METABOLIC CHANGES DURING AFTER-RIPENING IN *PRUNUS CERASUS* SEEDS

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ABBREVIATIONS

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
DNP	2,4-Dinitrophenol
EMP pathway	Embden-Meyerhof-Parnas pathway
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced)
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
PP pathway	Pentose phosphate pathway
RQ	Respiratory quotient
TCA cycle	Tricarboxylic acid cycle

ABSTRACT

Seed dormancy in _Prunus cerasus_ seed is primarily due to embryo dormancy. The emrbryo will produce a normal seedling when given 10 weeks of after-ripening. The seed coat tends to prevent or delay germination of a considerable number of these non-dormant embryos. Because of physiological differences, metabolic changes in cotyledons and embryonic axes were studied separately.

During after-ripening the lipid level of embryonic axes decreased whereas the dry weight, total sugar and rate of respiration increased. There was also a change in the pathway of glucose oxidation as indicated by a decreasing C6/C1 ratio. The increased participation of the pentose phosphate in the total respiration is believed to be due to an increase in the availability of NADP. In cotyledons similar but less pronounced changes were observed in respiration and substrate breakdown. Their dry weight decreased and the activity of the pentose phosphate pathway remained unchanged.

INTRODUCTION

A viable seed which fails to germinate under suitable environmental conditions is considered to be dormant. Delayed germination is advantageous to many plants in nature, as it enables the reproductive structure to withstand unfavourable environmental conditions, and in addition provides the necessary time interval for the dissemination of the seed and spread of the species.

Dormancy at the same time causes inconvenience to man in the testing of viability of seeds. Dormant seeds of horticulturally significant plants are problems to nurserymen and propagators. Plant breeders and geneticists, anxious to grow as many generations of a species or variety in as short a time as possible, are also faced with the problem of dormancy in seeds.

The general problem of dormancy has been extensively studied with such organs as buds, seeds and tubers. Dry seed is the most favourable material because it contains an embryonic plant in an arrested condition, fully equipped with its store of organic materials, minerals and growth factors and dependent upon the external environment only for water, oxygen, a favourable temperature and light. While the seed may be considered functionally to be a closed system, it can not be treated as a homogeneous system since it is structurally complex with physiological specialization closely associated with this structural

complexity. Generally speaking the potential for active synthesis and growth is restricted to the embryonic axis, while the cotyledons are primarily concerned with the storage and subsequent mobilization of reserve materials. For a real understanding of dormancy, therefore, the physiology of these two organs should be studied separately, while their interactions can subsequently be determined.

The changes from the dormant to the non-dormant state involves major alterations in the metabolism of embryonic tissues. Shifts in the pattern of metabolism (chemical differentiation) may be linked to the nucleic acids and may be of a similar nature to that found in the induction of flowering where nucleic acids appear to be an important controlling factor (7). The data in the literature concerning the influence of after-ripening treatment on such metabolic shifts, and their functional value, are very scanty. The present work, therefore, was initiated to clarify this important aspect and to seek evidence to explain mechanisms responsible for dormancy termination.

Cherry seed (<u>Prunus cerasus</u> L.) was chosen as a suitable experimental material since it contains a well developed embryonic axis, has a definite rest period and is readily available.

LITERATURE REVIEW

The phenomenon of seed dormancy has been a problem faced by man from his earliest attempts to cultivate plants. Within the last fifty years concerted attempts have been made to determine the specific factors involved in this plant growth mechanism. Dormancy can be attributed to a number of physical and biochemical factors which individually or in combination act as blocks to germination. During after-ripening the embryo undergoes certain metabolic changes at the completion of which germination will occur. Eckerson (20) defined after-ripening in the following statement:

> A few seeds have been studied which do not grow when all coats have been removed and the embryo put in good germinating conditions. Some change within the embryo is necessary before germination, that is, lengthening of the hypocotyl, can take place. This process is what we mean by 'after-ripening'.

This type of seed dormancy may be referred to as embryo dormancy.

Factors such as seed coat effects, gas exchange, light, temperature, moisture etc., affect the order and the speed of such metabolic changes. In some cases the presence of endogenous growth inhibiting compounds has been detected which have been shown to prevent or delay germination. The blocks to germination due to these factors are

elininated under natural conditions by time, temperature changes, leaching, decay of surrounding fruit tissue, and light. The physiological effects of these factors, individually or in combination, have been studied extensively.

It has been proposed that the seed coat delays germination because it offers mechanical resistance to the expansion of the embryo and other tissues (17), resists gaseous exchange (34), and may contain growth inhibitors (34). Crocker (16) reported that one of the most complete blocks to seed germination in a number of plant families including Malvaceae, Cannaceae and Solanaceae is seed coat impermeability to water. Crocker et al (17) showed that walnut embryos must exert enormous pressure to overcome the mechanical resistance offered by its shell to the expansion of the growing embryo. Wellington (62) arrived at similar conclusions and reported that covering layers ofwheat grains if not disrupted during after-ripening will prevent expansion of the embryo until the water absorbing capacity has been increased by the transfer of reserves from the endosperm. Miyamota et al (34) concluded that wheat seed dormancy is due to seed coat impermeability to oxygen and water and to their content of inhibitors. Conventional germination results could be obtained in a few days, greatly reducing the time required for after-ripening if the endocarp and other seed coverings were removed. The work of Corns and Schraa (14) indeed showed

that the removal of endocarp of silverberry seeds increased germination four-fold. Hilton *et al* (25) also reported that scarification of the seed coverings of *Prunus pennsylvanica* L, *Amelanchier laevis* Weig and *Sorbus aucuparia* with concentrated sulphuric acid for 10-20 minutes resulted in early and higher germination percentages.

Thornton (51) attributed the beneficial effects of removing the seed coats to improved gas exchange conditions. She used controlled gas mixtures of oxygen, carbon dioxide, nitrogen and hydrogen and reported that in cocklebur (Xanthium) secondary dormancy was induced in the moist seeds only when oxygen was absent from the atmosphere. The same worker (52) in a subsequent paper advanced the idea that secondary dormancy may have its inception in the accumulation of intermediate products that are formed by partial anaerobic respiration. Wareing and Fonda (60) have indicated that supplied oxygen was utilized for the removal of the inhibitors present in the embryos of cocklebur. Black (4) had similar findings with seeds of *Avena fatua*. Vegis (58) and Pollock (40) suggested that restricted supply of oxygen in dormant buds tends to limit the oxidative breakdown of pyruvate in the TCA cycle. The elevated levels of pyruvate are then utilized in the formation of compounds such as ethyl alcohol and acetaldehyde which act as growth

inhibitors (40). Besides the expected alteration in chemical reactions when seeds are held in conditions of partial oxygen deficiency, Steinitz (48) has reported an influence of after-ripening atmosphere upon chromosome reproduction and consequent cell division necessary for germination. These effects were observed in barley and no report is known where this variable has been studied on dicotyledonous seeds.

Black (4) indicated that in _Avena fatua_ the loss of dormancy by oxygen action, and the onset of secondary dormancy by anaerobiosis are correlated with changes in the content of an endogenous inhibitor, which blocks the accumulation of sugar and its utilization in growth. Naylor and Simpson (37) reversed the action of this inhibitor with gibberellic acid treatment. Thus a balance between inhibitory and stimulatory substances seems to be involved in the control of germination. Wareing and Villiers (61) supported this view and reported that dormancy breaking effects of chilling on _Fraxinus excelsior_ seed are not correlated with any reduction in the inhibitor, but with the appearance of promoters which counteract its action. LaCroix (37) reported that in _Polygonum pensylvanicum_ there is an intricate pattern of interaction between germination inhibitors and embryo dormancy that determines the overall seed dormancy. Naylor and Christie (36) and Kommedhal _et al_ (31)

reported that aqueous extracts of wild oats are inhibitory to seedling growth. Knowles and Zalik (29) have shown that embryo dormancy in seeds of Viburnum trilobum Marsh is caused by one or more growth inhibiting chemicals and the progress of germination will continue to be sluggish until the inhibitor is leached away or decomposed or otherwise inactivated. This normally is a function of time, temperature, humidity, and gaseous exchanges and the time may be shortened b y seed coat scarification to permit entry of water and the egress of leachate from within the protective seed covering. Hilton et al (25) reported that extracts from resting Amelanchier laevis Weig and Sorbus aucuparia L seeds revealed the presence of unidentified growth inhibitors which were presumed to be primary reasons for prolonged seed dormancy. Wheeler (63) showed that aqueous extract fractions from seeds of sugar beet have a growth promoting effect when applied, in small quantities, and will inhibit growth when large amounts of extracts are applied. Miyamota et al (34) isolated and crystallized an inhibitor from the wheat seed coat which in concentrations of 50 ppm inhibited the germination of wheat embryos. A number of naturally occurring inhibitors have been identified: ammonia produced by beet seeds, hydrogen cyanide from the seed constituent amygdalin, ethylene from ripe fruits,

essential oils, unsaturated lactones, coumarin, parascorbic acid, phthalids etc. Of these, unsaturated lactones are among the most active. The literature on germination inhibitors has recently been reviewed by Evenari (21) and Toole et al (54).

Certain seeds have specific light requirements which in all probability are linked to chemical reactions. Visible light may inhibit or favour germination of certain dormant seeds. Kinzel (27) reported that out of 964 species of seeds he studied, 672 were light stimulated and 258 were inhibited by light. Seeds such as corn, bean and clover germinate equally well in light and darkness. Navez (35) obtained evidence that light had a stimulatory effect on the production of growth substances. Skoog and Thimann (45) concluded that light favours the formation of growth substances in plants and darkness favour their physiological activity. Kahn (26) indicated that light sensitivity affects water uptake. Borthwick et al (8) studied the action of light on lettuce seed germination and found it to be influenced in a reversible manner by radiation in the red and far red. The main mechanism involved is the now well established low energy, reversible red/far red mechanism (54) in which the pigment phytochrome participates. The subject of light effects on dormancy has been reviewed in great detail by several authors (30,53,54).

Failure of seed germination may also be due to metabolic blocks to embryo growth, originating in the embryo itself or in tissues external to the embryo. In seeds of most deciduous tree fruits these metabolic changes occur in the main at temperatures above freezing, ranging from $1^{o}C$ to $15^{o}C$ (20,54,55). The induction or repression of enzyme activity or the competition of different enzymes for the same substrate or coenzyme are among the important factors governing the operation of metabolism. The metabolic changes taking place during after-ripening, therefore, must bring about or be due to alterations in the levels of the above mentioned factors. Eckerson (20) reported that in several species of Crataegus seeds, an early change noted was an increase in acidity, which was correlated with a greater water holding power. Catalase and peroxidase activity increased. Whether the acidity increase is of significance to dormancy or is merely correlative is not known. Green (23) has shown that it may lead to the liberation of enzymes. The increase in catalase and peroxidase activity during after-ripening was also reported from Flemion's (22) work with seeds of Sorbus aucuparia. Lasheen and Blackhurst(33) found that starch decreased while sucrose and reducing sugars increased during after-ripening period of blackberry. This carbohydrate transformation

took place very slowly when the seeds were after-ripened at room temperature, but was greatly accelerated at low temperature. The enzymatic activity of α and β amylase responsible for starch breakdown also showed the same trend. Srivastava and Meredith (46) suggested that gibberellic acid treatment had no effect on the levels of amylase, ascorbic acid and glutathione, but increased production of amylase. Naylor and Simpson (37) reported that natural inhibitors of germination in *Avena fatua* involved restriction of both accumulation and utilization of sugar in growth. In a subsequent paper they (44) concluded that gibberellic acid could overcome these metabolic blocks by inducing the synthesis of maltase or in some way activating the preformed enzyme. Klein (28) reported an increase in soluble nitrogen only in those lettuce seeds which germinated and not in those which remained dormant. Also the content of amino acids increased near germination. Factors such as light which promote lettuce seed germination decreased the protein/soluble nitrogen ratio while inhibitors such as coumarin prevented changes in this ratio. Herich (24) reported qualitative and quantiative changes in amino acid content when seeds were treated with gibberellic acid. In soybeans, a rise in protease activity in cotyledons in the early stages of germination has been reported by Tazawa and Hirokawa (50).

A decrease in lipid and a simultaneous increase in sugars was reported by Eckerson (20). It, however, is not clear if the lipid is converted to sugars via the glyoxylate cycle in the after-ripening seeds, as has been found in germinating castor bean endosperm (12).

Olney and Pollock (38) have shown that during after-ripening at 5°C, total nitrogen and phosphorus increased and were translocated from the storage organ to the potentially growing organs of the cherry seed. The translocation of phosphates, in excess of growth requirements, was found to be especially important and this accumulation increased with after-ripening time. The accumulation of phosphate compounds in embryonic organs after-ripened at 5°C showed a pattern consistent with the movement of inorganic phosphate and nucleotides into more stable protoplasmic compounds such as nucleic acids. In seeds kept at 25°C, and not progressing towards germination, the data suggested a breakdown of compounds of this type and accumulation of inorganic phosphate. The same workers (42) suggested that one of the after-ripening effects which promoted termination of dormancy was an increase in phosphate acceptors to maintain or increase the closeness of coupling between respiration and synthesis. They further opined that dormancy might result from a block in the system of energy metabolism. Pollock (41) obtained similar results in leaf

primordia of maple buds.

The data showing the changes in levels of coenzymes in after-ripening seed, which also play an important role in controlling the mode and speed of metabolism is very scanty. The significance of the changes in coenzyme levels, however, has been studied in germinating seedlings and various other plant organs. Yamamoto (64) reported that the ratio of endogenous NAD+NADH to NADP+NADPH levels was a rate limiting factor for the metabolism of various plant tissues. In the cotyledons of *Vigna sesquipedalis*, during germination, most of NAD was present in the oxidised form and NADP in the reduced form. He concluded that the NADP+NADPH/NAD+NADH quotient is low in storage organs (cotyledons and endosperm) and high in growing parts. Brown (10) also reported that in the seeds of *Pisum sativum* L during the last phase of germination (after 40 hr), leading to the emergence of radicle, NADP+NADPH/NAD+NADH ratio increased while AMP, ADP and ATP contents decreased.

Studies on the mechanism of respiration have shown that in seeds, as in other organs, glycolysis, the TCA cycle and the pentose phosphate cycle all seem to be operating (30). However, these pathways do not seem to be equally important at all stages. Prior to seed germination the direct oxidation of glucose phosphate has been shown to be the major oxidative pathway and its relative importance decreased

during growth and development of the seedling, giving way to glycolysis and the TCA cycle (13). Glycolysis and fermentation was reported (39) in pea cotyledons due to the existence of partial anaerobic conditions in this organ. Bradbeer and Colman (9) reported that TCA cycle activity did not seem to be critical in the after-ripening of Corylus avellana seeds. However, the results with acetate-2-C^{14} indicated that the active synthesis of aspartate and glutamate via the TCA cycle may be a necessary part of the progress of after-ripening. Beevers and Gibbs (3) examined various plant materials and in a great many of them showed clear evidence for the participation of the PP sequence. In general older aerial parts showed an increased traffic of glucose through the PP sequence; meristematic tissues were the only ones where glucose breakdown was predominantly by the EMP sequence. The functional importance of these alternate pathways in respiration, so far, is quite obscure.

MATERIALS AND METHODS

Seed of sour cherry, *Prunus cerasus* L. of the family Rosaceae was obtained from the 1962, British Columbia commercial crop. The material was dried and stored at 5°C. The same lot of seed was used in all the experiments reported. The sour cherry fruit contains a single seed enclosed by a thin seed coat and a hard endocarp ½-1 mm thick. The embryo is composed of a very large pair of cotyledons and a small well developed embryonic axis. The weight ratio of cotyledons to embryonic axis is approximately 100:1. The portion referred to as embryonic axis includes root primordium, hypocotyl and shoot apex.

RESPIRATION STUDIES

Embryos were isolated, at weekly intervals, from seeds after-ripening at 1°C in a moist, sand-peat mixture (1:1). These embryos were separated into embryonic axes and cotyledons which were used for respiration studies. Duplicate lots of 25 embryonic axes and 10 cotyledons were used in each experiment. Oxygen uptake at 25°C was used as a measure of respiration and was determined by a Warburg respirometer. The use of the apparatus is based on the principle that at constant temperature and constant gas volume any changes in the amount of a gas can be measured by changes in its pressure. The procedure and the details for oxygen uptake calculations were taken from Umbreit

et al (56). The tissue was incubated in 1 ml of potassium phosphate buffer, pH 5.5. Carbon dioxide was absorbed in 0.2 ml 20% potassium hydroxide in the central well of the Warburg flask.

Carbon dioxide determinations were carried out by the paired flask method of Dixon (18). The respiratory quotient (R.Q.) of the tissue was calculated as a ratio of carbon dioxide evolved to oxygen consumed. These data gave a rough indication of the substrate respired.

DETERMINATION OF PARTICIPATION OF PENTOSE PHOSPHATE (PP) PATHWAY AND EMP PATHWAY

To determine PP and EMP pathway contributions, the C6/C1 ratio was determined, both in embryonic axes and cotyledons, at weekly intervals of the after-ripening period. The seeds being after-ripened were washed with running water, their endocarp and seed coats were removed, and embryonic axes separated from cotyledons. The separated tissues were stored in water prior to the initiation of the experiment. All determinations were made in duplicate. The general procedure was the same as indicated for respiration studies except that 5 umoles (0.1 uc) glucose-1-C^{14} or glucose-6-C^{14} was added to the incubation mixture.

COLLECTION OF RADIOACTIVE CARBON DIOXIDE

Potassium hydroxide containing radioactive carbon

dioxide was removed from the center well at 1,2,4 and 8 hr after the start of the experiment. The absorbed carbon dioxide was converted to barium carbonate by the method described by Steele and Sfortunato (47) which in brief consists of transferring potassium hydroxide to a 15 ml centrifuge tube containing 8 ml of a 10% barium chloride and 1% ammonium chloride solution. The center well was washed twice with carbon dioxide free water and the wash added to the total solution in the centrifuge tube.

The barium carbonate precipitate was sedimented by low speed centrifugation at approximately 1500 rpm for two minutes and the supernatant discarded. The precipitate was resuspended in carbon dioxide free water, plated on weighed microporous porcelain discs and dried for 1½ hrs at 100°C. The samples were reweighed and the radioactivity determined by a continuous gas flow Geiger Counter. Counts were corrected for background and self absorption.

<u>EXTRACTION OF TISSUE</u>

The material used for C6/C1 ratio determinations, was removed at the end of the experiment and washed with water to remove adsorbed labelled glucose. The tissue was then killed by the addition of 20 ml of boiling 80% ethanol. Successive extractions of the tissue were made on a steam bath with 20 ml 80% ethanol, 20 ml 40% ethanol and 20 ml of

water followed by an additional 20 ml 80% ethanol. The tissue remaining after these extractions constituted the alcohol insoluble residue.

The combined alcohol extracts were evaporated to dryness under reduced pressure at 40°C. The residue was taken up in 20 ml water and extracted with an equal volume of ethyl ether. The ether phase contained the lipid fraction and the water phase the amino acid, organic acid and sugar fractions. These water soluble components were fractionated by means of ion-exchange resins.

PREPARATION OF ION EXCHANGE COLUMNS

Dowex 50-X8 (H^+) cation exchange resin (200-400 mesh) was placed in a beaker and suspended in three times its volume of water. The suspension was allowed to stand until the majority of the resin beads had settled; the upper liquid containing the fine resin particles and floating impurities was decanted. This step was repeated three to four times or until no fine particles were observed in the decanted liquid. The resin was then suspended in twice its volume of 2 N hydrochloric acid. This suspension was heated to 100°C with continuous stirring to prevent bumping, and then allowed to cool for one hour. The resin was allowed to settle and the yellow supernatant liquid decanted. This procedure was repeated until the supernatant

was clear. The resin was then poured into the column and eluted with twice its volume of 2N hydrochloric acid. The resin was then washed with distilled water until the effluent was neutral in reaction.

Dowex 1-X10 (Cl^-) anion exchange resin (200-400 mesh) was purified in the same manner indicated for cation exchange resin. It was then poured into a glass column 1 cm in diameter and converted to the formate form by eluting with 40 ml of 1 M sodium formate per 5 ml resin. The conversion is complete when the effluent does not form a precipitate with silver nitrate solution. To remove "throw" the resin was poured into a beaker, suspended in 2N formic acid and heated to 60-70°C. On cooling the supernatant was eluted with 10 ml of 0.1 N formic acid per 5 ml resin and then washed until the effluent water was neutral.

Glass columns 1 cm in internal diameter were used to receive the purified resin. A small piece of glass wool was covered by a 1 cm layer of fine glass beads. The resin was transferred to the column as a thick slurry via a 10 ml volumetric pipette with the tip removed. It was packed with slight air pressure, with care being taken to maintain liquid at all times above the resin. When the resin reached the desired depth, the air pressure was removed and glass beads 1 cm deep were placed on top of the resin.

FRACTIONATION OF WATER SOLUBLE COMPONENTS

The water soluble extracts were passed through a 6x1 cm column of Dowex 50-X8 (H^+) and Dowex 1-X10 (formate). The column effluent (neutral fraction) contained mainly sugars and is here-after called the sugar fraction. The amino acid fraction was eluted from the cation exchange resin with 50 ml 1N ammonium hydroxide, whereas the fraction composed mainly of organic acids was eluted from the anion resin with 40 ml 4 N formic acid followed by 20 ml 8 N formic acid.

The radioactivity of each fraction was measured on a liquid scintillation counter by dissolving ½ ml of the radioactive fraction in 5 ml of dioxane scintillation liquid prepared as follows:

Dioxane	833 ml
Ethylene glycol monomethyl ether (methyl cellosolve)	166 ml
Naphthalene	50 g/liter
2:5 Diphenyloxazole (PPO)	4 g/liter
1:4-bis (2-(5-phenyl)oxazolyl (POPOP)	100 mg/liter

After determination of total radioactivity, the sugar fraction was reduced to approximately ½ ml; spotted on Whatman #1 paper and separated by descending chromatography using ethyl acetate, acetic acid and water (6:2:4). Radioactive areas were determined by exposing a strip of the chromatogram to Kodak (No Screen) X-ray safety film for a period of two and one half months. The X-ray film

was separated from the chromatogram paper and processed using "Kodak D 11" developer and "Edwal Rapid" fixative.

The radioactive spots marked on the chromatogram paper were removed and radioactive sugars extracted by washing them in hot water. The relative radioactivity of individual sugars was determined by dissolving a ½ ml aliquot in dioxane-naphthalene solvent and counting by the liquid scintillation method.

The amino acids and organic acids were separated by paper chromatography using n-butanol, acetic acid and water (12:3:5) as the solvent. The radioactivity of the individual acids was determined in a manner similar to the one described for sugars.

COMBUSTION OF C^{14} LABELLED MATERIAL

The alcohol insoluble fraction was combusted to carbon dioxide, trapped as barium carbonate and assayed for radioactivity. Combustion was by the reagent of Vanslyke and Folch (57) in the apparatus of Stutz and Burris (49). In the procedure 5 ml of the acid mixture (equal parts by volume of concentrated sulphuric and phosphoric acid containing 1.5% w/v potassium iodate) was added to 1-2 mg of tissue in the presence of 50 mg of dry reagent (potassium iodate and potassium dichromate, 2:1 w/w). The mixture was heated in a nitrogen atmosphere and the evolved carbon

dioxide collected in 0.25 N barium hydroxide. The barium carbonate precipitate was plated and counted in the manner previously indicated. For determinations by the liquid scintillation method, the evolved carbon dioxide was trapped in 5 ml ethanolamine and ethylene glycol monomethyl ether (1:2) and radioactivity counted in toluene solvent prepared by mixing the following in one liter of toluene:

2:5 diphenyloxazole (PPO) .... 4 gm
1:4 bis (2-(50phenyl)oxazolyl)
(POPOP)................................50 mg

NICOTINAMIDE-ADENINE DINUCLEOTIDE PHOSPHATE (NADP) EFFECT ON GROWTH

The NADP solutions at concentrations of 0.5 mM, 1.0 mM, 1.5 mM and 2.5 mM were vacuum infiltrated into 11 week after-ripened embryos. The embryos serving as control were infiltrated with water. The treated embryos were placed on moist filter paper in autoclaved petri dishes and kept at room temperature for seven days, after which the total growth of primary roots and hypocotyl was measured.

QUANTITATIVE DETERMINATIONS OF SUGARS

Total sugars were estimated by the phenol method of Dubois et al (19). The method though non-specific is extremely sensitive and can be used to determine quantitatively as little as 1 ug sugar in a volume of three ml.

Samples of 500 mg of cotyledons and 30 mg of embryonic

axes were dried for 16 hrs at 80°C and used for sugar determinations. The hydrolysis was carried out by boiling the tissue for 16 hrs in 5% nitric acid (30 ml acid per 100 mg tissue). At the completion of hydrolysis, the nitric acid was neutralized with barium carbonate and the solution evaporated to dryness. Sugars were taken up in absolute alcohol and the barium nitrate removed by filtration. The filtrate was taken to dryness and the sugars redissolved in water. Three ml of this solution and 0.12 ml of 80% phenol were placed in a test tube; 5 ml of concentrated sulphuric acid was rapidly added and the tube contents thoroughly mixed. After cooling the optical density was determined at 490 mu and total sugars calculated. The reaction followed Beer's law in the concentration range of 1-180 ug sugar.

QUANTITATIVE DETERMINATIONS OF LIPIDS

Determinations of total lipids were carried out on both cotyledons and embryonic axes. The cotyledons were ground and a two gram sample extracted for four hours with skellysolve B (n-hexane) in a Soxhlet apparatus. The solvent was then partially evaporated under vacuum at 20°C and its last traces removed by heating for 1 hr at 100-105°C. On cooling the weight of the lipid was determined.

Samples of embryonic axes, 200-250 mg used for such extractions were not ground because of their small size.

This procedure was found to be superior to that of Bligh and Dyer (5) in that 20-25% higher yields were obtained.

FATTY ACID DETERMINATIONS

Fatty acids were determined according to the procedure of Craig and Murty (15). A sample of 50 mg oil was dissolved in five ml skellysolve F (petroleum ether b.p.30-60°C) and refluxed for 20 minutes with 5 ml of 0.02 N sodium methoxide. After cooling to room temperature, 0.5 ml of 0.2 N glacial acetic acid was added to inactivate the catalyst and the solvent was taken to dryness. The residue was throughly dissolved in a 10 ml mixture of equal quantities of water and skellysolve F. The ether layer was separated and traces of water removed by adding 1 gram of anhydrous magnesium sulphate. After two hours the supernatant was decanted and the solvent removed by evaporation and 1 hour of drying at 60°C.

Fatty acid esters were separated on an Aerograph Gas Chromatograph having a thermal conductivity detector. A copper column 4½ foot x ¼ inch was packed with 40-60 mesh firebrick coated with butanediol-1,4-succinate (4/1 w/w). In the analysis the column, injector and detector temperatures were kept at 196°, 210° and 230°C respectively and helium flow rate at 100 ml/minute. The quantitation of the recorded peaks was obtained by area measurements using a disc chart integrator.

RESULTS

Embryo dormancy, which is prevalent in seeds of *Prunus cerasus*, the chosen experimental material, can be overcome by 16 weeks of after-ripening treatment. In this study, metabolic changes occurring during after-ripening treatment were followed. Changes in pattern of respiration rates and in pathways of glucose breakdown were studied. The incorporation of labelled glucose into the alcohol soluble fraction relative to the insoluble residue was determined. The effects of NADP on growth and on the pathway of oxidation were studied. The quantitative and qualitative changes in lipid and sugar levels were also examined.

EMBRYO VIABILITY TESTS

Seeds were tested for viability to establish their suitability as an experimental material. Quadruplicate lots, each of 25 seeds were used for this test. After removing the endocarp and seed coat the embryos were placed in sterile petri dishes and incubated at room temperature for seven days. Some embryos showed elongation of the hypocotyl, but in most activity was exhibited as a greening and spreading apart of the cotyledons. Non-viable embryos discoloured or decayed during this time. The observed sluggish growth indicated embryo dormancy in the *Prunus cerasus* seeds. The very high viability of 92% suggested that the material could be used for after-ripening studies (Table 1).

TABLE 1. Viability test of dormant seeds of *Prunus cerasus*.

Sample Number	Viability Response (percent) Hypocotyl elongation	Cotyledonary greening	Cotyledonary spreading	Total
1	12	68	16	96
2	24	48	20	92
3	8	48	36	92
4	20	36	32	88
Mean	16	50	26	92

EFFECT OF AFTER-RIPENING ON THE GERMINATION OF SEEDS

Seeds were after-ripened at 1°C in a moist sand-peat mixture (1:1) and the germination results recorded. Duplicate lots of 50 embryos isolated from the after-ripened seeds were placed on moist filter paper at room temperature. Embryos which did not form seedlings within seven days were considered as being dormant. As shown in Table 2 the germination percentages were very low during the initial six weeks of after-ripening. The number of seedlings obtained from the seven to nine week after-ripened material was considerable, though the actual growth observed was slightly sluggish. The pattern of germination in the 10 to 16 week after-ripened material was quite similar indicating that the optimum length of after-ripening had been reached at approximately 10 weeks.

In a separate experiment, duplicate lots of 50 seeds were placed in moist soil at room temperature and the percent germination calculated from the emerged seedlings. The seeds which did not form seedlings within three weeks were considered as dormant. As shown in Table 2 no seedlings were obtained from the material after-ripened up to eight weeks. The maximum percent germination obtained by this test was far less than the identical tests conducted on isolated embryos; the actual values being 32% and 83% res-

TABLE 2. Growth condition and percent germination of after-ripening seeds of *Prunus cerasus*.

	Embryos on moist filter paper		Seeds in moist soil	
After-ripening (weeks)	Percent germination	Condition of growth	Percent germination	Condition of growth
0 to 3	-	-	-	-
4	4	sluggish	-	-
5	9	"	-	-
6	16	"	-	-
7	48	"	-	-
8	59	"	6	sluggish
9	64	"	16	normal
10	68	normal	20	"
11	76	"	18	"
12	73	"	16	"
13	79	"	28	"
14	82	"	26	"
15	77	"	28	"
16	83	"	32	"

pectively. It is considered that the seed coat and hard endocarp, in some way, are restricting the growth of the non-dormant embryos which account for relatively low percent germination observed in the seeds.

QUANTITATIVE DETERMINATIONS OF TOTAL SUGARS

Embryonic axes and cotyledons, isolated from 0, 7, 11 and 16 week after-ripened seeds, were hydrolysed with nitric acid and total sugars determined (Table 3). The results indicated that in embryonic axes the total sugar levels increased from 12.5% when dormant to 18.1% when fully after-ripened. The increase, however, was very small during the initial six weeks and became considerable during the last stages of after-ripening. Cotyledons also showed an identical trend though the actual increase when fully after-ripened was 22.4% as compared to 44.8% in embryonic axes. These changes in sugar levels in cotyledons and embryonic axes along with the changes in lipid content (which will be discussed later) indicated that lipid is probably converted to sugars in the cotyledons and translocated in this form to embryonic axes.

CHANGES IN DRY WEIGHT

The dry weights of the dormant and fully after-ripened embryonic axes and cotyledons were determined to estimate

TABLE 3. Total sugars in embryonic axes and cotyledons isolated from dormant and after-ripened seeds.

	Embryos		Cotyledons	
After-ripening (weeks)	Sugars percent dry-weight	Sugars as percent of dormant	Sugars percent dry-weight	Sugars as percent of dormant
0	12.5	100	4.9	100
7	12.9	103.2	5.0	102.0
11	15.2	121.6	5.3	108.2
16	18.1	144.8	6.0	122.4

the changes in the total solids. To avoid caramelization of sugars, drying was carried out in a vacuum oven at 70°C. As shown in Table 4 the dry weight of fully after-ripened cotyledons decreased by 9.5% and that of embryonic axes increased by 34.2 percent.

QUANTITATIVE AND QUALITATIVE DETERMINATIONS OF LIPIDS

The quantitative determinations of lipids were made on isolated embryonic axes and cotyledons which had undergone 0, 7, 11 and 16 weeks after-ripening. The percent lipid obtained is given in Table 5. The results indicated that in cotyledons the lipid levels decreased with the increase of after-ripening time. The actual amounts were 44.4% when dormant and 40.4% when fully after-ripened. The rate of disappearance, although continuous, was maximum after 11 weeks after-ripening. In embryonic axes the lipid levels remained unchanged during the initial six weeks and then dropped from 14.1 to 8.7 over the remaining 10 weeks of after-ripening.

The lipids extracted from dormant and 16 week after-ripened cotyledons were hydrolysed and fatty acids were determined (as methyl esters) by gas liquid chromatography. As shown in Table 6 oleic and linoleic fatty acids are the main components of the lipids extracted at various stages of after-ripening. On the completion of a 16 week after-

TABLE 4. Changes in dry weight of dormant and 16 week after-ripened cotyledons and embryonic axes.

Tissue	Number used	Weight per 100(gm) Dormant	After-ripened	Percent change
Cotyledons	260	2.63	2.39	- 9.5
Embryonic-axes	195	0.040	0.054	+ 34.2

TABLE 5. Quantitative determinations of lipids in the embryonic axes and cotyledons isolated from dormant and after-ripened seeds of *Prunus cerasus*.

	Cotyledons		Embryonic Axes	
After-ripening (weeks)	Percent lipid dry-weight	Lipids as percent of dormant	Percent lipid dry-weight	Lipids as percent of dormant
0	44.4	100	14.1	100
7	43.6	98.2	13.9	98.6
11	42.8	96.3	10.4	73.8
16	40.4	90.9	8.7	61.8

TABLE 6. Composition of lipids and percent utilization of fatty acids in dormant and after-ripened seeds of *Prunus cerasus*.

	After-ripening (weeks)							
	0		11			16		
	Percent composition	Total (mg)	Percent composition	Total	Percent utilization	Percent composition	Total	Percent utilization
Lipid/100 mg dry weight		44.40		42.80			40.40	
Palmitic	8.1	3.24	7.8	3.00	7.4	7.4	2.69	17.0
Stearic	0.6	0.24	0.5	0.19	20.8	0.5	0.17	29.2
Oleic	48.6	19.42	47.4	18.27	6.0	46.8	17.01	12.4
Linoleic	42.7	17.06	44.3	17.06	0.0	45.3	16.49	3.5

ripening period the percent utilization of stearic acid was maximum, and that of linoleic minimum, the values being 29.2% and 3.5% respectively. The quantity of lipids extracted from embryonic axes was too small to be used for fatty acid determinations.

RESPIRATION AND R.Q. STUDIES

Weekly changes in respiration and R.Q. were determined at 25°C with both cotyledons and embryonic axes. Respiratory differences are expressed as percentages of the respiration rates for dormant tissues (Table 7). The respiration rate of the embryonic axes, during the first six weeks of after-ripening, remained almost unchanged from the initial level of 0.284 ul per hour per embryonic axis. The sharp increase during the seventh to tenth weeks was followed by a relatively constant rate until the end of the fifteenth week and a further moderate increase during the sixteenth week. The basic values of dormant and 16 week after ripened embryonic axes were observed to be 0.28 ul and 1.1 ul per hour per embryonic axis respectively. Thus the embryonic axes of fully after-ripened seeds showed a 392% increase in respiration rate. Calculated on a fresh weight basis the respiration rate increased by 305%. The cotyledons showed a parallel increase of 226% in respiration rate.

TABLE 7. Rates of oxygen uptake of embryonic axes and cotyledons from after-ripening seeds of *Prunus cerasus*.

After-ripening period (weeks)	Oxygen Uptake					
	ul/hr/embryonic axis		ul/hr/100 mg fresh weight of axes		ul/hr/100 mg fresh weight of cotyledons	
0	0.284	(100)*	32.3	(100)	8.3	(100)
1	0.292	(103)	33.6	(104)	8.5	(102)
2	0.308	(108)	35.7	(110)	8.3	(100)
3	0.284	(100)	32.3	(100)	8.9	(107)
4	0.268	(95)	32.8	(101)	8.4	(101)
5	0.280	(98)	34.0	(105)	9.3	(112)
6	0.312	(109)	35.6	(110)	9.5	(114)
7	0.640	(225)	69.6	(215)	14.2	(171)
8	0.652	(230)	69.4	(214)	13.8	(166)
9	0.756	(266)	75.0	(232)	15.8	(190)
10	0.836	(294)	86.0	(266)	16.9	(203)
11	0.844	(297)	85.6	(265)	17.0	(205)
12	0.852	(300)	83.9	(260)	16.0	(203)
13	0.868	(305)	84.8	(262)	16.6	(200)
14	0.832	(293)	84.1	(260)	17.5	(211)
15	0.832	(293)	87.1	(270)	18.7	(225)
16	1.112	(392)	98.6	(305)	18.8	(226)

*Figures in brackets are rates expressed as percentages of dormant. The incubation mixture consisted of 1.0 ml .05 M potassium phosphate buffer pH 5.5 and 25 embryonic axes. Central well contained 0.2 ml 20% KOH to absorb CO_2.

The hourly rate of respiration of 100 mg sample of dormant embryonic axes was 32.3 ul as compared to 8.3 ul for the same weight of cotyledons. In fully after-ripened tissues these figures rose to 98.6 ul and 18.8 ul respectively. This rate of respiration of embryonic axes (4 to 5.5 fold higher than that of cotyledons) indicated that this structure was the main site of metabolic activity in the embryo.

R.Q. determinations were utilized to obtain an indication of the respiratory substrate. The data of RQ determinations which were made at weekly intervals of after-ripening are given in Table 8. The results are averages of duplicate lots. In embryonic axes an RQ of higher than unity was observed during the first six week period which later declined and remained in the range of 0.93 to 0.96 until the completion of the 16 week after-ripening period. In cotyledons the RQ remained unchanged and varied in the range of 0.82 to 0.90.

DETERMINATION OF PARTICIPATION OF PP AND EMP PATHWAYS

Determinations of C6/C1 ratios were used to estimate the relative contributions of the PP and EMP pathway of glucose oxidation in the isolated tissues of both embryonic axes and cotyledons. C-1 is the first carbon to be released as carbon dioxide from glucose entering the PP sequence.

TABLE 8. Respiratory quotients of isolated embryonic axes and cotyledons of *Prunus cerasus* seeds.

After-ripening (weeks)	Embryonic Axes ul/hr/100 mg			Cotyledons ul/hr/100 mg		
	O_2 uptake	CO_2 evolution	RQ	O_2 uptake	CO_2 evolution	RQ
0	32.3	40.7	1.26	8.3	7.4	0.89
1	33.6	40.6	1.21	8.5	7.5	0.88
2	35.7	46.1	1.29	8.3	7.0	0.85
3	32.3	38.1	1.18	8.9	7.7	0.87
4	32.8	40.0	1.22	8.4	7.5	0.89
5	34.0	41.1	1.21	9.3	8.0	0.86
6	35.6	44.1	1.24	9.5	8.3	0.87
7	69.6	65.4	0.94	14.2	12.6	0.89
8	69.4	62.5	0.90	13.8	12.3	0.89
9	75.0	69.0	0.92	15.8	13.9	0.88
10	86.0	80.0	0.93	16.9	14.9	0.88
11	85.6	81.3	0.95	17.0	14.9	0.88
12	83.9	80.5	0.96	16.9	15.0	0.89
13	84.8	81.4	0.96	16.6	14.8	0.89
14	84.1	80.7	0.96	17.5	14.7	0.84
15	87.1	81.0	0.93	18.7	15.3	0.82
16	98.6	93.7	0.95	18.8	15.6	0.83

The rate of conversion of C-6 to carbon dioxide would be dependent on whether the triose produced in PP pathway were converted to pyruvate or recycled; in either event its appearance, relative to that of C-1 would be considerably delayed. If the glucose molecule were broken down by the EMP pathway, which includes equilibration at the triose level, C-1 and C-6 of glucose would appear as the methyl carbon of the pyruvate. In the subsequent oxidative breakdown of pyruvate C-1 and C-6 will be evolved as carbon dioxide, indistinguishably in the third cycle of TCA cycle.

Paired flask respiration experiments were conducted using glucose-1-C^{14} and glucose-6-C^{14} in the incubation medium. Evolved carbon dioxide was collected in 0.2 ml of 20% potassium hydroxide, which was changed at intervals of 1, 2,4 and 8 hours. Determinations of the radioactivity of the carbon dioxide collected during each time interval were made and the C6/C1 ratio calculated. As none of the ratios varied significantly during the eight hour period the cumulative radioactivity for the eight hour period is presented in Table 9.

In embryonic axes the C6/C1 values during the initial six weeks after-ripening period remained unchanged and in the order of 0.95. In the seventh week this value dropped abruptly to 0.65 and then gradually declined to 0.53 at the end of the 16 week after-ripening period. In cotyle-

TABLE 9. Weekly changes in C6/C1 ratios of embryonic axes and cotyledons during after-ripening at 5°C. The data are expressed on a fresh weight basis.

After-ripening (weeks)	Embryonic Axes Counts/min/100 mg.			Cotyledons Counts/min/100 mg.		
	glucose $-1-C^{14}$	glucose $-6-C^{14}$	C6/C1	glucose $-1-C^{14}$	glucose $-6-C^{14}$	C6/C1
0	3106	2859	0.92	170	108	0.63
1	2867	2669	0.93	182	113	0.63
2	2933	2818	0.96	185	125	0.66
3	2834	2630	0.93	181	112	0.63
4	3084	2989	0.97	157	102	0.65
5	3568	3386	0.95	240	158	0.67
6	3324	3160	0.95	215	145	0.67
7	3170	2062	0.65	213	139	0.66
8	3443	2339	0.68	252	165	0.66
9	3318	2190	0.66	227	157	0.69
10	3707	2338	0.63	248	159	0.64
11	3814	2325	0.61	238	153	0.65
12	4493	2654	0.59	233	152	0.65
13	4304	2624	0.61	242	151	0.62
14	8832	4941	0.56	244	163	0.67
15	9080	4809	0.53	230	148	0.64
16	8995	4769	0.53	315	195	0.62

values are expressed on a per hour basis and are the means of four CO_2 collection times of duplicate samples consisting of 10 cotyledonary pairs or 25 embryonic axes.

dons from dormant and after-ripened seeds these ratios remained relatively constant in the range of 0.62 to 0.67.

EFFECT OF GLUCOSE LABELLING POSITION ON THE INCORPORATION OF C^{14} INTO VARIOUS FRACTIONS OF EMBRYONIC AXES AND COTYLEDONS

Dormant and 16 week after-ripened embryos were used to determine the distribution of C^{14} derived from C1 and C6 labelled glucose. Immediately after C6/C1 determinations, the tissue was killed in alcohol and fractionated into the alcohol soluble and insoluble components. Distribution of radioactive components derived from glucose-1-C^{14} and glucose-6-C^{14} are presented in Tables 10 and 11.

In dormant embryonic axes 10.5% of supplied glucose was retained in the amino acid, organic acid and sugar fractions of the tissue; this value tripled when they were fully after-ripened. The C^{14} which was incorporated in the insoluble fraction increased from approximately 0.03% to nearly 9.0% when fully after-ripened; an increase in the range of 270 to 300 fold (Table 10).

A similar trend of glucose utilization was recorded for cotyledons (Table 11). The total retention was 31.7% to 35.6% when dormant and 65.5% to 73.2% when fully after-ripened. The increase in the radioactive components of the insoluble fraction was 15 to 17 fold, which was far less than that obtained with embryonic axes.

TABLE 10. Distribution of C^{14} in fractions of embryonic axes after eight hours of glucose-C^{14} feeding.

Position of glucose label	Percent Radioactivity									
			C^{14} Retained in the Tissue							
	Unused sub-strate	$C^{14}O_2$ evolu-tion	Sugars	Amino acids	Organic acids	Insoluble fraction	Column losses	Total	Other experiment losses	Total Recovery
Dormant embryonic axes										
C-1	85.3	3.6	5.8	2.8	1.9	0.030	0.47	10.2	0.9	98.63
C-6	85.4	3.3	5.8	2.1	1.9	0.026	0.67	10.5	0.8	98.53
After-ripened embryonic axes (16 weeks)										
C-1	55.8	11.4	11.0	4.6	3.5	8.6	2.2	29.9	2.9	94.90
C-6	56.4	6.0	12.8	6.6	3.7	9.0	2.6	34.7	2.9	94.50

The incubation media contained 25 embryonic axes 0.9 ml .05M phosphate buffer pH 5.5 and 5 micromoles (0.1 µc) of labelled glucose dissolved in 0.1 ml water.

TABLE 11. Distribution of C^{14} in fractions of cotyledons after eight hours of glucose-C^{14} feeding.

Position of glucose label	Percent of Added Radioactivity									
			C^{14} Retained in the Tissue							
	Unused sub-strate	$C^{14}O_2$ evolu-tion	Sugars	Amino acids	Organic acids	Insoluble fraction	Column losses	Total	Other experiment losses	Total Recovery
Dormant cotyledons										
C-1	59.7	8.3	20.6	4.6	4.3	0.8	1.4	31.7	0.3	98.3
C-6	58.7	5.2	22.3	5.8	4.4	1.2	1.9	35.6	0.5	97.6
After-ripened cotyledons										
C-1	11.0	21.0	31.9	8.9	5.7	15.6	3.4	65.5	2.5	94.1
C-6	12.2	12.5	35.0	11.6	7.1	15.8	3.7	73.2	2.1	94.2

The incubation media contained 20 cotyledons, 0.9 ml 0.05M phosphate buffer pH 5.5 and 5 micromoles (0.1 μc) of labelled glucose dissolved in 0.1 ml water.

There was a quantitative variation in the radioactivity of the various fractions of embryonic axes, which was dependent on the position of the glucose labelling. In general there was a greater diversion of carbon six of glucose than of carbon one (Table 12). This tendency was much more pronounced in non-dormant embryonic axes. The amino acid fraction of after-ripened embryonic axes, for example contained 43.5% more radioactivity when glucose-6-C^{14} was the substrate rather than glucose-1-C^{14} (Table 12). The pattern of labelling of cotyledonary fractions, as dependent on substrate label position, was similar to that of embryonic axes (Table 12).

The percent radioactivity in the sugars extracted from 12 week after-ripened cotyledons is given in Table 13. The results indicated a much higher radioactivity of sucrose when glucose-6-C^{14} was fed rather than glucose-1-C^{14}.

EFFECT OF 2-4 DINITROPHENOL (DNP) ON RESPIRATION

In embryonic axes from dormant and six week after-ripened seed the EMP pathway of oxidation is predominant. As after-ripening continues, the participation of the PP pathway of oxidation increases. With these relationships of oxidation pathways, one would expect a greater respiration increase during the first six week of after-ripening

TABLE 12. C^{14} incorporation into various fractions expressed as percent of initial radioactivity and as a ratio.

Position of glucose label		C^{14} incorporation (percent of added radioactivity and C6/C1 x 100)							
		Sugars		Amino acids		Organic acids		Insoluble fraction	
Embryonic axes									
Dormant	C-1	5.8	100	2.0	105	1.9	100	0.030	86.7
	C-6	5.8		2.1		1.9		0.026	
After-ripened	C-1	11.0	116.4	4.6	143.5	3.5	105.7	8.6	104.6
	C-6	12.8		6.6		3.7		9.0	
Cotyledons									
Dormant	C-1	20.6	108.2	4.6	126.0	4.3	102.3	0.8	150
	C-6	22.3		5.8		4.4		1.2	
After-ripened	C-1	31.9	109.7	8.9	130.3	5.7	124.5	15.6	101.3
	C-6	35.0		11.6		7.1		15.8	

TABLE 13. The percentage distribution of radioactivity of the sugar fraction among the various sugars isolated from 12 week after-ripened cotyledons incubated with glucose-1-C^{14} and glucose-6-C^{14}.

Position of glucose label	Sample	Sucrose	Glucose	Fructose	Unidentified.
C-1	1	45.6	13.0	16.6	24.8
	2	45.2	13.8	15.8	25.2
Mean		45.4	13.4	16.2	25.0
C-6	3	60.2	13.3	13.6	12.9
	4	65.2	11.9	11.0	11.9
Mean		62.7	12.6	12.3	12.4

if the supply of ADP (a necessary and often limiting factor in EMP activity) were increased. More ADP can be made available by uncoupling the phosphorylation step of terminal oxidation with DNP. Embryos from dormant and after-ripened seed of Prunus cerasus were treated with DNP and respiration increases determined. The level of DNP addition was 10^{-4}M; a concentration which gave maximum stimulation as determined by preliminary experiments.

The rate of respiration of each lot of 50 embryonic axes isolated from 0, 6, 11 and 16 week after-ripened seeds was measured for two hours; DNP was then added to bring the final concentration in the system to 10^{-4} M. Respiration was measured for an additional two hours in the presence of DNP. The data expressed as means of duplicate lots are presented in Tables 14 and 15. With dormant and six week after-ripened embryonic axes increase in respiration due to DNP addition was identical. The response to DNP, however, decreased with advance of after-ripening time (Table 14). The results of a similar study with 0, 8 and 16 week after-ripened cotyledons are given in Table 15. The percent respiratory increase for dormant and non-dormant cotyledons was approximately equal. Unlike embryonic axes, after-ripening did not suppress the DNP response.

EFFECT OF INFILTRATED NADP ON GROWTH

The enzyme glucose-6-phosphate dehydrogenase, responsible

TABLE 14. Effect of DNP (10^{-4} M) on oxygen uptake of embryonic axes isolated from dormant and after-ripened seeds of *Prunus cerasus*.

After-ripening (weeks)	Oxygen uptake ul/hr/sample			*DNP stimulation (percent)
	- DNP		+ DNP	
	0-2 hours	2-4 hours	2-4 hours	
0	19.0	20.0		
	19.2		38.2	94.2
6	19.5	21.3		
	18.3		36.3	90.8
11	61.1	61.0		
	62.3		86.7	39.4
16	67.7	66.7		
	66.3		79.0	20.5

DNP stimulation percent =

$$\frac{\text{DNP respiratory increase minus change in control x 100}}{\text{Initial rate of respiration}}$$

The incubation mixture consisted of 2 ml .05 M potassium phosphate buffer pH 5.5. After two hours .5 ml of 5×10^{-4}M DNP was tipped into the main compartment to yield a final concentration of 10^{-4}M DNP. An equal amount of water was added to control flasks.

TABLE 15. Effect of DNP (10^{-4}M) on oxygen uptake of cotyledons isolated from dormant and after-ripened seeds of <u>Prunus</u> <u>cerasus</u>.

After-ripening (weeks)	Oxygen uptake ul/hr/sample			*DNP stimulation (percent)
	- DNP		+ DNP	
	0-2 hours	2-4 hours	2-4 hours	
0	37.4	38.3		
	36.5		48.2	29.5
8	57.0	59.2		
	55.6		71.9	25.8
16	79.9	83.3		
	78.6		106.0	30.7

DNP stimulation percent =

$$\frac{\text{DNP respiratory increase minus change in control} \times 100}{\text{Initial rate of respiration}}$$

The incubation mixture consisted of 2 ml .05 M potassium phosphate buffer pH 5.5. After two hours .5 ml of 5×10^{-4} M DNP was tipped into the main compartment to yield a final concentration of 10^{-4} M DNP. An equal amount of water was added to control flasks.

for oxidation of glucose-6-phosphate to 6-phosphogluconate is NADP specific. NADP availability is frequently limited and, therefore, its levels may control the rate of PP pathway of oxidation (2). Experiments were initiated to determine if NADP is actually limiting to growth of embryos from partially after-ripened seed. From the seventh to the tenth week of after-ripening, the oxidative activity of the PP pathway increased in embryonic axes. This change could have been due to increased levels of NADP. Embryos showed a marked increase in capacity for growth after this same period of after-ripening treatment. This suggested a relationship between growth and NADP concentration. To confirm this aspect, NADP at concentrations of 0.5 mM, 1.0 mM, 1.5 mM and 2.5 mM was vacuum infiltrated for one hour in 11 week after-ripened embryos and the embryos were grown on a moist filter paper. After seven days the length of the primary root and hypocotyl was measured and compared with the control to determine the NADP effect on growth. Mean values of duplicate lots of 20 seeds are given in Table 16. The treated embryos not only showed an early and vigorous growth but also exhibited a substantial growth increment over the control. The maximum response was observed in the embryos infiltrated with 1.5 mM NADP solution. The confirmation of NADP infiltration and its

TABLE 16. Effect of infiltrated NADP on growth of radicles of 11 week after-ripened embryos isolated from *Prunus cerasus* seeds.

NADP solution (mM)	Mean length of radicles (mM)			
	Sample 1	Sample 2	Mean	Increase over control
0.0	20.1	17.7	18.9	0
0.5	22.9	20.3	21.6	2.7
1.0	23.6	25.6	24.6	5.7
1.5	29.8	32.0	30.9	12.0
2.5	22.3	24.7	23.5	4.6

Number of embryos per sample = 20.

Volume of NADP solution used = 10 ml for each sample.

L.S.D. (5%) = 4.37 mm.

participation in metabolism was indicated by a significant drop in C6/C1 ratio in the treated seeds (Table 17).

TABLE 17. Effect of infiltrated NADP (1.5 mM) on C6/C1 ratio of four week after-ripened embryos isolated from <u>Prunus cerasus</u> seeds.

Embryos Control			Embryos (Treated)		
Counts/min/100 mg.			Counts/min/100 mg.		
Glucose $-1-C^{14}$	glucose $-6-C^{14}$	C6/C1	glucose $-1-C^{14}$	glucose $-6-C^{14}$	D6/C1
323	259	0.80	403	213	0.53
408	298	0.73	497	323	0.64
380	256	0.67	600	324	0.54
495	378	0.76	520	236	0.45
514	319	0.62	554	290	0.52
483	252	0.52	524	268	0.51
Mean		0.68			0.53

$C^{14}O_2$ was collected at one hour intervals from each sample of five embryos which were vacuum infiltrated with 10 ml of 1.5 mM NADP. Incubation medium consisted of 0.7 ml of 0.05 M potassium phosphate buffer pH 5.5 and 0.3 micromoles (.075 uc) of labelled glucose dissolved in 0.3 ml water.

Analysis of variance for differences between groups of C6/C1 ratios.

F (calculated) = 6.34

F (.05) = 4.96

DISCUSSION

Seed dormancy in *Prunus cerasus* is primarily due to embryo dormancy. The embryo will produce a normal seedling when given 10 weeks of after-ripening. The seed coat tends to prevent or delay germination of a considerable number of these non-dormant embryos. The role of the embryonic axes and the cotyledons differs greatly in processes leading to the termination of dormancy. Physiologically the embryonic axes are more active and play an important part in synthesis and growth, while the cotyledons are comparatively inert and are responsible for the supply of reserve materials to the after-ripening embryos. Because of these physiological differences, metabolic changes in these organs were studied separately.

During after-ripening, the embryonic axes increased their rate of respiration, dry weight and sugar levels and also changed their pathways of glucose oxidation. The increase in PP pathway of oxidation may have been due to increase in NADP levels during the cold treatment. NADP, which is a coenzyme of glucose-6-phosphate and isocitrate dehydrogenases, then could have accelerated the PP and the TCA cycles to supply growth substances, structural components and energy required for the embryo to break dormancy. In cotyledons similar but less pronounced changes were observed in respiration and substrate breakdown.

Their dry weight decreased and the activity of the PP pathway remained unchanged at all stages of after-ripening. Indirect evidence of the operation of the glyoxylate cycle was also obtained.

On the basis of preliminary tests (Table 1) embryos of the test material were found to be high in viability. This viability was expressed by cotyledonary greening or by very small hypocotyl elongation. Similar behaviour was observed in embryos isolated from seeds after-ripened up to eight weeks. This indicated that embryo dormancy was prevalent in the _Prunus cerasus_ seeds. Embryos from 10-16 week after-ripened seeds showed normal seedling growth on moist filter paper; the maximum germination attained was 83% (Table 2). When the seeds from the same lots were allowed to grow in a moist sand peat mixture, the percentage emergence was only 32% when the seeds were given a 16 week after-ripening treatment. The decreased emergence could have been due to the action of seed coverings in offering mechanical resistance to the expansion of the embryo; their acting as a barrier to gaseous exchange or supplying growth inhibitors to the embryo.

In embryonic axes, at the completion of a 16 week after-ripening period, the levels of sugars (Table 3) and dry weight (Table 4) increased and that of lipids decreased

(Table 5). When the amount of glucose which had been respired is taken into consideration together with increased sugar levels, it seems probable that lipids in the cotyledons were being converted into sugars and translocated to the embryonic axes. Added support to this assumption came from the simultaneous decrease in lipid and dry weight levels of cotyledons during that time (Tables 4 and 5).

The rate of respiration of embryonic axes remained almost unchanged during the initial six weeks of after-ripening. A sharp increase in respiration during the seventh to tenth weeks was followed by a relatively constant rate until the end of the fifteenth week. A further moderate increase occurred during the sixteenth week (Table 7). On an average, embryonic axes from fully after-ripened seeds showed a rate of respiration four times higher than that of dormant embryonic axes.

Cotyledonary respiration also followed a similar trend. The rate after 16 weeks of after-ripening, however, was only slightly more than twice the initial level. The rate of respiration of embryonic axes per unit fresh weight was four times higher than cotyledons when dormant and seven times higher when fully after-ripened. This indicated that the embryonic axes were a more active tissue than the cotyledons and the major site of metabolic changes. Pollock and Olney (42) also working on _Prunus cerasus_, showed a 70% increase in respiration in embryos and almost 600% increase in

in both embryonic axis and leaf primordia during a 16 week after-ripened treatment at 5°C. The respiratory increases in embryonic axes and leaf primordia were reported to be linear with after-ripening time. It, however, must be mentioned that the reported respiratory rate, measured at 25°C, should actually be considered as the respiratory capacity of the tissue and that the real rate of respiration at the usual after-ripening temperature of 1°C would be considerably lower than the one presented (Table 7). Not only will the yield of $C^{14}O_2$ be less at 1°C than at 25°C but the C6/C1 ratio (Table 9) might also be different because it is probable that one or both of the alternate pathways may not be operating at a maximum capacity at 1°C. Since the usual temperature at which the after-ripened seeds are normally grown is about 25°C, the results would depict the changes which have taken place in seeds that are ready to germinate.

The observed respiratory increase could have been due to the increased levels of enzymes, coenzymes and other rate limiting factors. The increased contribution of PP oxidation and a simultaneous respiratory increase during the seventh week indicated that probably NADP was a limiting factor prior to this period. Increased activity of the TCA cycle requires a continuous supply of pyruvate, thus the rate of glycolysis must be accelerated. This

may have resulted from an increased level of ADP if the results of DNP experiments are considered. DNP will increase ADP levels by uncoupling oxidative phosphorylation. Glycolytic reactions which are not directly affected by DNP response strikingly to the ADP increase and are greatly stimulated. The DNP response in dormant and non-dormant cotyledons was the same, indicating that factors other than ADP might be responsible for this respiratory increase. With embryonic axes, on the other hand, a greater respiratory increase in non-after-ripened than in fully after-ripened seeds was obtained.

It is suggested that the effect of low temperature was to increase the NADP and ADP levels in embryonic axes and/or the synthesis and translocation of these factors from the cotyledons. The dormancy termination may, therefore, be associated with the elevated levels of NADP and ADP which presumably stimulate metabolic cycles to supply intermediates and energy for the breaking of dormancy.

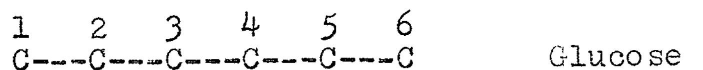
RQ values higher than unity observed in embryonic axes during the initial six week after-ripening period may have been due to a partial anaerobic metabolism (Table 8). The products of anaerobic metabolism may have been acting as growth inhibitors as has been suggested by several authors (34,59). The RQ values which were slightly

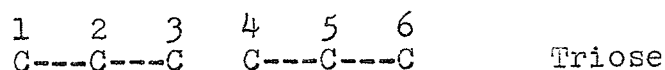
lower than unity during 7 to 16 week indicated that the substrate for respiration in embryonic axes during this stage was mainly sugars with some contribution of fat. This conclusion is further strengthened by the results obtained from the changes in the lipid levels. No change in lipid levels was observed during the first seven weeks (Table 5) indicating that lipid was being spared as a substrate for respiration during this time. The lipid levels dropped after seven weeks indicating their participation in respiration as shown by an RQ drop from 1.2 to 0.95.

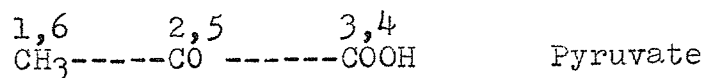
In cotyledons, however, the RQ was always below one (Table 8) suggesting that fat at all stages of after-ripening was a partial substrate for respiration. This caused the observed continuous decline in lipid levels in this tissue (Table 5).

Use was made of C6/C1 ratios, as suggested by Bloom and Stetten (6), to determine the relative contributions of EMP and PP pathways of oxidation. If glucose is broken down by glycolysis which includes equilibration at the triose level, pyruvate would be produced in which carbon atoms C-1 and C-6 of the glucose would appear as the methyl carbons of the acid, C-2 and C-5 as the carbonyl carbons. Carbons 1, 2 and 3 of the original glucose would, therefore, be indistinguishable from 6,5 and 4 respectively and in the subsequent oxidative breakdown of the pyruvate, each

member of a pair would appear in carbon dioxide at the same rate as its partner.







The pair C-3 and C-4 would appear first as carbon dioxide followed by C-2 and C-5 and then C-1 and C-6. Thus, if comparable samples of tissue were respiring on exogenously supplied glucose-1-C^{14} and glucose-6-C^{14} respectively, the contribution of C^{14} to the carbon dioxide given off would be the same in each case. If, on the other hand, glucose is metabolised by way of the PP pathway, carbon dioxide from glucose-1-C^{14} would be expected to be initially higher in C^{14} than that from the glucose-6-C^{14} since C-1 of glucose is the first to be converted to carbon dioxide. Provided that no assimilation of carbon residues containing different amounts of C-1 and C-6 occurred, the total yield of the radioactive carbon dioxide from the two glucose samples would be the same when oxidation was complete regardless of the path of breakdown. In short term experiments, however, a C6/C1 ratio of near unity would indicate that pathways other than the EMP are playing a very minor

part in glucose breakdown, whereas a ratio of less than unity would implicate participation of the PP pathway.

In this study the C6/Cl ratios obtained at 1, 2, 4 and 8 hours were almost the same and, therefore, their means were taken for presentation (Table 9). In embryonic axes the drain of glucose through the PP pathway appeared to be small during the initial six week period of after-ripening. Observed C6/Cl values, which were very close to unity, indicated the EMP to be the major system of metabolism during this time. In the seventh week the significant drop of this ratio to 0.65 indicated a rapid change of EMP break-down to PP oxidation. This ratio further dropped gradually to 0.53 by the end of the 16 week after-ripening time. The C6/Cl values of dormant as well as fully after-ripened cotyledons were found to be the same. These values varied from 0.65 to 0.69, indicating that the rate of glucose breakdown through the PP pathway remained essentially unaltered (Table 9).

Although currently the C6/Cl method is widely used to study metabolic pathways of glucose breakdown, the method has been criticized in several aspects. Of particular importance is the loss of certain intermediates into cellular constituents which precludes the appearance of certain carbons as carbon dioxide. This differential effect on the carbon of glucose may under or over estimate the PP

contribution. The retention of C6 would also lead to an over estimation of the PP pathway since the C-6 unit may either accumulate as intermediates or be incorporated into cellular constituents such as pentosans. As a result C-6 of glucose is incompletely converted into carbon dioxide. These considerations are directly involved in the present study, since there was a slightly greater incorporation of C-6 than C-1 into the amino acids, sugars and insoluble fraction (Table 10 and 11). This may have resulted in a slight over estimation of the PP contribution both in cotyledons and embryonic axes (Table 9).

Other methods of estimating precisely the contributions of the pathways to total glucose utilization have been proposed and evaluated (1, 43). Each of these involves assumptions, and none seems to be generally acceptable or applicable. In some of these methods the fate of the five carbon unit is not considered and it is assumed that the only carbon dioxide to appear from the pentose phosphate pathway is the original C-1 of the glucose, whereas it is quite clear that any glucose resynthesized by the pentose phosphate pathway would now have a different carbon atom in position one and this would be released as carbon dioxide when the glucose molecule re-entered the sequence. A further complication is that pyruvate, which may be drained

through triose from preceeding cycles of the PP sequence, would have a changing complement of the original glucose carbons. This introduces further hazards into the calculations of the alternate sequence.

Because of these considerations other evidence was sought to strengthen the metabolic trend as depicted by C6/C1 ratio. Supporting evidence came from the observed respiratory increase due to DNP addition to the embryonic axes and cotyledons given different after-ripening treatments. Two moles of ADP are required for each mole of pyruvate produced from supplied glucose if it is metabolised through the EMP pathway; the breakdown through the PP pathway is independent of this requirement. This indicates that EMP is more dependent on ADP and thus will be accelerated strikingly if additional ADP is made available to the system. On the contrary if the PP pathway is the major route, the increase in respiration would not be as great and the system will be accelerated to a degree depending on the extent to which the EMP pathway is contributing to the over all metabolism. In the actual procedure, therefore, ADP levels of embryonic axes which had undergone 0, 6, 11 and 16 weeks of after-ripening were raised by treating the embryos with DNP and the percent increase in respiration calculated. The data as shown in Table 14 indicated that during the initial six week after-ripening

period, the response due to DNP was three to four times higher with zero and six week after-ripened embryonic axes than with those that had been after-ripened for 11 to 16 weeks. It is concluded from these results that the EMP pathway is far more important in the first six weeks of after-ripening than in the remaining after-ripening period when the PP pathway contributed to a greater extent to total respiration. On the same basis after-ripened cotyledons which showed an identical C6/C1 ratio through out after-ripening also showed an equal response to DNP treatment (Table 15). The increased levels of ADP with increased after-ripening time as reported by Pollock and Olney (42), or rapid turn over of ATP/ADP during the advanced stages of after-ripening could have also made the EMP pathway less responsive to an increase in ADP levels. If so, then the DNP would have less effect on increasing oxygen uptake as after-ripening time increased.

The major route of amino acid formation in plants is via α-ketoglutaric acid, an intermediate in the TCA cycle. Thus if the EMP pathway is operative, then α-ketoglutarate will be equally labelled from C1 and C6 of the supplied glucose and as will the amino acids formed from this precursor. Further evidence which supported the observed changes in pathways of oxidation comes from the study of C^{14} incorporation into amino acids from glucose-6-C^{14} as

compared to that from glucose-1-C^{14} (Table 12). This drain of C6 into the amino acids of after-ripening embryonic axes can be explained if we consider that C1 of the supplied glucose was lost before reaching the TCA cycle. In other words, PP oxidation increased in importance as after-ripening progressed. In dormant embryonic axes where the EMP pathway is the prevalent pathway of oxidation, as shown by C6.C1 ratio and DNP response, the loss of C1 was negligible and therefore, the contribution to amino acids of C1 and C6 of glucose was essentially the same.

In cotyledons, where the contribution of the PP pathway of oxidation is significant but relatively constant throughout the after-ripening period, the greater contribution to amino acid labelling of C6 from glucose relative to C1 was also constant throughout the after-ripening period. Thus although the contribution of individual pathways cannot be precisely estimated, the present study showed that in cotyledons, in addition to the EMP pathway, breakdown via the PP sequence plays an important role in glucose dissimilation. In embryonic axes the EMP pathway is prevalent during the initial six weeks of after-ripening followed by a greater role of the PP pathway in subsequent after-ripening.

The question of what determines the extent of diver-

sion of glucose-6-phosphate to the PP pathway is not well understood. Presently two mechanisms are thought to be responsible (2). The first is the availability of NADP or the rate of re-oxidation of NADPH, which might well limit diversion of glucose through the pentose phosphate sequence. The dehydrogenases responsible for oxidation of glucose-6-phosphate to 6-phosphogluconate and further to ribulose-5-phosphate are NADP specific and thus in its absence their activity is limited and so is the PP contribution. The second mechanism involves the inhibitory effects of intermediates (6-phosphogluconate and erythrose-4-phosphate) on the glucose-6-phosphate isomerase. Thus when the cycle becomes operative, its intermediates help to increase the participation by inhbiting glucose-6-phosphate isomerase activity.

If NADP is a limiting factor to the initial increase in the PP pathway of oxidation then it may be assumed that the levels of this coenzyme may have increased in one of two possible ways. Phosphorylation of NAD due to the increased activity of NAD kinase or the oxidation of NADPH. The second reason appears more probable. Yamamoto (64) found that the majority of the NADP in cotyledons of _Vigna sesquipedalis_ was in the reduced state. He attributed this to the partial anaerobic conditions of the tissue. In the early after-ripening stage of _Prunus cerasus_ seeds par-

tial anaerobic conditions may exist as suggested by high RQ values. These conditions could make the transfer of electrons from NADPH ot oxygen difficult and thus NADP levels would remain low.

Brown (11) indicated that in Cucurbita pepo seed the permeability of the inner coat was greatest when the membrane was slightly less than completely saturated with water. Following this reasoning, therefore, it appears possible that as after-ripening advanced, the barrier to gaseous exchange imposed by the seed coverings may have decreased due to water uptake or slight rupturing of the endocarp. The rupture in the endocarp becomes considerable after 10-11 weeks of after-ripening period. The increased gaseous exchange may have facilitated an increased conversion of NADPH to NADP. NADP appears to be related to growth since when it was vacuum infiltrated into 11 week after-ripened embryos, a 64% growth increment over the control was observed (Table 16).

The C6/C1 ratio in cotyledons was lower than unity from the very beginning of the after-ripening period indicating comparatively higher NADP levels than in embryonic axes. The NADP in this tissue may be utilized for conversion of reserve materials such as lipids to transportable forms such as sucrose via the glyoxylate cycle. Although direct evidence was not obtained to indicate the function-

ing of the glyoxylate cycle in this tissue, the following observations may support the possibility of its operation. It was observed (Table 13) that there was an increased activity of C6 in the isolated sucrose. If the synthesis of sucrose was directly from labelled glucose, then the radioactivity in sucrose would be independent of glucose label position. High radioactivity in sucrose from C6 relative to C1 labelled glucose may indicate synthesis via a reversal of glycolysis. However, the increased activity in sucrose from C6 could also be due to the resynthesis of hexose from intermediates of the PP pathway of oxidation. Other evidence comes from the changes in the levels of lipids and sugars of the embryonic axes and cotyledons. In embryonic axes and cotyledons an increase in sugar levels with a corresponding decrease in lipid levels was observed (Tables 3 and 5). A similar trend in germinating castor bean slices was reported by Canvin and Beevers (12) and was found to be due to the conversion of fats to sucrose via the glyoxylate cycle.

Most natural fats contain mixtures of triglycerides, although in particular species one or another fatty acid usually predominates. The fats from _Prunus cerasus_ seeds were mainly composed of oleic and linoleic containing triglycerides. Although palmitic and stearic acids were present in small amounts, percentage-wise they were utilized

at a faster rate. The available evidence does not indicate any orderly discrimination of oleic and linoleic acids utilization (Table 6). It must, however, be pointed out that the dry weight of the seeds at the same time decreased and thus the actual utilization of the fatty acids was uncer estimated to some extent. It is not known whether this mode of utilization of fatty acids has anything to do with breaking dormancy.

In general during after-ripening, the cotyledons must remain intact with embryonic axes for the growth of the embryo. This suggests that certain essential growth factors must be translocated from the cotyledons to the embryonic axes. In *Prunus cerasus* seeds the weight of embryonic axes increased by 34.1% and that of cotyledons decreased by 9.5%. It is considered that certain materials are being transported from the cotyledons to the embryonic axes. The cotyledons are rich in fats, which are converted to sucrose via the glyoxylate cycle and the reversal of glycolysis. This sucrose is supplied to the after-ripening embryonic axes, where it may be utilized for respiratory processes or for the biosynthesis of growth substances and structural materials.

On the basis of the results presented, a hypothetical scheme for the biochemical changes occurring in this seed

is presented. It appears that embryos of _Prunus cerasus_ are dormant because of a lack of certain growth factors required for germination. During after-ripening certain metabolic changes take place both in embryonic axes and cotyledons. In cotyledons the high lipid level is utilized in a conversion to sugars via the glyoxylate cycle and reversal of glycolysis. These sugars with other materials are translocated to embryonic axes. It is unlikely that substances from the embryonic axes will move to any extent against this main translocation stream, thus changes in metabolism of the embryonic axis are unlikely to have any direct effect on metabolism of cotyledons. In the embryonic axes, it appears that moisture, oxygen and low temperature during the initial six week after-ripening period speed up enzymatic activity and oxidation of reduced pyridine nucleotides. Due to the oxidation of NADPH or to its _de novo_ synthesis during this period, the increased level of NADP brings about a striking increase in PP activity. The ample availability of this NADP also increases the activity of isocitric dehydrogenase. The result is an acceleration of both PP pathway and TCA cycles. The intermediates of these cycles appear to be linked to the synthesis of certain growth substances which are required to overcome the inhibitory block responsible for dormancy. During the seventh week, the metabolic shift from the EMP to the PP

pathway suggests that intermediates of this latter cycle might be playing a dominant role in the synthesis of these growth factors.

SUMMARY

Seed dormancy in *Prunus cerasus* is primarily due to embryo dormancy. An after-ripening treatment of 7-10 weeks resulted in a termination of embryo dormancy but intact seeds failed to germinate completely due to effects of the endocarp and seed coats.

In embryonic axes during the first six weeks of after-ripening, lipids, sugars, dry weight and respiratory rate remained unchanged. During this period the EMP was the predominant pathway of glucose oxidation.

Possibly due to the effects of increased levels of NADP, there was an abrupt change in metabolic activity at the seventh week of after-ripening. At this time, studies of C6/Cl ratio, 2-4 DNP effect on respiration and contribution of C6 and Cl of glucose to the amino acids fraction indicated the PP to be the major pathway of glucose breakdown. These changes were accompanied by a rapid increase in respiratory rate, a decrease in lipid level, and an increase in sugars and dry weight.

A similar, but less marked increase in respiration occurred in cotyledons, but the C6/Cl ratios remained constant at approximately 0.65. Indirect evidence indicated that lipids were being oxidized via the glyoxylate cycle and were being translocated in some form to supply increased respiratory requirements of the embryonic axes.

It is suggested that the changes in pathway of oxidation are linked in some manner to growth factor production which leads to a termination of embryo dormancy.

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