnesium and various sulphates whose toxic effects on germination and growth are reduced (*Kolodziejek & Patikowski* 2015) (Box 13).

Galium cracoviense (Rubiaceae) seed germination is influenced by temperature, light, soil moisture, oxygen and active calcium (Ca^{++}) concentration. The most important factor is temperature. During winter dormancy, the seed of this plant tolerates calcium carbonate in large amounts without viability loss. After winter, the germination starts provided that the limestone concentration is reduced. Prolonged high concentrations of calcium negatively affect germination (*Kolodziejek & Patikowski* 2015).

Conclusion and phenomenological aspects

Origin and metamorphoses of limestone

'Limestone' has a cosmic origin, its archetype coming from the Sun. The etheric current generator of substances that gave birth to it is later than that of silica. It penetrated into the fluid proteinaceous atmosphere which surrounds the Earth, bringing about the appearance of the chemical elements and inducing the effects we see today. Unlike silica (radiating current with surface: two dimensions), 'limestone' is in the form of mist or clouds (volume: three dimensions). The limestone of this fluid protein atmosphere is subject to **oscillations**, turning it from vapour to liquid and vice versa, according to constantly ascending and descending movements. In addition, the limestone leaves the protein atmosphere and goes down to the Earth which it infiltrates continuously, making it more and more dense.

This 'limestone' process occurs at the same time as a phenomenon related to the protein atmosphere. It densifies in places, somehow coagulates, generating structured forms which swim in this atmosphere. They become the supports of the first animals (Urtiere, in German). Their consistency is that of a gel, even cartilage. Limestone shows a particular attraction for these gels or cartilaginous masses. It impregnates these animal forms with its earthly forces, drawing them down towards the Earth and creating earthly entities. The limestone, whose origin is cosmic, has somehow stolen animals from the sky (*Steiner* 1-12-1923-GA 232). These animal forms tend to introduce limestone into their solid primordia to form exoand endoskeletons in the course of evolution. After the death of the animals, calcareous deposits form which in the course of time become limestone mountains (*Steiner* 28-10-1923 GA 230, *Steiner* 1 et 2-12-1923 GA 232, *Steiner* 17-2-1923 GA 349, *Benesch & Wilde* 1983).

Limestone: the remains of living organisms after their death

In reality, most living organisms fix calcium in various forms of 'limestone' which returns to the surroundings after their death. Plants return calcium carbonate directly, but more often by the detour of calcium oxalate. The plant's CaOx results from the introduction into its life cycle of calcium released into the environment by erosion, and from its combination with oxalic acid produced by photosynthesis from CO_2, the degradation product of carbon substrates.

As a result, limestone rock is the remains of living organisms after their death. This secondary rock is witness to not only animal life, but also that of past lives of bacteria, algae and plants. In other words, limestone rock is a dead deposit between a form of past life and a form of future life (*Steiner* 17-2-1923 GA 349, *Gargaud et al.* 2009).

Supporting or limiting life

In plants, excess calcium is excluded from metabolic processes in the form of insoluble calcium carbonates and oxalates. Dead materials, excluded from the processes of life and ejected to their surroundings or to the periphery of the organisms that produce them include stromatolites, coccoliths, mollusc shells, tree corals and even eggshells of birds. This also applies to vertebrate skeletons (endoskeletons), considered as internalized fragments of an earthy nature, as insoluble mineral formations whose vitality is weak. According to Steiner and Wegman (GA 27 1925), the human being already dies in its skeleton.

But paradoxically, these dead structures protect the life of the organisms that produce them. In plants, the insoluble calcium (CaOx; $CaCO_3$) avoid the toxicity due to excess calcium, protecting the photosynthetic apparatus. Calcium consolidates the cell wall, which forms a kind of exoskeleton round the aged vegetal cell. These deposits protect the plant from herbivores (predators). In animals, they protect life with the exoskeleton or the egg shell. When required, these calcium deposits of plants and vertebrates (endoskeletons) constitute calcium reservoirs as, on the scale of the universe, are the calcareous rocks. Active calcium (soluble) can be released from limestone to fulfil its missions in the service of life. Limestone is a dead deposit between a form of past life and a form of life of the future (*Steiner* 17-2-1923 GA 349).

Limestone rock is hostile to life, killing the etheric or, at the very least, reducing the vigour of the etheric and favouring the effects of the astral (*Steiner* 13-6-1924 GA 327). Nothing grows on calcareous rock. It prevents seed germination. A calcareous substrate limits vegetative growth but promotes the expression of the floral impulse. Even defence mechanisms against herbivores partly result from this negative character.

Active calcium (ionized, soluble) is indispensable for the proper function of major physiological processes governed by cellular signalling, in which calcium is the main co-ordinator. Its role can only be envisaged within certain concentration limits, oscillating between low and high concentrations, between deficiency and toxicity. In these phenomena we see the expression of calcium's genius: on the one hand its antagonistic effects on growth and assimilation processes (cf. limitation of Tp growth; reduction of the elasticity of the cell wall; decreased stomatal conductance, transpiration and photosynthesis), on the other hand, its protective effects on life and the transmission of life (consolidation of the cell wall, protection against stress, movements, reproductive function).

Between, on the one hand, inert calcium deposits that are inhospitable to life and, on the other hand, soluble, mobile active forms of calcium indispensable to life processes, the oscillation seems universal. It appears in all aspects studied at the levels of the geosphere and biosphere, notably of the plant.

Environmental limestone as mediator of cosmic forces

Soil 'limestone' is a receptor of the forces of planets below the Sun, namely Venus, Mercury and Moon. It is also the mediator of these forces to the benefit of the plant, allowing it to attain flowering, to become a reproductive plant, to reach a level that goes beyond itself. Limestone satisfies the plant's desire to become an animal. Put another way, the transmitted astral forces support the floral impulse. Through them the plant becomes able to reproduce and form fertile seeds. These forces contain, limit and transform the vegetative plant. The plant is just touched by them, leading it to produce flowers and colours. Steiner said that it expresses something of a psychic nature[28]. The expression of these forces is correlated with the Sun's annual cycle (*Steiner* 13 and 14-6-1924 GA 327-10, *Benesch & Wilde* 1983).

A weakened reception of these cosmic forces generates all kinds of diseases that can be corrected by a supplement of 'living limestone'[29] such as the oak bark used in biodynamic agriculture (*Steiner* 13-6-1924 GA 327, *Masson* 2015). But only co-operation with silica allows for achieving satisfactory development of the plant. This is illustrated by the growth of fruit. Fruit, becoming food in the highest sense, ripens under the care of silica and the planets above the Sun (Saturn, Jupiter and Mars) which promote the formation of substances, proteins, lipids sugars and aromas. On the other hand, the production of fertile seeds (reproductive function) is under the influence of forces below the Sun transmitted by limestone (*Steiner* 14-6-1924 GA 327).

Glossary

(*Morère & Pujol* 2003, *Brice* 2011, *Meyer et al.* 2013, *Jouy & de Foucault* 2016)

Abiotic: factors that are not dependent on living organisms, such as wind, frost, day length, salinity, drought, etc.
Abscicic acid: is a plant hormone (phytohormone) whose name conveys its stimulating effect on the shedding of an organ (leaf, fruit, etc.), and whose action mainly comprises inhibiting growth and prolonging dormancy of buds and seeds.
Acidicline: plant preferring slightly acid soils.
Antiport: membrane transporter coupling the movement of two ions or molecules in opposite directions.
Apoplasm: comprises cell walls, cavities of dead cells and intercellular spaces. It represents the main route for the circulation of water and mineral substances. By 'apoplasm' we mean the cell wall, the space between the wall and the plasma membrane and the xylem* vessels (limited to the wall, dead cell, tube without cell).
Aquaporin: small transmembrane protein functioning as a channel for the diffusion of water.
Biotic: ecological factors involving living organisms.
Calcium channel: transmembrane protein complex allowing the passage of calcium ions.
Calvin synthesis: all the biochemical reactions leading to the first stable compound (glyceraldehyde-3-P) after the incorporation of CO_2 on a pre-existing carbon substrate during photosynthesis as such.
Caspary band: suberized or lignified band around the transverse or radial walls of endodermis* cells, especially in the roots.
Central nuclei: haploid nuclei localized at the centre of the embryonic sac which participates in the second fertilization generating the endosperm
Chloroplasts: plastids containing chlorophyll; the chloroplast is the seat of photosynthesis.
Connective: tissue located in the extension of the stamen filament connecting the pollen locules of the anther.
Cryptochrome: flavoproteins (yellow pigments) receiving blue light (λ = 400 - 500 nm) and UV-A (λ = 320 – 400 nm)
Cytosol: more or less liquid foundation substance of the cell in which float the organelles of the cytoplasm (mitochondria, chloroplasts, etc.)
Divalent cations: (from Greek *cata-*, 'downwards' and *iôn*, 'which goes') a cation moves towards the cathode during electrolysis; calcium (written Ca^{++}) is an ion possessing 2 positive charges, able to make two bonds with an anion (negatively charged); e.g. Ca^{++}, the calcium cation, has 2 charges; it can bind to 2 chloride anions each possessing one negative charge (Cl^-); sodium (Na^+) is a monovalent cation able to form only one bond.
Elicitor: substance responsible for inducing specific defence reactions in plants.

---

[28] 'The flower is an immobilized butterfly, the butterfly is a flower set free' (Steiner GA 230-26-10-1923). The butterfly, flower without a stem, which flutters...' (*Gérard de Nerval*, Les Odelettes 1831).

[29] Produced by life, as we should say.

Embryonic sac: haploid cellular structure of Angiosperm ovum containing several nuclei including the female gamete called oosphere* and two central nuclei*; the fusion of one of the male gametes with the oosphere yields the embryo, the fusion of the second with the central nucleus yields the endosperm*.
Endocytosis: membrane transport mechanism enabling cells to absorb various materials from the exterior surroundings in the form of solutes, or represented by vesicles which form by invagination of the plasma membrane.
Endodermis: internal cellular seating of the sheath surrounding the central cylinder in roots and certain shoots and leaves.
Endoplasmic reticulum: membrane system forming a network in the cytosol and participating in protein synthesis.
Endosperm: triploid reserve tissue surrounding the seed embryo of Angiosperms and providing its food at the time of germination.
Exocytosis: allows eukaryote cells to expel to outside the contents of secretory vesicles.
Generalist species: a species that grows in a wide variety of soils.
Glycan: polymer of different kinds of sugar.
Golgi apparatus: organelles formed of stacks of small flattened sacs from which protrude vesicles; the Golgi apparatus is often located near the nucleus and plays an important part in the synthesis of the components of the wall.
Hydraulic conductivity: the process water flows in the tissues.
Hyphae: filaments without chlorophyll constituting the fungal thallus.
Lacunar parenchyma: situated between the palisade tissue of the leaf's inferior epidermis; its lacunar character facilitates gaseous diffusion.
Ligand: molecule that binds to a specific receptor and triggers biological effects.
Macronutrient: a quantitatively significant nutrient essential to the plant.
Median lamella: layer primarily made of pectin at the junction of the walls of two adjacent cells.
Mesocarp: middle layer of the wall of fruit (cherry), situated between the epicarp (skin) and endocarp (nucleus).
Mesophyll: tissue situated between the upper and lower epidermis of a leaf, also called 'leaf parenchyma'.
Mitochondrion: organelle surrounded by two membranes, contained within the cytosol, playing an essential part in the cell's respiratory processes.
Mucopolysaccharide: macromolecule comprising three long linear chains made of disaccharides one of whose sugars carries an amine group (e.g. glucosamine) and the other a carboxyl group (e.g. uronic acid*).
Mycorrhiza: symbiotic association between a fungus and the root cells of a plant.
Neutrocline: a plant showing a preference for neutral soils.
Nucleoplasm: nuclear constituent of eukaryotic cells, largely comprising a concentrated solution of proteins and ribonucleic acid bathing the chromatin and nucleoli.
Oosphere: female gamete contained within the embryonic sac.
Palisade tissue: chloroplast-rich tissue comprising elongated cells arranged perpendicular to the surface of a leaf.
Parenchyme: foundation tissue comprising living cells providing various functions.
Photolysis of water: decomposition of water occurring in the chloroplast allowing the transformation of solar energy into chemical energy and into reductive substances necessary for Calvin synthesis (photosynthesis).
Plasmadesmata: cytoplasmic bridges between the cytoplasm of two adjacent cells.
Plasmid: DNA molecule present in the cytoplasm of bacteria in addition to the main genome, carrier of its own information, able to replicate itself and to transmit itself.
Primary wall: wall formed during the growth of a cell.
RUBISCO: an acronym for ribulose-bisphosphate carboxylase/oxygenase, the first enzyme of the Calvin cycle; it is probably the most important enzyme of the living world; this enzyme can catalyse the carboxylation or oxidation of ribulose-bisphosphate according to the value of the ratio of tissue concentrations of CO_2 and O_2. If $[CO_2] > [O_2]$, the enzyme will work as a carboxylase. If $[CO_2] < [O_2]$, the enzyme will work as an oxidase.
Second messenger: a substance released in the cytoplasm after the binding of an elicitor* or a ligand* with its membrane receptor. Second messengers are involved in the activation of enzyme systems and in the transduction of signals. Ca^{++} is an important second messenger.
Secondary phloem: processed sap conductive tissue differentiating from the cambium.
Stomatic conductance: the process of exchanges at the level of the stoma.
Stomium: zone of thin-walled cells through which the dehiscence of a sporangium or an anther is effected, thus allowing the release of spores or pollen. The stomium is located under the mechanical annulus in ferns, and between pairs of pollen sacs in seeded plants.
Suffrutescent: a herbaceous plant whose base is persistent and woody.

Symbiosis: cohabitation between living organisms with reciprocal benefits, e.g. lichens, mycorrhizae, Fabaceae root nodule bacteria.

Symplasm: assemblage of cells of the same tissue linked by plasmadesmata*

Transduction: transmission of a signal coming from outside the cell through the plasma membrane leading to a physiological response.

Transpiration: the process of water loss in the form of vapour into the atmosphere from plants mainly at the level of the stomata.

Trichomes: collective noun for a group of hairs or a coating of hair.

Uniseriate cells: cells arranged in a single row.

Uronic acid: acid resulting from the oxidation of the primary alcohol function of a sugar; e.g. glucuronic acid resulting from the oxidation of glucose.

Xylem: tissue providing for the flow of crude sap (wood).

References

Aeschimann D., Lauber K., Moser D.M., Theurillat J.P. (2004) Flora Alpina. Haupt, Berne.

Amaudric du Chaffaut S. (2008) La terre et la vie, quatre milliards d'années d'histoire. CRDP de l'Académie de Grenoble.

Ascough G.D., Nogemane N., Mtshali N.P., van Staden J. (2005) Flower abscission : environment control, internal regulation and physiological responses of plants. South African Journal of Botany 71(3&4), 287-301.

Atkinson C.J. (2014) Is xylem sap calcium responsible for reducing stomatal conductance after soil liming ? Plant Soil, 382, 349-356.

Augereau J.M. (2008) Les plantes médicinales In Hallé F et Lieutaghi P Aux origines des plantes, Tome 2. Fayard

Bahr A., Ellström M., Schnoor T.K., Påhlsson L., Olsson P.A. (2012) Long-term changes in vegetation and soil chemistry in a calcareous and sandy semi-natural grassland. Flora, 207, 379-387.

Barth J. G. (2019) Silicon, the long forgotten element of plants. Elemente der Naturwissenschaft N° 110 p.32-53.

Bauer P., Elbaum R., Weiss I.M. (2011) Calcium and silicon mineralization in land plants : transport, structure and function. Plant Science 180, 746-756.

*Benesch F., Wilde K.(*1983) Kiesel, Klak, Ton. Prozesse in mineral, Pflanze, Tier und Mensch. Verlag Urachhaus, Stuttgart.

Birkeland C.(2015) Coral reefs in the anthropocène. In Birkeland C, Coral reefs in the anthropocene, Springer Science + Business Media Dordrecht.

Bothe H. (2015) The lime-silicate question. Soil biology & biochemistry 89, 172-183.

Bouillard J.C. (2016) Les minéraux, sciences et collections. CNRS Editions, Paris.

Brice F. (2011) Les mots de la botanique. Actes Sud.

Brown S.L., Warwick N.W.M., Prychid C.J. (2013) Does aridity influence the morphology, distribution and accumulation of calcium oxalate crystals in Acacia (Leguminosae : Mimosoideae) ? Plant Physiology and Biochemistry 73, 219-228.

Campbell N., Reece J.B. (2004) Biologie. De Boeck, Bruxelles

Charpentier M., Oldroyd G.E.D. (2013) Nuclear calcium signaling in Plants 1. Plant Physiology 163(2), 496-503.

Choi W.G., Swanson S., Gilroy S. (2011) Calcium, mechanical signaling and Tip growth. In Luan S, Coding and decoding of calcium signals in plants. Springer, Berlin, Heidelberg.

Chytry M., Danihelka J., Axmanova I., Bozkova J., Hettenbergerova E., Li C.F., Rosbrojova Z., Sekulova L., Ticht L., Vymazalova M., Zeleny D. (2010) Floristic diversity of an eastern mediterranean dwarf shrubland : the importance of soil pH. Journal of Vegetation Science 21, 1125-1137.

Clarkson D.T. (1965) Calcium uptake by calcicole and calcifuge species in the genus Agrostis L. Journal of Ecology Vol 53, N° 2, 427-435.

Collin-Bellier C., Isambert M., Philippe M. (2010) Plantes, calcaire et calcium du sol. La Garance Voyageuse N°90.

Craddock C., Lavagi I., Yang Z. (2012) New insights into Rho signaling from plant ROP/Rac GTPases. Trend in cell biology vol 22 N°9 , 492-501.

Cukerzis J. (1984) La biologie de l'écrevisse (*Astacus astacus* L.). INRA publications, Versailles.

Dauer J.M., Perakis S.S. (2014) Calcium oxalate contribution to calcium cycling in forests of contrasting nutrient status. Forest Ecology and Management 334, 64-73.

Dayod M., Tyerman S.D., Leigh R.A., Gilliham M. (2010) Calcium storage in plants and the implications for calcium biofortification. Protoplasma 247, 215-231.

Diederich K, Riggers U (2003) Die Arnika. Der Merkurstab 56, N°2, 61-76.

Domingues L.S., Ribeiro N.D., Andriolo J.L., Possobom M.T.D.F., Zemolin A.E.M. (2016) Growth, grain yield and calcium, potassium and magnesium accumulation in common bean plants as related to calcium nutrition. Acta Scientiarum. Agronomy, Maringá v 38, N°2, 207-217.

Driscoll C.T., Lawrence G.B., Bulger A.J., Butler T.J., Cronan C.S., Eagar C., Lambert K.F., Likens G.E., Stoddard J.L., Weathers K.C. (2001) Acidic deposition in the northeastern United States : sources and inputs, ecosystem effects and management strategies. Bioscinec Vol 51 N°3, 180-198.

Du E., Dong D., Zeng X., Sun Z., Jiang X., de Vries W. (2017) Direct effect of acid rain on leaf chlorophyll content of terrestrial plants in China. Science of the Total Environment, 605-606, 764-769.

Ducerf G. (2015) L'encyclopédie des plantes bio-indicatrices. Vol 2 (3° édition), Editions Promonature 71110 Briant

Ducerf G. (2017) L'encyclopédie des plantes bio-indicatrices. Vol 1 (5° edition) et Vol 3 (2° edition), Editions Promonature 71110 Briant

Dumé G., Gauberville C., Mansion D., Rameau J.C., Bardat J., Bruno E., Keller R. (2018) Flore forestière française, tome 1, Plaines et collines. Centre National de la propriété forestière, Institut pour le développement forestier.

Epstein E. (1994) The anomaly of silicon in plant physiologie. Proc.Natl. Acad. Sci. USA Vol.91, 11-17.

Escudero A., Palacio S., Maestre F.T., Luzuriaga A.L. (2015) Plante life on gypsum : a review of its multiple facets. Biological reviews, 90, 1-18.

Franceschi V.R., Nakata P.A. (2005) Calcium oxalate in plants : formation and function. Annu. Rev. Plant Biol. 56, 41-71.

Faheed F., Mazen A., Abd Elmohsen S. (2012) Physiological and ultrstructural studies on calcium oxalate crystal formation in some plants. Turkish Journal of Botany, 37, 139-152.

Gargaud M., Martin H., Lopez-Garcia P., Montmerle T., Pascal R. (2009) Le soleil, la terre, la vie, la quête des origines. Belin, Paris.

Gehlig R. (1994) Kiesel, Kalium, Calcium und Wasser als Leitelemente in den Naturreichen. Ein Bild von Lebensvorgängen in der Gesteinwelt. Tycho de BraheJahrbuch für Goetheanismus. Tycho Brahe Verlag, Niefern-öschelbronn.

Gilliham M., Dayod M., Hocking B.J., Xu B., Conn S.J., Kaiser B.N., Leigh R.A., Tyerman S.D. (2011) Calcium delivery and storage in plant leaves : exploring the link with water flow. Journal of Experimental Botany Vol.62, N°7, 2233-2250.

Goguel J. (1959) La Terre. Encyclopédie de la Pléiade, Gallimard, Paris

Gounelle M. (2017) Les météorites. Que sais-je N° 3859, Presses Universitaires de France.

Grassé P.P., Doumenc D. (1993) Zoologie. 1. Invertébrés. Masson, Paris.

He H., Veneklaas E.J. Kuo J., Lambers H. (2014) Physiological and ecological significance of biomineralization in plants. Trends in Plant Science, Vol. 19, N°3, 166-174.

Himschoot E., Beeckman T., Friml J., Vanneste S. (2015) Calcium is an organizer of cell polarity in plants. Biochimica et Biophysica Acta 1853, 2168-2172.

Hopkins W.G. (2003) Physiologie végétale. De Boeck Université, Bruxelles.

Hubbard D.K. (2015) Reef biology and geology – not just a matter of scale. In Birkeland C, Coral reefs in the anthropocene, Springer Science + Business Media Dordrecht.

Huheey J., Keiter J.E., Keiter E.A. (1997) Inorganic chemistry : principles of structure and reactivity. Prentice Hall Editors (New Jersey, USA).

I*AAP - International Association of Anthroposophic Pharmacists* (2017) Anthroposophic pharmaceutical Codex. Med Pharm Scientific Publishers, Stuttgart.

Jamin A., Yang Z. (2011) Interactions between calcium and ROP signaling regulate pollen tube tip growth. In Luan S. Coding and decoding of calcium signals in Plants. Springer, Heidelberg, New York.

Jefferies R.L., Willis A.J. (1964) Studies on the calcicole-calcifuge habit : I. Methods of analysis of soil and plant tissues and some results of investigations on four species. Journal of Ecology, 52, 1, 1231-1238.

Jefferies R.L., Willis A.J. (1964a) Studies on the calcicole-calcifuge habit : II. The influence of calcium on the growth and establishment of four species in soil and sand cultures. Journal of Ecology, 52, 3, 691-707.

Johnsen O. (2016) Guide Delachaux des minéraux. Delachaux et Niestlé, Paris.

Jouy A., de Foucault B. (2016) Dictionnaire illustré de Botanique. Biotope, Mèze.

Kleiber T., Calomme M. & Borowiak K. (2015) The effect of choline-stabilized orthosilicic acid on microelements and silicon concentration, photosynthesis activity and yield of tomato grown under Mn stress. Plant Physiology and Biochemistry 96, 180-188.

Kolodziejek J., Patykowski J. (2015) The effect of temperature, light and calcium carbonate on seed germination and radicle growth of the polycarpic perennial *Galium cracoviense* (Rubiaceae), a narrow endemic species from southern Poland. Acta biologica cracobiensia, series botanica 57/1 : 70-81.

Labidi S., Jeddi F.B., Tisserant B., Debiane D., Rezgui S., Grandmougin-Ferjani A., Lounès-Hadj Sahraoui A. (2012) Role of arbuscular mycorrhizal symbiosis in root mineral uptake under $CaCO_3$ stress. Mycorrhiza, 22, 337-345.

Le Y., Jingzhe J., Chan Z.,Linrong J., Nenghui Y., Yusheng L., Guozheng Y., Ee L., Changlian P., Zhenghui h., Xinxiang P. (2010) Glyoxylate rather than ascorbate is an efficient precursor for oxalate biosynthesis in rice. Journal of Experimental Botany 61, 6, pp1625-1634.

Leurquin J. (2010) Plantes et calcaire. Les Naturalistes de Charleroi.be.

Liu T.W., Wu F.H., Wang W.H., Chen J., Li Z.J., Dong X.J., Patton J., Pei Z.M., Zheng H.L. (2011) Effects of calcium on seed germination, seedling growth and photosynthesis of six forest tree species under simulated acid rain. Tree Physiology 31, 402-413.

Ma J.F., Miyake Y. et Takahashi E.(2001) Silicon as a beneficial element for crop plants. In Datnoff LE, Snyder GH et Korndörfer GH Silicon in agriculture. Elsevier, New York.

Ma J.F., Yamaji N. et Mitani-Ueno N. (2011) Transport of silicon from roots to panicles in plants. Proc. Jpn. Acad., Ser B87, 377-385.

Mann S. (2001) Biomineralization, principles and concepts in bioinorganic materials chemistry. Oxford

Mansfield T.A., Hetherington A.M., Atkinson C.J. (1990) Some current aspects of stomatal physiology. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 55-75.

Masson P. (2015) Guide pratique pour la biodynamie. Editions Biodynamie Services, F-71250 Château.

Mazars C., Thuleau P., Cotelle V., Brière C. (2011) Calcium Signaling and homoeostasis in nuclei. In Luan S, Coding and decoding of calcium signals in plants. Springer, Berlin, Heidelberg.

McAinsh M.R., Pittman J.K. (2009) Shaping the calcium signature. New Phytologist, 181, 275-294.

McCormick P.V., Gibble R.E. (2014) Effects of soil chemistry on plant germination and growth in a northern everglades peatland. Wetlands 34 : 979-988.

Mehrabanjoubani P., Abdolzadeh A., Sadeghipour H.R. et Aghdasi M. (2015) Silicon affects transcellular and apoplastic uptake of some nutrients in plants. Pedosphere 25 (2), 192-201.

Meyer S., Reeb C., Bosdeveix R. (2013) Botanique, biologie et physiologie végétales. Maloine, Paris.

Morère J.L., Pujol R. (2003) Dictionnaire raisonné de biologie. Editions Frison-Roche, Paris.

Muller-Parker G., D'Elia C.F., Cook C.B. (2015) Interactions between corals and their symbiotic algae. In Birkeland C, Coral reefs in the anthropocene, Springer Science + Business Media Dordrecht.

Murray R.K., Bender D.A., Botham K.M., Kennelly P.J., Rodwell V.W., Weil P.A. (2013) Biochimie de Harper. De Boeck, Bruxelles.

Nakata P.A. (2015) An assessment of engineered calcium oxalate crystal formation on plant growth and development as a step toward evaluating its use to enhance plant defense. Plos ONE 10(10):e0141982. Doi:10.137/journal.pone.0141982.

Perakis S.S., Maguire D.A., Bullen T.D., Cromack K., Waring R.H., Boyle J.R. (2006) Coupled nitrogen and calcium cycles in forests of the Oregon coast range. Ecosystems 9, 63-74.

Perakis S.S., Sinkhorn E.R. (2011) Biochemistry of a temperate forest nitrogen gradient. Ecology 92(7), 1481-1491.

Perakis S.S., Sinkhorn E.R., Catricala C.E., Bullen T.D., Fitzpatrick J.A., Hynicka J.D., Cromack K. (2013) Forest calcium depletion and biotic retention along a soil nitrogen gradient. Ecological Applications, 23(8), 1947-1961.

Pinto E., Ferreira I. (2015) Cation transporters/channels in plants : tools for nutrient biofortification. Journal of Plant Physiology Vol 179, 64-82.

Raman V., Horner H.T., Kahn I.A. (2014) New and unusual forms of calcium oxalate raphide crystals in the plant kingdom. Journal of Plant Research127, 721-730.

Rameau J.C., Mansion D., Dumé G., Gauberville C. (2008) Flore Forestière Française-Tome 3 : Région méditerranéenne. Institut pour le développement forestier, Ministère de l'Agriculture et de la Pêche, Paris.

Reduron J.P. (2008) Ombellifères de France. Bulletin de la Société Botanique du Centre-Ouest, N° spécial, F-16200-Jarnac.

Rickles R.E., Miller G.L. (2005) Ecologie. Editions De Boeck Université, Bruxelles.

Rorison I.H. (1960) Some experimental aspects of the calcicole-calcifuge problem : I. The effects of competition and mineral nutrition upon seedling growth in the field. Journal of Ecology Vol 48, N°3, 585-599.
Rothwell S.A., Dodd I.C. (2014) Xylem sap calcium concentrations do not explain liming-induced inhibition of legume gas exchange. Plant Soil 382, 17-30.
Salisbury E.J. (1920) The significance of the calcicolous habit. Journal of Ecology, Vol 8, N°3, 202-215.
Schilperoord P. (2011) Metamorphosen im Pflanzenreich. Lesen im Buch der Verwandlungen. Verlag Freies Geistesleben, Stuttgart.
Serdar B., Demiray H. (2012) Calcium oxalate crystal types in three oak species (Quercus L.) in Turkey. Turk. J. Biol. 36, 386-393.
Simonis W.C. (1975) Wege zum Heilpflanzen-Erkennen. Mellinger Verlag, Stuttgart.
Simpson J.F.H. (1938) A chalk flora on the lower greensand : its use in interpreting the calcicole habit. Journal of Ecology Vol 26, N°1, 218-235.
Singh S., Katzer K., Lambert J., Cerri M., Parniske M. (2014) CYCLOPS, a DNA-biding transcriptional activator, orchestrates symbiotic root nodule development. Cell Host & Microbe 15, 139-152.
Steiner R., Wegmann I. (1925) Données de base pour un élargissement de l'art de guérir. Triades 1978, GA 27.
Steiner R. (1905) Grundelemente der Esoterik. Rudolf Steiner Verlag, Dornach 1987, GA 93a.
Steiner R. (1914) Okultes Lesen und okultes Höhren. Rudolf Steiner Verlag, Dornach 3. Auflage 2003, GA 156.
Steiner R. (1922) Menschenfragen und Weltenantworten. Rudolf Steiner Verlag, Dornach 2. Auflage 1987, GA 213.
Steiner R. (1923) Der Mensch als Zusammenklang des schaffenden, bildenden, gestaltenden Weltenwortes. Rudolf Steiner Verlag, Dornach 6. Auflage 1985, GA 230.
Steiner R. (1923) Mysteriengestaltung. Rudolf Steiner Verlag 4. Auflage Dornach 1987, GA 232.
Steiner R. (1920) Médecine et science spirituelle. Editions anthroposophiques romandes, Yverdon-les-Bains, 5° édition1976, GA 312.
Steiner R. (1924) Pédagogie curative. Editions anthroposophiques romandes, Yverdon-les-Bains 5 édition 1976, GA 317.
Steiner R. (1924) Geisteswissenschaftliche Grundlagen zum Gedeihen der Landwirtschaft. Rudolf Steiner Verlag, Dornach 8. Auflage 1999, GA 327.
Steiner R. (1980) Vom Leben des Menschen und der Erde. Über das Wesen des Christentums. Rudolf Steiner Verlag, Dornach 2. Auflage 1980, GA 349.
Stephens W.E. (2012) Whewellite and its key role in living systems. Geology Today Vol. 28, N°5, 180-185. University Press.
Suty L. (2015a) Les végétaux : les relations avec leur environnement. Editions Quae, Versailles.
Suty L. (2015b) Les végétaux : des symbioses pour mieux vivre. Editions Quae, Versailles.
Thomas R., Busti D., Maillart M. (2016) Petite flore de France. Editions Belin.
Tuteja N., Mahajan S. (2007) Calcium signaling network in plants. Plant Signaling and Behavior, 2, 2, 79-85.
Webb A.A.R., Robertson F.C. (2011) Calcium signals in the control of stomatal movements. In Luan S, Coding and decoding of calcium signals in plants. Springer, Berlin, Heidelberg.
Wenk E.H., Dawson T.E. (2007) Interspecific differences in seed germination, establishment and early growth in relation to preferred soil type in an alpine community. Artic, Antarctic and Alpine research Vol 39, N°1, 165-176.
Zhu J.K. (2016) Abiotic signaling and responses in plants. Cell 167, 6, 313-324.