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Anaerobiosis and Carbohydrate Status of the Embryonic Axis of Germinating Cucumber Seeds

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Abstract. **The effect of anaerobiosis, imposed during germination of 'Calypso' cucumber (*Cucumis sativas L.*) seeds, was studied. Anaerobic conditions inhibited reserve mobilization from the cotyledons and dry weight gain by the embryonic axis. Within the embryonic axis, lipid degradation was stopped and use of all readily metabolizable carbohydrate reserves was strongly stimulated. By 48 hr of exposure to an anaerobic environment, the axis was nearly depleted of endogenous carbohydrate reserves. Aerobically germinating seeds accumulated a massive concentration of hexose sugars within the axis during the same time period. Thus, growth inhibition within cucumber seeds during anaerobiosis may result in part from carbohydrate deprivation of the embryonic axis.**

Anaerobic conditions markedly inhibit growth of germinating seeds of many species. Upon imbibition of water by seeds, O_2 must diffuse through an aqueous barrier to reach the embryo, and the early phases of germination are often characterized by a fermentation process that is not necessarily detrimental to germination if anaerobiosis does not persist too long (2). The influence of temperature on O_2 solubility in water strongly influences the amount of O_2 available to the embryo. At higher temperatures, there is a greater need for O_2 to support more rapid growth of the embryo, and, at the same time, there is less available due to its diminished solubility (2). Under conditions of prolonged excess soil water resulting in an anaerobic soil atmosphere, seed germination and stand establishment may be adversely affected. It has been demonstrated that genetic progress is possible for improved low-temperature germination in cucumber (6, 8). Perhaps genetic variation may exist for low-O_2 germination as well; it is desirable to more fully understand the basis of anaerobic inhibition of seed germination.

A recent study suggested that the success of seeds of various species to germinate at very low O_2 concentration may be related to the nature of the seed food reserve (1). Whereas seeds containing predominantly a starch food reserve were able to germinate slowly at low-O_2 concentration, seeds with a predominant lipid food reserve germinated very poorly. In lipid-containing seeds, the adenylate energy charge dropped sharply at low-O_2 concentration, but dropped to a much lesser extent in starch-containing seeds.

During anoxia there is a sharp rise in the concentration of fructose-2,6-bisphosphate in the lipid containing endosperm of castor bean (3). This regulatory metabolite strongly stimulates glycolysis by activating pyrophosphate-dependent phosphofructokinase. The conversion of lipids to glucose is inhibited by anaerobic conditions because of the requirement in β-oxidation for O_2 for the regeneration of oxidized cofactors. This anaerobic inhibition of glucose formation is further reinforced due to inhibition by fructose-2, 6-bisphosphate of fructose-1,6-bisphosphatase, a key enzyme in the reversal of carbon flow through glycolysis during gluconeogenesis (3).

Thus, under prolonged anaerobiosis, with gluconeogenesis inhibited and glucose consumption stimulated, it is possible that the embryonic axis of a lipid-containing seed might become deprived of metabolizable carbohydrate, which, in turn, might contribute to decreased growth. The predominant food reserve in mature cucumber seeds is lipid (7) rather than starch. This study was undertaken to examine embryonic axis growth as well as carbohydrate and lipid status of aerobically and anaerobically germinating cucumber seeds.

Seeds of 'Calypso' cucumber were visually selected for uniformity of size. Samples of 35 seeds were placed into petri dishes between moist Whatman no. 1 filter papers and aerobically germinated in darkness at 25°C. An anaerobic treatment was initiated 24 hr after imbibition. The petri dishes containing the germinating seeds were placed in desiccators that then were flushed thoroughly with N_2 gas through fittings in a rubber stopper in the glass desiccator lid. A beaker containing a 20% (by weight) KOH solution and a fluted filter paper were placed in each desiccator to absorb CO_2 gas produced by the seeds. Seeds were sampled from the aerobic treatment before imbibition of water and at 12-hr intervals thereafter up to 72 hr after imbibition. Anaerobic seeds were sampled at 36, 40, 60, and 72 hr after imbibition. At each sampling time, three petri dishes were taken from each treatment and treated as triplicate samples. These dishes were placed on ice, where seed coats were removed as necessary and each seedling was divided into cotyledon and embryonic axis tissue. This sampling required ≈0.5 hr. The samples were frozen and subsequently lyophilized. Dried samples of tissues were weighed, and the embryonic axis tissue ground to a powder at

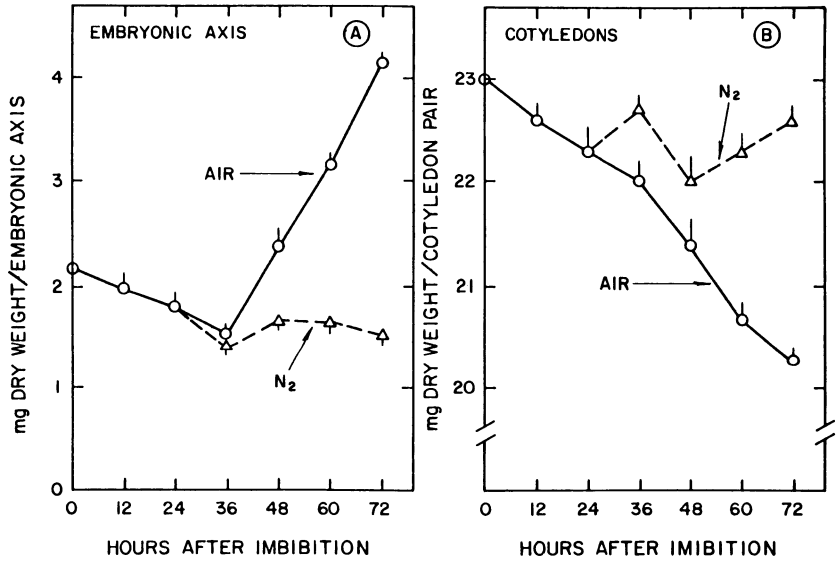

Fig. 1. Comparison of dry weight changes in (**A**) the embryonic axis and (**B**) the cotyledons of cucumber seeds germinating under aerobic (Air) or anaerobic (N_2) conditions. Vertical bars represent 1 SE of the mean of three dishes of seeds.

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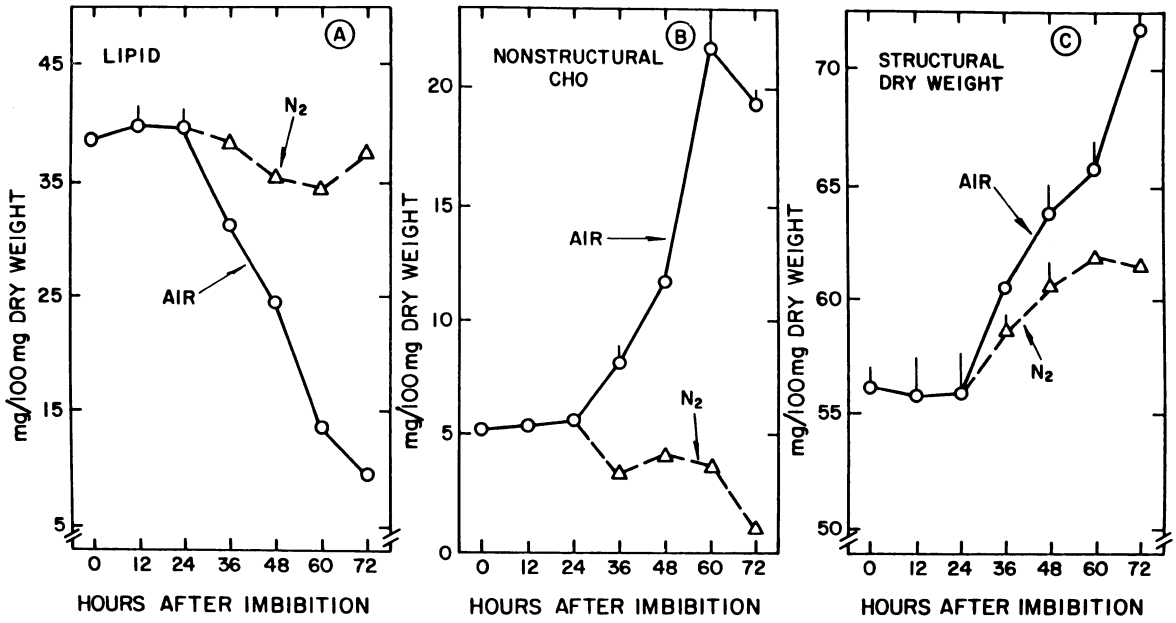

Fig. 2. Changes in the concentrations of (**A**) lipid, (**B**) nonstructural carbohydrate, and (**C**) structural dry weight in the embryonic axis of cucumber seeds germinating under aerobic (Air) and anaerobic (N_2) conditions. Nonstructural carbohydrate represents the sum of starch, raffinose saccharides, sucrose, and hexose sugars, and this fraction represents readily metabolizable carbohydrate. Structural dry weight is the residual fraction of dry weight not accounted for by lipid and nonstructural carbohydrate. Vertical bars represent 1 SE of the mean of three dishes of seeds.

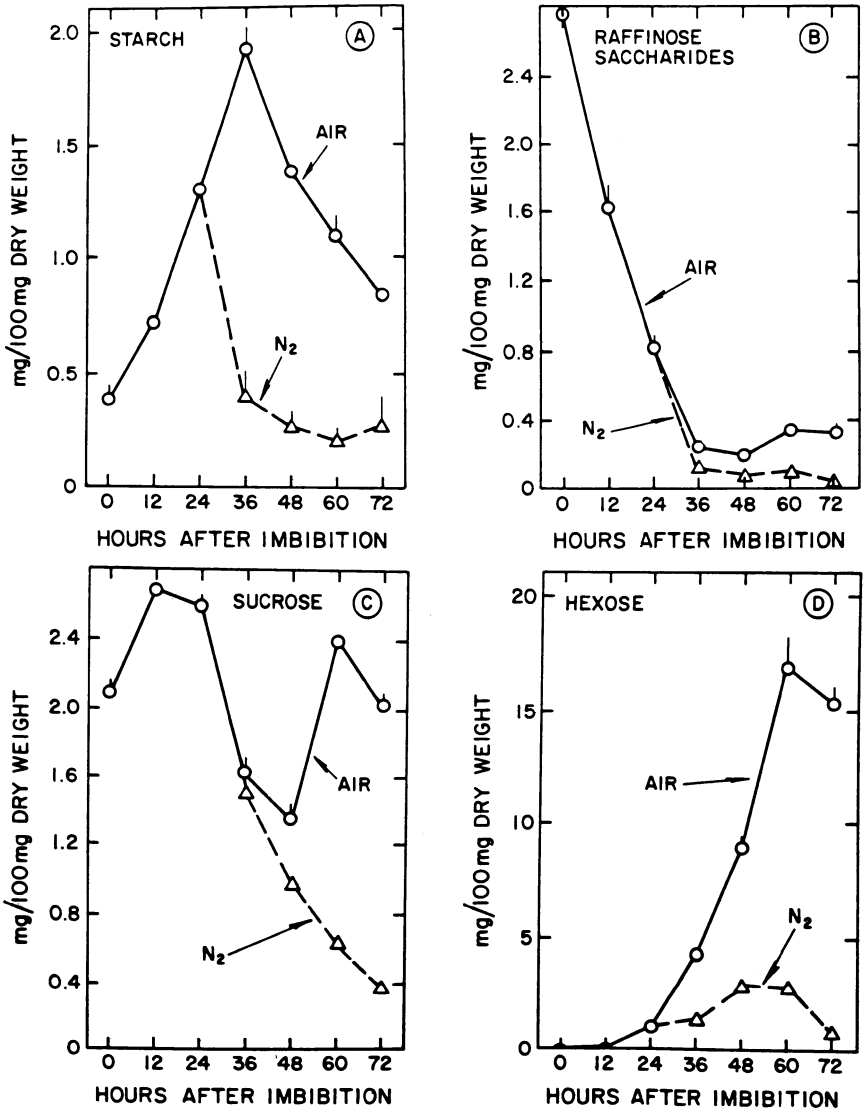

Fig. 3. Changes in the concentrations of (**A**) starch, (**B**) raffinose saccharides; (**C**) sucrose, and (**D**) hexose sugars in the embryonic axis of cucumber seeds germinating under aerobic (Air) or anaerobic (N_2) condtions. Vertical bars represent 1 SE of the mean of three dishes of seeds.

room temperature in a mortar.

Lipid was extracted with petroleum ether from 50 mg of the powdered embryonic axis tissue as described for soybean seeds (5). Soluble sugars were then extracted from the defatted tissue residue with 80% aqueous ethanol (v/v). Stachyose, raffinose, and sucrose were measured using high-performance liquid chromatography (5) and hexose sugars (glucose and fructose) were measured from this extract using an enzymatic assay (4). After the removal of soluble sugars, starch in the tissue residue was gelatinized and then digested to glucose with amyloglucosidase (Sigma), and glucose was measured enzymatically (4).

The overall effect of anoxia on dry weight partitioning between cotyledons and the embryonic axis of germinating cucumber seeds is shown in Fig. 1. During the first 36 hr of germination in air, the embryonic axis of cucumber seedlings lost dry weight (Fig. 1A). Thereafter, dry weight increased linearly, up to at least 72 hr after imbibition. In air, the cotyledons lost dry weight continuously, presumably representing in large part mobilization of stored reserves to the growing axis (Fig. 1B). Gain in dry weight by the embryonic axis was largely inhibited by the imposed anoxia at 24 hr (Fig. 1A), and dry weight loss by the cotyledons was largely eliminated by anoxia (Fig. 1B).

The embryonic axis of mature cucumber seeds contained a very significant lipid reserve comprising almost 40% of the dry weight of the axis tissue (Fig. 2A). This reserve decreased in concentration rapidly in aerobically germinating seeds while anoxia prevented this decline (Fig. 2A). Nonstructural carbohydrate, representing readily metabolizable carbohydrate, increased markedly after the first 24 hr of germination in aerobic seeds. In contrast, nonstructural carbohydrate declined in anaerobic seeds to a value of less than 1% of the dry weight of the

embryonic axis by 72 hr (Fig. 2B). The residual fraction of the axis dry weight, termed structural dry weight, increased to a much greater extent in aerobic seeds than in anaerobic seeds (Fig. 2B).

More detailed changes in the components that comprise the nonstructural carbohydrate fraction of the embryonic axis are shown in Fig. 3. Starch concentration in the mature embryonic axis was very low (Fig. 3A). Starch was synthesized for the first 36 hr after imbibition within the embryonic axis of seeds germinating in air, and starch declined rapidly thereafter. Anaerobiosis caused a sharp decline in starch concentration. Raffinose saccharides, i.e., stachyose plus raffinose, rapidly declined in concentration during the first 36 hr after imbibition, and anaerobiosis resulted in a slightly lower concentration of these sugars than in aerobic conditions (Fig. 3B). Two peaks in sucrose concentration were observed in aerobic seeds, but anaerobiosis resulted in a marked decline in sucrose (Fig. 3C). By far, the greatest change in carbohydrate observed was a substantial increase in hexose sugar in the aerobic embryonic axis (Fig. 1D). This increase was markedly inhibited in seeds under anoxia. By 72 hr after imbibition, the hexose concentration was <1 mg/100 mg dry weight of embryonic axis, whereas this value was >15 mg/100 mg for the aerobic seeds.

Low metabolizable carbohydrate in the embryonic axis does seem to be a factor resulting from as little as 48 hr of anaerobic conditions (Fig. 2B). Several factors contributed to the lack of metabolizable carbohydrate. Mobilization of reserves from the cotyledons to the growing axis was markedly inhibited by anoxia, and lipid degradation within the embryonic axis itself was also strongly inhibited by anoxia (Figs. 1A and 1B, Fig. 2A). Simultaneously, imposition of anaerobic conditions resulted in a marked depletion in all of the readily available carbohydrate pools within the embryonic axis. Based on these observations, it is plausible that growth inhibition under anaerobiosis may result in part from a lack of carbohydrate for carbon-backbones needed for the many anabolic syntheses in new cells and tissues leading to structural dry weight gain of the axis. A lack of carbohydrate might also contribute to the lower adenylate energy charge in anaerobic seeds (1).

Unlike lipid degradation, starch degradation was not inhibited in the axis of germinating cucumber seeds (Fig. 2A and Fig. 3A). It would be of interest in future studies to compare soluble carbohydrate provision to the growing axis of starch containing as well as lipid-containing species of seeds.

Literature Cited

1. Al-Ani, A., F. Bruzan, P. Raymond, V. Saintges, J. Marcleblanc, and A. Pradet. 1985. Germination, respiration and adenylate energy charge of seeds at various oxygen partial pressures. Plant Physiol. 79:885–890.
2. Come, D. and T. Tissaoui. 1973. Interrelated effects of imbibition, temperature and oxygen on seed germination, p. 157–169 In: W. Heydecker (ed.). Seed ecology. Proc. 19th Easter School in Agr. Sci., Univ. Nottingham 1972. Vol. 9.
3. Kruger, N.J. and H. Beevers. 1985. Synthesis and degradation of fructose 2,6–bisphosphate in endosperm of castor bean seedlings. Plant Physiol. 77:358–364.
4. Pharr, D.M., S.C. Huber, and H.N. Sox. 1985. Leaf carbohydrate status and enzymes of translocate synthesis in fruiting and vegetative plants of *Cucumis sativus* L. Plant Physiol. 77:104–108.
5. Saravitz, D.M., D.M. Pharr, and T.E. Carter, Jr. 1987. Galactinol synthase activity and soluble sugars in developing seeds of four soybean genotypes. Plant Physiol. 83:185–189.
6. Staub, J.E., J. Nienhuis, and R.L. Lower. 1986. Effects of seed preconditioning treatments on emergence of cucumber populations. HortScience 21:1356–1359.
7. Trelease, R.N., W.M. Becker, P.J. Gruber, and E.H. Newcomb. 1971. Microbodies (glyoxysomes and peroxisomes) in cucumber cotyledons. Plant Physiol. 48:461–475.
8. Wehner, T.C. 1984. Estimates of heritabilities and variance components for low-temperature germination ability in cucumber. J. Amer. Soc. Hort. Sci. 109:664–667.

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Embryo Development in 'Tonda Gentile delle Langhe' Hazelnut

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Abstract. **'Tonda gentile delle Langhe' hazelnut bloomed from 22 Dec. to 19 Jan. Fertilization occurred about 5 months later, in the last 10 days of May. In the first days of June, the embryo was globular whereas the free nuclear endosperm had started to become cellular. Around the middle of June the embryo was heart-shaped and the endosperm was entirely cellular. In the following 2 weeks, the endosperm became vacuolated and then disintegrated. Cotyledons formed (torpedo stage) and grew quickly, filling the ovule by the end of June. The seed had its final shape and dimension by 20 July, but the embryonal axis continued elongating until nut maturity, 15 to 30 Aug. Mature embryos contained six to eight leaf primordia, the apical meristem, and the radicle.**

Reproductive development in hazelnut (*Corylus avellana* L.) has previously been described only to the early stages of embryo sac development. Davis (2) and Thompson (4) report that in the first stages of development the endosperm is free nuclear and becomes cellular shortly after the embryo begins to grow. Reports on the time interval between first division of the endosperm and first mitotic division of the zygote range from 4 to 8 days (3–5).

This paper presents the development of the ovule, endosperm, and embryo in 'Tonda gentile delle Langhe' from the stage of the embryo sac being ready for fertilization, to nut maturity (mid-August). 'Tonda gentile delle Langhe' is the most important cultivar in Italy for excellent quality of nuts and its production is mainly destined for processing. In North Italy bloom and pollination of 'Tonda gentile delle Langhe' can occur from the last 10 days of December to the first days of February, depending on environmental conditions. According to Benson (1) 3 to 4 months elapse between pollination and fertilization in *Corylus*.

In 1984, developing fruits were collected at the Nasio Farm in Cravanzana (Cuneo, Italy), from 18-year-old plants. Female flowers bloomed from 22 Dec. to 19 Jan. Thirty fruit clusters were picked every second day from 21 May to 30 July and then weekly until 30 Aug. All samples were fixed in formalin–acetic acid–alcohol (FAA) for 1 day and then treated with 8 N NaOH from 1 to 8 days, depending on stage of nut development. Nuts were sectioned at 10 to 15 μm and stained with safranin-fast green or with the periodic acid-Schiff's reaction. For each developmental stage, ovule, nucellus, embryo sac, embryo, and embryonal axis length were measured using 15 ovules and the average value was calculated.

On 21 May the ovule was about 0.7 mm

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