This is to certify that the

dissertation entitled

Characterization of Secondary Dormancy

in Apple Seeds

presented by

Jocelyn Ann Ozga

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Horticulture

Frank G. Dennis Jr.
Major professor

Date 11/17/88

CHARACTERIZATION OF SECONDARY DORMANCY IN APPLE SEEDS

BY

Jocelyn Ann Ozga

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1988

ABSTRACT

CHARACTERIZATION OF SECONDARY DORMANCY IN APPLE SEEDS

BY

JOCELYN ANN OZGA

When stratified apple seeds are stressed, secondary dormancy is induced. High temperature, low oxygen tension, high levels of ethylene and exogenous ABA were characterized as stress factors in inducing secondary dormancy.

Exposure to 25-35°C inhibited subsequent germination of stratified apple seeds at 20°; however, only 35° inhibited embryo germination. As time of stratification at 5° increased, the seeds and embryos became less sensitive to high temperature induction of dormancy. High temperature did not completely negate the effect of a prior exposure to low temperature unless full germination potential of the seed had been achieved prior to the heat treatment.

Anaerobiosis reportedly breaks the dormancy of non-stratified apple embryos. Low O_2 treatments (0.3-0.5% and <0.16% O_2) were tested for effectiveness on both non-stratified and stratified apple seeds and embryos, and on stratified seeds in which secondary dormancy had been

induced by exposure to 30°C for 6 days. A small promotive effect was observed in non-stratified, but not in stratified embryos, however, the treatments inhibited germination of stratified seeds. Neither treatment broke secondary dormancy in intact seeds. And germination of embryos excised from them was often inhibited.

Exposure to 10^{-6} to 10^{-3} M ABA or to 3 or 6 days at 30°C inhibited stratified seed and embryo germination, and the effects of the two treatments were additive. However, no correlation was found between endogenous ABA content of seed tissues (testa, cotyledons, or embryonic axis) and either the duration of incubation at 5°, or the induction of secondary dormancy by heat stress (30°C for 0, 3 or 6 days).

The observations made by others that ethylene (C_2H_4) is required for germination of stratified embryos of apple was not confirmed. Neither removal of ethylene from the atmosphere nor inhibition of ethylene action by norbornadiene or silver thiosulfate appreciably affected germination of fully stratified seeds or embryos at 20° or 30°C. The addition of ethylene either had no effect or reduced germination of fully stratified seeds or embryos; therefore, C_2H_4 does not appear to be essential for germination of apple seeds or embryos.

ACKNOWLEDGEMENTS

I would like to express my deep appreciation to my major professor, Frank Dennis, for his encouragement, direction, and participation in the completion of this thesis and for being a great human being.

I would also like to thank my family for their support during the making of this thesis.

My deepest gratitude belongs to my husband Dennis, for all his assistance, encouragement, sacrifice, and love.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

LITERATURE REVIEW

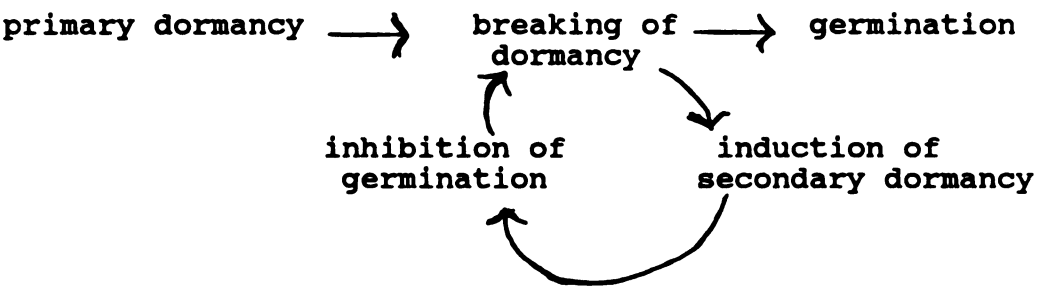
Introduction

Environmental factors play an important role in seed germination. Firstly, imbibition of the seed is necessary to initiate an active metabolism. Such metabolic activity requires O_2 and will occur only within certain temperature limits. However, many seeds are unable to germinate even when placed in the presence of water and oxygen at permissible temperatures. This inability to germinate, known as dormancy, will be addressed in this review.

Definition of dormancy

Dormancy is the suspension of growth of any plant structure containing a meristem. Classification into primary and secondary dormancy is based on the phase in the life-cycle of a seed in which the induction occurs. Primary dormancy prevents germination during development and maturation on the mother plant and may continue for some time after shedding or harvesting of the seeds. Secondary dormancy develops after dispersal or harvest in seeds that are primarily non-dormant or have emerged partly or fully from primary dormancy (Karssen, 1980/81). Under natural

conditions, secondary dormancy increases the probability that once the seed germinates, the seedling will survive.



No evidence exists that proves that a seed entering secondary dormancy returns to a physiological state identical to its state during primary dormancy. Visser (1956b), stored stratified apple seeds for various periods of time at 25°C. As storage time increased, longer periods at a dormancy breaking temperature (3°C) were required to restore the initial germination capacity of the embryos. This was also the case in Impatiens glandulifera seeds (Mumford, 1988). These data support the hypothesis that primary and secondary dormancy are not identical states. However, if dormancy is quantitative, longer periods merely increase the depth of dormancy; therefore, secondary dormancy could be argued to be identical to primary dormancy. Other researchers did conclude that primary and secondary dormancy were identical (Thornton, 1945; Abbott, 1955); however, they presented no firm evidence to verify their claim.

This thesis will emphasize the factors and mechanisms involved in the induction and release of secondary dormancy

in apple seeds.

Apple seed morphology

The mature apple seed consists of the following parts (Luckwill, 1952; Harrington, 1923):

1. Embryo: embryonic axis with two large fleshy cotyledons containing the bulk of the stored food reserves.
2. Endosperm layer: a whitish membranous layer of endosperm tissue to which a very thin nucellus layer adheres closely.
3. Inner integument: a thin, translucent brownish layer of tissue without any opening.
4. Outer integument: a thick, brown and fibrous layer with the open micropyle.

The outer integument constitutes approximately 33%, the inner integument and endosperm layer 11%, and the embryo 56% of the seed fresh weight (Harrington, 1923).

The inner and outer integuments collectively are called the seed coat in this thesis.

Conditions which induce secondary dormancy

Induction of secondary dormancy under natural conditions in the field has been observed in seeds of many wild species of plants. The increase in dormancy is often part of cyclical changes that follow a seasonal pattern (Karssen, 1980/81).

In controlled conditions, induction of secondary dormancy occurs in seeds when exposed to one or more of a number of environmental stresses. High temperature induces secondary dormancy in seeds of many species (Stokes, 1964). Abbott's (1955) experiments with apple seeds demonstrated that exposure to temperatures above 17^{o}C induced secondary dormancy in partially chilled seeds; temperatures below 17^{o}C were effective in breaking dormancy. At 17^{o}C, defined as the "compensation temperature", germination capacity of the partially chilled seeds remained the same. This implied that both dormancy breaking and dormancy inducing processes are in equilibrium at that temperature. Imbibition in an osmoticum imposes secondary dormancy in seeds of lettuce (Kahn, 1960) and Chenopodium bonus-henricus (Khan and Karssen, 1980). Low oxygen tension induces secondary dormancy in seeds of Xanthium canadense (Davis, 1930) and embryos of apple (Malus domestica) (Come and Tissaoui, 1968). In addition, exogenous abscisic acid inhibits germination in non-dormant apple embryos (Rudnicki and Pieniazek, 1973), and induces secondary dormancy at 10^{-4} M after a 15 day exposure (Durand et al., 1973).

Induction of secondary dormancy can be prevented in certain cases. Sisymbrium officinale seeds escaped light-induced, and Polygonum persicaria dark-induced, dormancy when treated with nitrate (Karssen,1980/81a and b). In some cases simultaneous treatment with light or growth regulators (e.g. gibberellin) counteracts the dormancy-inducing factors

such as high temperature and osmotica (Khan and Karssen, 1980; Khan, 1980/81). High levels of oxygen and ethylene prevent secondary dormancy in Xanthium (Esashi et al., 1978); in contrast, apple embryos did not enter a dormant phase when subjected to high temperature under anaerobic conditions (Bulard, 1986). Lettuce and Rumex crispus seeds escaped high temperature and dark-induced dormancy when held under anaerobic conditions (Karssen, 1980/81a; LeDeunff, 1973).

Mechanisms of secondary dormancy induction

Various hypotheses have been proposed to explain secondary dormancy in seeds. One of the earliest hypotheses suggested that a limited supply of oxygen to the embryo was responsible for germination inhibition and secondary dormancy induction (Crocker, 1906). In 1930, Davis suggested that a decrease in growth promotors or increase in inhibitors may have a role in secondary dormancy induction, and, in 1982, Bewley and Black proposed changes in membranes as a possible mechanism in the regulation of dormancy.

Limited availability of oxygen to embryos

Crocker (1906) found that upper seeds of Xanthium canadense germinate readily on exposure to an atmosphere of 100% O_2; seeds held in air did not. He concluded that the inhibition of germination was due to the seed coat restricting the supply of oxygen to the embryo; seeds displayed no dormancy if the seed coat was removed. Davis

(1930) was able to induce dormancy in the cocklebur embryo by embedding the moist seeds in clay or immersing them in agar for two months at 27°-30°C under conditions where presumably a low supply of oxygen was available to the seeds. The dormancy was subsequently overcome by removal of the seed coats or moist storage at 5° for three months.

Visser (1956b) studied the effect of high temperature on respiratory activity (O_2 consumption and CO_2 production) in apple seeds. Partially chilled seeds were successively subjected to temperatures from 3° to 30°C for 2 hours at each temperature. With increasing temperature the respiratory intensity of both seeds and embryos increased. However, at 25-30° the respiratory quotient of the intact seeds was higher than that of the excised embryos. Visser suggested that restriction of O_2 uptake by the seed coat increased with temperature. If intact, partially stratified seeds were germinated at 25°, about 60% of seeds entered secondary dormancy. However, if the seed coat was removed and the endosperm ruptured, particularly at the radicle end, high germination levels were obtained (Visser, 1956a).

From these data, Visser proposed that the comparatively high respiration, combined with the lower O_2 availability to the embryos in intact seeds at high temperatures, are the primary factors responsible for the development of secondary dormancy.

Secondary dormancy was induced in 5 days at 25°C in partially stratified Tatarian maple seeds (Nikolaeva, 1969).

A period greater than 8 days (< 92 days) at low temperatures was required for seeds to respond to the transfer to higher temperatures in the above manner. After transfer of partially stratified seeds to high temperature (25^{o}) for 2 hours, a sharp intensification of respiration (O_2 consumpted) followed and the RQ value rose above unity. The initial high respiration and RQ fell after 5 days, and after 16 days, they had dropped to the level of dormant seeds. Nikolaeva proposed that secondary dormancy was induced by high respiration rates of the embryo under conditions of greatly impeded gas exchange, which reduced the supply of O_2 to the embryo.

Come and his colleagues (1972), following this line of inquiry, suggested that oxygen is consumed by the testa enclosing the embryo. In apple, oxygen consumption by the testa was attributed to the oxidation of various phenolics present such as phloridzin, chlorogenic acid, and para-coumarylquinic acid. They suggested that when apple seeds are exposed to elevated temperatures, oxygen becomes less soluble in water and oxidation of the phenolic compounds in the seed coat increases. These factors reduce availability of O_2 to the embryo, resulting in induction of secondary dormancy.

Come and Tissaoui (1968,1972) directly measured the effect of low O_2 tension on apple embryos. A 42 day exposure to O_2 tensions of <0.16% had no effect on germination of partially stratified embryos, but exposure to

higher O_2 levels (0.16 to 0.84%) induced secondary dormancy. However, a 42 day period of O_2 deprivation for apple embryos is not physiologically meaningful when only 8 days at 30°C were required for secondary dormancy induction in embryos (Perino and Come, 1977). Moreover, embryos exposed to 0.5% O_2 for 7 days do not enter into secondary dormancy (Come and Ralambosoa, 1979).

Levels of growth inhibitors

Abscisic acid. Since its chemical identification in 1965 (Ohkuma et al.), abscisic acid has become the most studied growth inhibitor in plants. Attempts to correlate the level of ABA with the induction of dormancy in seeds have resulted in conflicting evidence. Freshly harvested seeds of species that exhibit primary dormancy, such as ash (Fraxinus americana) and hazelnut (Corylus avellana) (Sondheimer et al., 1968; Williams, et al., 1973) contain relatively high levels of ABA.

Balboa-Zavala and Dennis (1977) studied the seed maturation of two apple cultivars with respect to their ABA levels and germinability. In developing 'Golden Delicious' seeds, ABA levels in the embryonic axis, as measured by electron capture gas chromatography (EC-GC), were highest when germinability was highest, with both declining as the seed matured. In 'McIntosh', ABA levels remained relatively constant (400-600 ng/g fresh wt.) during the period when germinability declined from 60% to 0%. ABA levels

subsequently rose to very high levels (1300 ng/g fresh wt.), after the seeds had become dormant. However, the concentrations of ABA in embryonic axes reported by Balboa-Zavala and Dennis (1977) are approximately 500 times greater than values obtained using gas chromatography combined with mass spectrometry (GC-MS) (Subbaiah, 1987), making these data questionable.

The most consistent evidence available for the involvement of abscisic acid in the induction of seed dormancy comes from the studies on ABA-deficient mutants of Arabidopsis thaliana (L.) Heynh. Mutant lines of Arabidopsis characterized by high transpiration rates and a lack of seed dormancy were found to contain low levels of ABA in leaves and mature seeds in comparison with the wild-type plants (Karssen, et al., 1983). In the wild-type seeds, the ABA concentrations reached 200-500 ng per g fresh weight, whereas in the mutants the concentration never exceeded 10 ng per gram. The wild-type seeds were dormant at maturity but the mutant seeds were not. Reciprocal crosses of wild type (Aba/Aba) and ABA-deficient mutants (aba/aba) were made in order to produce seeds with the Aba/aba embryo genotype in both a wild-type and an Aba-type mother plant. Dormancy did not develop when both parents were ABA-deficient, but it developed fully in the heterozygous F1 seeds, irrespective of the genotype of the mother plant. Therefore, Karssen et al. (1983) concluded (a) that the development of dormancy is regulated by the

genotype of the embryo and is not a maternal effect, and (b) that the probability that ABA and dormancy induction are not causally related is very low because genetic analysis has shown that only a single gene is involved in this mutation (Koornneff et al. 1982). The data of Karssen et al. (1983) are very strong evidence that embryonic ABA is associated with primary dormancy in Arabidopsis; however, can we conclude that induction of dormancy is the same in herbaceous species as in a woody species, or in primary dormancy as in secondary dormancy?

The possibility that ABA is responsible for the initiation of secondary dormancy is equivocal. Balboa-Zavala and Dennis (1977) also measured ABA levels in partially stratified apple seeds in which secondary dormancy was induced by exposure to 1 week at 27°C. ABA levels decreased during the period of high temperature in whole seeds; however, ABA levels in the embryonic axis, cotyledons, and seed coats were not measured separately.

ABA prevents only the very last phase of germination, that involving cell expansion, in Chenopodium album (Karssen, 1976). Radicle growth in this seed can be divided temporally into 3 stages. The radicle splits the outer integument in stage 1, but the inner testa remains intact; in stage 2, further growth occurs, still inside the intact inner integument, then, in stage 3, the radicle bursts through this coat. Application of ABA to germinating seeds does not stop them from reaching stages 1 and 2, but

prevents the onset of stage 3. Studies with ^{14}C-labelled ABA demonstrated that a substantial amount of applied inhibitor entered the seeds within 16 hours from the start of imbibition, well before seeds had reached stage 1.

Studies on Sinapis alba (Schopfer et al., 1979) and Haplopappus gracilis (Galli et al., 1980) also suggest a late action of abscisic acid. ABA remains an effective inhibitor as long as it is applied just before the initiation of radicle elongation.

The late action of ABA could explain the inhibition of germination of fully stratified apple seeds, since they are poised for radicle elongation before exposure to dormancy inducing conditions. However, secondary dormancy can also be induced in partially stratified seeds that would not be poised for radicle elongation in the same conditions.

At which level, and via what mechanism, ABA exerts its inhibitory influence in the developing or mature seed is an interesting line of inquiry. Galli, et al. (1979) observed that ABA at $10^{-5}M$ completely inhibited the emergence of radicles from Haplopappus gracilis seeds, and this effect was prevented effectively by fusicoccin (FC), a fungal toxin which promotes H^+/K^+ exchange in plant membranes. However, FC had limited effect in preventing ABA inhibition of post-germinative growth. ABA at this later stage of germination inhibited DNA synthesis as demonstrated by the incorporation of [^3H] thymidine into nuclei. Because FC did not reverse this inhibition, the authors suggested that two different

mechanisms are involved in the ABA inhibition of H. gracilis germination. One mechanism, which is reversed by FC, interferes with K^+ uptake at the level of cell membranes; the other, which is not, interferes with the synthesis of nucleic acids and/or proteins.

Studies by Jacobsen and Beach (1985) on the inhibition of Ga_3-induced synthesis of α-amylase by ABA in barley (Hordeum vulgare) aleurone cells has led to the conclusion that ABA antagonizes GA action in two major ways, one preventative and one promotive. It prevents GA action at the level of transcription by suppressing GA-induced mRNA synthesis. The promotive action involves the ABA-induced increase in mRNAs and protein, which in one case is proposed to be related to the production of an -amylase inhibitor (Ho, 1988).

Ethylene. Another growth regulator with a possible role in secondary dormancy induction is ethylene. Sinska and Gladon (1984) suggested that ethylene was required for germination of apple embryos. If this is the case, inducers of secondary dormancy may act in apple seeds by reducing ethylene levels, resulting in inhibition of germination. Secondary dormancy of sunflower seeds (Helianthus annuus L.) induced by 5 days at 45°C, was partially prevented when seeds were presoaked at 5° for 22 hours with 2-chloroethylphosphonic acid (ethephon). However, C_2H_4 (55 ul/l) and ACC (2.5mM) were not effective (Corbineau, et al., 1988).

Changes in membranes

A body of information supports the possibility that processes controlled by the state of membranes are involved in the regulation of dormancy (Bewley and Black, 1982). Membranes undergo sharp transitions in their physical state at particular temperatures. Enzymes associated with them also undergo abrupt changes in activity. Factors which induce dormancy may do so through changes in membranes and membrane-bound enzymes. However, the nature of these processes remains speculative.

Maintenance of secondary dormancy

Investigations on the mechanisms involved in the maintenance of secondary dormancy have led to an understanding of the processes that occur during this period, but they do not define which process or processes are responsible for this state. In the Visser study (1956b) when partially chilled apple seeds were stored for up to 8 weeks at 25^{o}C under moist conditions, both respiratory activity and germination capacity in excised embryos decreased. Therefore, high respiratory activity in the embryos was not necessary for the maintenance of secondary dormancy.

Autoradiographic studies have shown that protein synthesis occurs in the axis, scutellum, coleorhiza, and aleurone layer of dormant wild oat grains. The amount of synthesis in the dormant embryos, however, is not substantially different from that in the embryos of after-

ripened grains in the first 12-24 hours of imbibition (Chen and Varner, 1970; Cuming and Osborne, 1978). In dormant grains, an analysis of membrane proteins indicated that they continuously turn over at a relatively high rate, although no new classes of membrane proteins were produced (Cuming and Osborne, 1978a and b). The authors suggested that this continuous generation and recycling of cellular membranes is necessary to maintain the metabolic integrity of the hydrated cells of the dormant embryos.

Evidence that chemical inhibitors are responsible for the maintenance of dormancy is equivocal. In some cases, dormant seeds have higher levels of abscisic acid (ABA) than non-dormant seed, as in Fraxinus (Sondheimer et al., 1968). However, no such differences occur between non-dormant Avena sativa and the highly dormant A. fatua (Berrie et al., 1979) or among species and strains of Pyrus with different degrees of dormancy (Dennis et al., 1978).

Release of secondary dormancy

Secondary dormancy of apple seeds is broken by low temperature stratification. Although no reports on alternative methods for breaking secondary dormancy have been published for apple seeds, factors such as light and combinations of growth regulators reportedly overcome secondary dormancy in other species. High temperature dormancy in lettuce can be relieved by a combination of 20% oxygen and 40-80% carbon dioxide (Thornton, 1936) or exposure to respiratory inhibitors (Khan and Zeng, 1985).

Osmotically induced dormancy of Chenopodium bonus-henricus is overocme by light and by gibberellin A4 and A7 (Khan and Karssen, 1980). Secondary dormancy induced by darkness and high temperature in lettuce seeds is broken by various combinations of light, gibberellin, cytokinin, and ethylene (Bewley, 1980; Khan, 1980/81).

Summary

Secondary dormancy in seeds reflects a general insensitivity to external and, perhaps, internal inducers of germination. The non-specificity of both the inducing agents and the inhibitory condition suggests a very general change in cell metabolism or subcellular structure, but what these changes may be is not known. Since this dormancy can be fairly easily manipulated under laboratory conditions, further investigation in this field could prove very fruitful in understanding the biochemical basis of dormancy.

The goals of this thesis were to a) confirm and extend the observations of the effects of temperature on the induction and release of secondary dormancy in apple seeds, b) define the roles of apple seeds and embryos with respect to the induction of secondary dormancy by low O_2 tension, c) define the role of abscisic acid in heat stress induced secondary dormancy in apple seeds, and d) define the role of C_2H_4 in apple seed germination and secondary dormancy induction.

Literature Cited

Abbott, D.L. 1955. Temperature and the dormancy of apple seeds. Rept. XIV Intern. Hort. Congr., Netherlands. 2A:746-753.

Balboa-Zavala, O. and F.G. Dennis, Jr. 1977. Abscisic acid and apple seed dormancy. J. Amer. Soc. Hort. Sci. 102:633-637.

Berrie, A.M.M., Buller, D., Don, R. and W. Parker. 1979. Possible role of volatile fatty acids and abscisic acid in the dormancy of oats. Plant Physiol. 63:758-764.

Bewley, J.D. 1980. Secondary dormancy (skotodormancy) in seeds of lettuce (Lactuca sativa cv. Grand Rapids) and its release by light, gibberellic acid and benzyladenine. Physiol. Plant. 49:277-280.

Bewley, J.D. and M. Black. 1982. Physiology and Biochemistry of Seeds, Vol.2. Springer-Verlag, New York. pp.207-212.

Bulard, C. 1986. Conditions of induction of secondary dormancy in apple after breaking of primary dormancy by anaerobiosis and low temperature. Physiol. Plant. 67:279-284.

Chen, S.S.C., and J.E. Varner. 1970. Respiration and protein synthesis in dormant and after-ripened seeds of Avena fatua. Plant Physiol. 46:108-112.

Come, D. and T. Tissaoui. 1968. Induction d'une dormance embryonnaire secondaire chez le pommier (Pirus malus L.) par des atmospheres tres appauvries en oxygene. C.R. Acad. Sci. Paris 266:477-479.

Come, D. and T. Tissaoui. 1972. Interrelated effects of imbibition temperature and oxygen on seed germination. p.157-168. In: W. Heydecker (ed) Seed Ecology. Pennsylvania State University Press, Univ. Park, PA.

Come, D. and J. Ralambosoa. 1979. Influence simultanee de la lumiere et de l'oxygene sur la germination de l'embryon de pommier non-dormant. C.R. Acad. Sc. Paris 289:869-871.

Corbineau, F., Rudnicki, R.M., and D. Come. 1988. Induction of secondary dormancy in sunflower seeds by high temperature. Possible involvement of ethylene biosynthesis. Physiol. Plant. 73:368-373.

Crocker, W. 1906. Role of seed coats in delayed germination. Bot. Gaz. 42:265-291.

Cuming, A.C. and D.J. Osborne. 1978a. Membrane turnover in imbibed and dormant embryos of the wild oat (Avena fatua L.) I. Protein turnover and membrane replacement. Planta 139:209-217.

Cuming, A.C. and D.J. Osborne. 1978b. Membrane turnover in imbibed and dormant embryos of the wild oat (Avena fatua L.) II. Phospholipid turnover and membrane replacement. Planta 139:219-226.

Davis, W.E. 1930. Development of dormancy in seeds of cocklebur (Xanthium). Amer. J. Bot. 17:77-87.

Dennis, F.G. Jr., Martin, G.C., Gaskin, P. and J. MacMillan. 1978. Hormones in pear seeds. II. Levels of abscisic acid, dihydrophaseic acid, and their metabolites in relation to seed dormancy in several Pyrus species. J. Amer. Soc. Hort. Sci. 103:314-317.

Durand, M., Thevenot, C. and D. Come. 1973. Influences de l'acide abscissique sur la germination et la levee de dormance des embryons de Pommier (Pirus Malus L.). C. R. Acad. Sci. Paris D 277:53-55.

Esashi, Y., Okazaki, M., Yanai, N., and K. Hishinuma. 1978. Control of the germination of secondary dormant cocklebur seeds by various germination stimulants. Plant and Cell Physiol. 19:1497-1506.

Galli, M.G., Miracca, P., and E. Sparvoli. 1979. Interaction between abscisic acid and fusicoccin during germination and post germination growth in Haplopappus gracilis. Plant Sci. Lett. 14:105-111.

Galli, M.G., Miracca, P., and E. Sparvoli. 1980. Lack of inhibiting effects of abscisic acid on seeds of Haplopappus gracilis (Nutt.) Gray pregerminated in water for short times. J. Exp. Bot. 31:763-770.

Harrington, G.T. 1923. Respiration of apple seeds. J. Agr. Res. 23:117-131.

Ho, T.D. 1988. Regulation of gene expression by ABA in barley aleurone layers. 13th International Conf. Plant Growth Substances. p.23 (Abstract)

Jacobsen, J.V. and L.R. Beach. 1985. Evidence for control of transcription of α-amylase and ribosomal RNA genes in barley aleurone protoplasts by gibberellic acid and abscisic acid. Nature 316:275-277.

Kahn,A. 1960. An analysis of "dark-osmotic inhibition" of germination of lettuce seeds. Plant Physiol. 35:1-7.

Karssen, C.M. 1976. Uptake and effect of abscisic acid during induction and progress of radicle growth in seeds of Chenopodium album. Physiol. Plant. 36:259-263.

Karssen, C.M. 1980/81a. Environmental conditions and endogenous mechanisms involved in secondary dormancy of seeds. Israel J. Bot. 29:45-64.

Karssen, C.M. 1980/1981b. Patterns of change in dormancy during burial of seeds in soil. Israel J. Bot. 29:65-73.

Karssen, C.M., Brinkhorst-van der Swan, D.L.C., Breekland, A.E., and M. Koornneef. 1983. Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of Arabidopsis thaliana (L.) Heynh. Planta 157:158-165.

Khan, A.A. 1980/1981. Hormonal regulation of primary and secondary seed dormancy. Israel J. Bot. 29:207-224.

Khan, A.A. and C.M. Karssen. 1980. Induction of secondary dormancy in Chenopodium bonus-henricus L. seeds by osmotic and high temperature treatments and its prevention by light and growth regulators. Plant Physiol. 66:175-181.

Khan, A.A. and G. Zeng. 1985. Dual action of respiratory inhibitors: inhibition of germination and prevention of dormancy induction in lettuce seeds. Plant Physiol. 77:817-823.

Koornneeff, M., Jorna, M.L., Brinkhorst-van der Swan, D.L.C., and C.M. Karssen. 1982. The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of Arabidopsis thaliana (L.) Heynh. Theor. Appl. Genet. 61:385-393.

Le Deunf, Y. 1973. Interactions entre l'oxygene et la lumiere dans la germination et l'induction d'une dormance secondaire chez les semences de Rumex crispus L. Note C.R.Acad. Sci. Serie D 276:2381-2384.

Luckwill, L.C. 1952. Growth-inhibiting and growth-promoting substances in relation to the dormancy and after-ripening of apple seeds. J. Hort. Sci. 27:53-65.

Mumford, P.M. 1988. Alleviation and induction of dormancy by temperature in Impatiens glandulifera Royle. New Phytol. 109:107-110.

Nikolaeva, M.G. 1969. Physiology of deep dormancy in seeds. p. 146-162 Israel Program for Scientific Translations Press, Jerusalem.

Ohkuma, K., Addicott, F.T., Smith, O.E., and W.E. Thiessen. 1965. The structure of abscisin II. Tetrahedron Lett. 29:2529-2535.

Perino, C. and D. Come. 1977. Influence de la temperature sur les phases de la germination de l'embryon de Pommier (Pirus malus L.). Physiol. Veg. 15:469-474.

Rudnicki, R. and J. Pieniazek. 1973. The effect of abscisic acid on stratification of apple seeds. Bull. Acad. Pol. Sci. 21:149-154.

Schopfer, P., Bajracharya, D., and D. Plachy. 1979. Control of seed germination by abscisic acid. I. Time course of action in Sinapis alba L. Plant Physiol. 64:822-827.

Sinska, I. and R.J. Gladon. 1984. Ethylene and the removal of embryonal apple seed dormancy. HortScience. 19:73-75.

Sondheimer, E., Tzou, D.S., and E.C. Galson. 1968. Abscisic acid levels and seed dormancy. Plant Physiol. 43:1443-1447.

Stokes, P. 1964. Temperature and seed dormancy, pp746-803, In: Handbuch der Pflanzenphysiologie, Volume 15. Ruhland, W. (Ed.), Springer, Berlin.

Subbaiah, T. 1987. Abscisic acid relationships in apple seed dormancy. Ph.D. Thesis, Cornell University.

Thornton, N. 1936. Carbon dioxide storage. IX. Germination of lettuce seeds at high temperatures in both light and darkness. Contrib. Boyce Thompson Inst. Plant Res. 8:25-40.

Thornton, N. 1945. Importance of oxygen supply in secondary dormancy and its relation to the inhibiting mechanism regulating dormancy. Contr. Boyce Thompson Inst. Pl. Res. 13:487-500.

Visser, T. 1956a. The role of seed coats and temperature in after-ripening, germination and respiration of apple seeds. Proc. K. Ned. Akad. Wet. 59:211-222.

Visser, T. 1956b. Some observations on respiration and secondary dormancy in apple seeds. Proc. K. Ned. Akad. Wet. 59:314-324.

Williams, P.M., Ross, J.D., and J.W. Bradbeer. 1973. Studies in seed dormancy. VII. The abscisic acid content of the seeds and fruits of Corylus avellana L. Planta 110:303-310

SECTION I

The Effect of Temperature on the Induction and Release of Secondary Dormancy in Apple Seeds

The Effect of Temperature on the Induction and Release of Secondary Dormancy in Apple Seeds

Introduction

Partially stratified apple seeds can be induced into secondary dormancy by high temperature and release from this dormancy by restratification at low temperature (Abbott, 1955; Visser, 1956). Visser (1956) held apple seeds at 25°C to induce secondary dormancy following cold stratification. As the time of storage at 25°C increased, longer periods at 3°C were required to restore the initial germination capacity of the excised embryos. However, he did not establish whether high temperature completely negated the promotive effects of chilling. In order to further characterize the conditions inducing and breaking secondary dormancy, seeds stratified at 5°C for 6,8, and 12 weeks were exposed to temperatures ranging from 15 to 40°, then germinated at 20°. In a second experiment, seeds were stratified for varying periods of time prior to exposure to 30°, the restratifed at 5°.

Materials and Methods

Apple seeds (*Malus domestica* cv. Golden Delicious) were removed from fruit at harvest, dried, and stored at 5°C. Subsequently, seeds were soaked overnight in water, rinsed, placed in Petri plates containing filter paper wetted with 0.5% Captan solution, then held in darkness at 5°C for 0 to

12 weeks before exposure to higher temperatures. Embryos were considered germinated when the radicle reached a length of 3 mm or longer. Seeds induced into secondary dormancy by high temperature were restratified at 5°C to check viability.

Both experiments were arranged in a completely random design. An analysis of variance (ANOVA) was performed and Duncan's Multiple Range Test (DMRT) used to determine mean separation.

Experiment 1. Induction of secondary dormancy. Seeds were stratified for 6, 8, or 12 weeks, then placed at 15, 20, 25, 30, 35, or 40°C for 1 week prior to transfer to 20° for germination (5 replications of 10 seeds each). Embryos were dissected from half the seeds on transfer to 20°. Germination was evaluated during a 10-day period at 20° in darkness.

Experiment 2. Release from secondary dormancy. Seeds were stratified at 5°C for 3, 6, or 9 weeks. Control seeds were germinated at 20° immediately on removal from 5°. All other seeds were transferred from 5° to 30° for 6 days, then returned to 5° for 0, 3, 6, or 9 weeks. Following the last exposure to 5°, all seeds, or embryos dissected from similarly treated seeds, were placed at 20° for germination. Six replications of 10 seeds or embryos were used per treatment.

The results of both experiments are presented as the sum of the mean daily percentage germination after 12 days

(Sum 12; 1200 indicates 100% germination on the first day), according to the method of Timson (1965).

Results

Experiment 1. Induction of secondary dormancy. Germination of seeds increased with stratification time, reaching nearly 100% after 12 weeks at 5°C, provided subsequent temperature did not exceed 30° (Table 1). In contrast, almost all embryos germinated, even after only 6 weeks of stratification, at all temperatures except 35°.

Exposure to temperatures greater than 20°C inhibited germination of seeds stratified for 6 or 8 weeks, whereas a temperature of 35° was required to inhibit those stratified for 12 weeks (Table 1). Germination at 35° was negligible in seeds stratified for 6 or 8 weeks. Embryo germination was inhibited at 35°, regardless of stratification time. Both seeds and embryos were killed by exposure to 40° for 1 week, although radical protrusion occurred in a few cases (data not shown). More than 90% of the seeds induced into secondary dormancy by 25, 20, and 35°C germinated on restratification at 5° (data not shown).

Interaction between duration of stratification at 5°C and temperature of exposure after stratification was significant at $P < 0.01$ for seed germination, indicating that inhibition occurred at 25° and 30° only when stratification time was less than 12 weeks (Table 1). This interaction was not significant for embryos excised

following exposure to 15, 20, 30, and 35°.

Experiment 2. Release from secondary dormancy. Germination capacity of both seeds and embryos increased as the duration at 5°C increased from 0 to 9 weeks. Although the seed versus embryo comparison was not tested critically, as separate ANOVAs were performed, removal of the seed coat consistently increased germination capacity except in seeds stratified continuously for 9 weeks.

High temperature treatment prior to stratification did not appreciably affect response to stratification in either seeds or embryos (compare first column vs. first line in each set of data), although Sum 12 at 6 weeks was significantly higher for both following the 30°C treatment (Table 2). In contrast, exposure to 6 days at 30° following stratification reduced germination capacity in both seeds and embryos. Exposure to high temperature eliminated the effects of prior chilling only in seeds stratified for 9 weeks; those chilled for 3 or 6 weeks following high temperature treatment germinated consistently better than those chilled for the same periods of time, but not exposed to high temperature (Table 2; Figure 1). Interaction was not tested because of missing data, but is clearly evident in Figure 1, which suggests that high temperature inhibition of germination was more effective in seeds in which the chilling requirement had been saturated.

Discussion

The data for experiment 1 confirm those of Abbott (1955) and Visser (1956) in demonstrating that high temperatures induce secondary dormancy in apple seeds. Temperatures of 25° and 30°C were equally effective in inhibiting germination of seeds, but neither was effective in embryos following stratification for 6 weeks or longer. Holding seeds at 35°, on the other hand, reduced subsequent germination at 20° of both seeds and of embryos dissected from them. As stratification proceeded, the seeds and embryos became less sensitive to high temperature induction of dormancy. The data in Table 1 suggest that seeds are more sensitive to the induction of dormancy by high temperature than are embryos. This is probably more apparent than real, however, for the seed coat acts as a physical barrier to germination of the embryos. Seed coat removal therefore allows germination to occur despite relatively unfavorable conditions. Had an osmoticum been used to restrict germination, inhibitory effects on embryo germination might have been apparent at 25° and 30°.

In experiment 2, exposure to 30°C, which induced secondary dormancy in seeds and embryos stratified for 3 or 6 weeks, did not completely negate the promotive effect of the previous stratification period. In contrast, exposure to high temperature eliminated the effects of prior chilling in seeds stratified for 9 weeks.

These data indicate that high temperature may not

completely negate the promotive effect low temperature exposure has on seed or embryo germination unless full germination potential of the seed has been achieved prior to the heat treatment.

Literature Cited

Abbott, D.L. 1955. Temperature and the dormancy of apple seeds. Rept. XIV Intern. Hort. Congr., Netherlands. 2A:746-753.

Timson, I. 1965. New method of recording germination data. Nature 207:216-217.

Visser, T. 1956. Some observations on respiration and secondary dormancy in apple seeds. Proc. K. ned. Akad. Wet. 59:314-324.

Table 1. The effect of time of stratification of seeds at 5°C and subsequent exposure to higher temperatures for 1 week on germination of seeds and embryos during 10 days at 20°C.

Weeks at 5°C	Subsequent temp. (°C)	Germination (%)	
		Seeds	Embryos
6	15	36bcd[y] (26)[z]	100a
	20	49bc (48)	100a
	25	26d (8)	100a
	30	22de (2)	96a
	35	2f (2)	34c
8	15	86a (78)	100a
	20	89a (87)	100a
	25	40bcd (16)	100a
	30	54b (16)	100a
	35	6ef (4)	34c
12	15	98a (98)	100a
	20	100a (100)	100a
	25	96a (82)	--
	30	94a (62)	100a
	35	32cd (32)	54b
Time at 5°C X Temperature		**	n.s.

-- Missing treatment

[z] % germination after 7 days prior to transfer to 20°

[y] Within seeds or embryos, means followed by the same letter are not significantly different from one another by DMRT, $P < 0.05$.

** Significant at the 5% level ; n.s. = not significant

Table 2. The effect of stratification at 5°C before and after exposure to 30° for 6 days on germination of apple seeds and embryos after 12 days at 20°.

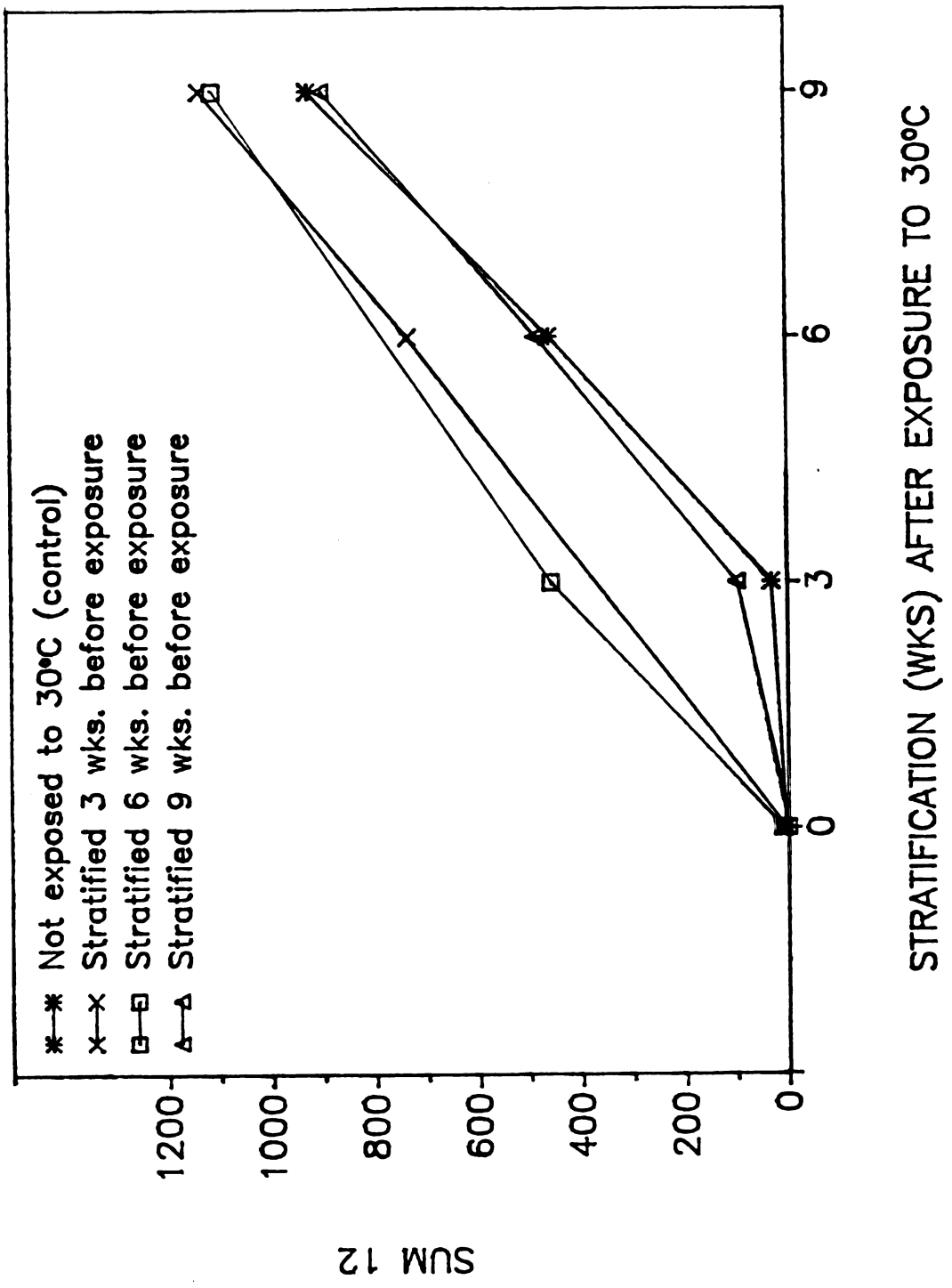
Stratification at 5°C (wks) before exposure to 30°C	Sum 12				
		Stratification at 5°C (wks) after exposure to 30°C			
	control[z]	0	3	6	9
			Seeds (Sum 12)		
0	4e[y]	0e	8e	637c	951b
3	31e	20e	-	733c	1132a
6	459d	10e	456d	-	1107a
9	923b	0e	97e	483d	896b
			Embryos (Sum 12)		
0	483g	277h	668f	1052bc	1139ab
3	663f	527g	-	1125ab	1194a
6	936d	808e	1157a	-	1175a
9	1113ab	216h	979cd	1104ab	1183a

[z] not exposed to 30°C

[y] Within seeds or embryos, means followed by the same letter are not significantly different from one another by DMRT, $P < 0.05$.

Figure 1. The effect of stratification at 5°C, before and after exposure to 30°C for 6 days, on germination of apple seeds and embryos.

FIGURE 1


Not exposed to 30°C (control)
Stratified 3 wks. before exposure
Stratified 6 wks. before exposure
Stratified 9 wks. before exposure
SUM 12
0
200
400
600
800
1000
1200
0
3
6
9
STRATIFICATION (WKS) AFTER EXPOSURE TO 30°C

SECTION II

Germination Responses of Apple Seeds vs. Embryos to High Temperature and Low Oxygen Tension

Germination Responses of Apple Seeds vs. Embryos to High Temperature and Low Oxygen Tension

Abstract. Anaerobiosis is reported to break the dormancy of non-stratified apple embryos. Both anaerobiosis (<0.16% O_2) and low O_2 treatments (0.3-0.5% O_2) were tested for effectiveness on both non-stratified and stratified apple seeds and embryos, and on stratified seeds in which secondary dormancy had been induced by high temperature. A small promotive effect was observed in non-stratified, but not in stratified embryos, and the treatments inhibited germination of stratified seeds. Neither treatment broke secondary dormancy in intact seeds, and often inhibited the germination of embryos excised from them. The possible reasons for the difference in response between seeds and embryos to low O_2 tension are discussed.

Tissaoui and Come (6) using apple seeds immediately removed from the fruit, reported that 7 days of anaerobiosis at 20°C broke dormancy in non-stratified embryos, and that 42 days of anaerobiosis did not induce secondary dormancy in stratified embryos (1,2). Come and Ralambosoa (3) also reported that 7 days of exposure to 0.5% O_2 did not induce secondary dormancy in non-dormant embryos. However, effects of low O_2 tension on the dormancy status of intact apple seeds has not been reported. We repeated the work of Tissaoui and Come on embryos, and observed the germination response of dormant and non-dormant intact seeds following exposure to high temperature and low oxygen tension.

Materials and Methods

Seed source and methods of stratification and germination. Seeds were removed from 'Golden Delicious' fruit at harvest, dried, and stored at 5°C. Subsequently, the seeds were soaked overnight in water, rinsed, placed in 150 X 15 mm Petri plates (400 seeds/plate) on filter paper wetted with 0.5% Captan solution, and stratified at 5° in the dark for 0,3,6,and 9 weeks. Half the seeds were then exposed to 6 days at 30°C to induce secondary dormancy. In one experiment, non-dried seeds were removed from fruit stored at 5° for approximately 23 weeks and used without drying. To test germination following various treatments, seeds or embryos were held on moist filter paper in Petri plates at 20°C. Embryos were considered germinated whenever radicle

length was 3mm or more.

Experimental conditions. In experiment 1 (<0.16% O_2), non-stratified seeds, partially stratified seeds, and seeds exhibiting secondary dormancy were placed in a 125 ml flask (30 seeds per replication; 3 replications per treatment) containing 10 ml Captan solution (0.5%). The flasks were placed on an apparatus made with copper tubing and humidified nitrogen gas was passed through the flasks at 0.4-0.5 ml/min for 6 days at 20°. Oxygen content of the effluent gas stream was measured daily using a flow-through O_2 electrochemical cell and was always below 0.16%. After 6 days the seeds were removed from the low O_2 environment, embryos were dissected from half the seeds, and both seeds and embryos were placed at 20° in air for germination.

In experiment 2 (0.3-0.5% oxygen), non-stratified seeds, partially stratified seeds, and seeds exhibiting secondary dormancy were placed in 60 X 15 mm Petri plates (6 replications of 15 seeds per treatment) on filter paper moistened with 4 ml Captan solution (0.5%). The plates were placed in a desiccator (2.5 L volume), the air was evacuated, and the desiccator was flushed with nitrogen gas 3 or more times. The final oxygen concentration varied from 0.3 to 0.5%. The desiccators were placed at 20° or 30°C for 6 days. Seeds were then removed from the desiccators, embryos dissected from half the seeds, and the seeds and embryos were placed at 20° in air for germination.

Evaluation of germination. The results are presented as the sum of the mean daily percentage germination after 12 days (Sum 12; 1200 indicates 100% germination on the first day), according to the method of Timson (5).

Seeds induced into secondary dormancy by high temperature or low O_2 were restratified at 5° for 12 weeks to check viability.

Statistical analysis. Experiments were arranged in a completely random design. A one-way analysis of variance was performed and Duncan's Multiple Range Test (DMRT) was used to determine mean separation. A 3 X 2 X 2 factorial analysis (stratification time X temperature X atmosphere) was performed and interactions tested.

Results

Stratification at 5° in air increased Sum 12 in both seeds and embryos germinated at 20° (Tables 1 and 2). Subsequent exposure to 30° almost completely inhibited germination of seeds and reduced that of excised embryos in 9 of 12 comparisons. The effect upon embryos was more pronounced in the first experiment than in the second.

Exposure to oxygen levels less than 0.16% (Table 1) at 20° for 6 days reduced germination of seeds and embryos stratified for 9 weeks, but promoted germination of non-stratified embryos. Oxygen tension between 0.3-0.5% was also inhibitory to seed germination following 6 and 9 weeks stratification, but did not affect embryos excised from

similarly-treated seeds (Table 2).

Low O_2 levels were ineffective in overcoming secondary dormancy induced by high temperature; in fact, germination of embryos was inhibited in 4 of 6 cases (Tables 1 and 2).

The response of non-stratified embryos to low O_2 tension was much less than expected based upon the data of Tissaoui and Come (6). However, they did not dry the seeds before use. We therefore compared the response of non-dried seeds that had been immediately removed from fruit stored at 5°C for approximately 23 weeks with those of seeds which were dried on removal from the fruit and stratified for 9 weeks in Petri plates. In general, germination was lower in dried than in non-dried seeds, although germination of control embryos was similar (Fig. 1). Holding seeds of both lots, or embryos of dried seeds, in air at 30°C markedly reduced subsequent germination at 20°. Exposure to low oxygen did not stimulate germination significantly in either seeds or embryos regardless of seed source, and was ineffective in breaking secondary dormancy. In fact, anaerobiosis significantly reduced germination is seeds held at 20°.

Low oxygen tension (0.3-0.5% O_2) did not affect the germination capacity of embryos from seeds held in Petri plates at 20°C (non-stratifying temperature) for 3, 6, and 9 weeks (data not shown).

Seeds induced into secondary dormancy by high temperature or low O_2 remained viable, as they germinated on

restratification at 5°C (data not shown).

Significant interaction ($P < 0.01$) between stratification time (at 5°) and temperature following stratification in experiment 1 indicated that seed and embryo germination at 20°C increased with increasing stratification time, whereas exposure to 30° for 6 days inhibited germination of embryos greatly and seeds totally, regardless of the length of stratification. In experiment 2, seeds responded as in experiment 1 (significant interaction at $P < 0.01$), however, there was no significant interaction between stratification time and temperature for embryos.

Interaction between stratification time and atmosphere for seeds in experiment 1 and 2 was also significant ($P < 0.01$). Germination capacity of seeds held in air increased with increasing time of stratification, whereas germination of seeds exposed to 6 days of anaerobiosis was totally inhibited regardless of stratification time. This interaction was also significant ($P < 0.05$) for embryos in experiment 2.

Temperature X atmosphere interaction was significant ($P < 0.01$) for seeds in both experiments. Germination capacity at 20° increased with increasing stratification time at 5°, but little or no increase occurred when stratified seeds were exposed to 30°. This interaction was also significant ($P < 0.01$) for embryos in experiment 1. Exposure to high temperature reduced germination capacity of embryos except

after 3 weeks of stratification. In experiment 2, significant interaction ($P < 0.05$) in embryos was due to a greater inhibition of germination of embryos exposed to 30^{o} followed by low O_2 than of embryos exposed to 30^{o} alone.

Finally, the triple interaction (stratification time X temperature X atmosphere) was significant for seeds ($P < 0.01$) and embryos ($P < 0.05$) in experiment 1 and seeds ($P < 0.01$) in experiment 2, indicating that the effect of low O_2 in inhibiting germination increased with time of stratification in seeds held at 20^{o}, but the effect was small or negligible in seeds held at 30^{o} (Figure 2 and 3). Low O_2 did not affect embryo germination at $20^{o}C$, however, at 30^{o}, germination was inhibited and embryos stratified for 3 and 6 weeks were more sensitive to low O_2 inhibition than those stratified for at 9 weeks (Figure 2).

Discussion

Stratified seeds responded differently from excised embryos following exposure to low oxygen tension. The endosperm acts as a mechanical barrier to germination and the seed coat contains compounds that are inhibitory to germination (4). Thus excising embryos from non-stratified or partially stratified seeds markedly improves germination. Low O_2 greatly reduced germination of seeds but only slightly reduced or had no effect on germination of embryos after 6 and 9 weeks stratification. This response may be mediated by the outer seed coverings. Oxygen-requiring

enzymes may break down endosperm tissue near the radical and the low O_2 tension could severely reduce their activity. On the other hand, embryo vigor may be reduced by high temperature; thus isolated embryos are capable of germination but those enclosed in the seed coat are not.

Our results differ from those of Tissaoui and Come (6) in that the dormancy-breaking effect of anaerobiosis on non-stratified embryos was small. This could be due to seed source, differences in depth of dormancy, or negation of response by drying.

Anaerobiosis (< 0.16%) did not break high temperature-induced secondary dormancy in apple seeds. Since (a) exposure of stratified seeds to 30°C for 6 days does not completely negate the promotive effects of previous chilling, and (b) anaerobiosis does not stimulate germination in stratified embryos, anaerobiosis may only be effective in stimulating germination of embryos that have neither been dried nor exposed to dormancy-breaking temperatures.

Literature Cited

1. Come, D. and T. Tissaoui. 1968. Induction d'une dormance embryonnaire secondaire chez le pommier (Pirus malus L.) par des atmospheres tres appauvries en oxygene. C.R. Acad. Sci. Paris 266:477-479.

2. Come, D. and T. Tissaoui. 1972. Interrelated effects of imbibition temperature and oxygen on seed germination. p.157-168. In: W. Heydecker (ed) Seed Ecology. Pennsylvania State University Press, Univ. Park, PA.

3. Come, D. and J. Ralambosoa. 1979. Influence simultanee de la lumiere et de l'oxygene sur la germination de l'embryon de pommier non-dormant. C.R. Acad. Sci. Paris 289:869-871.

4. Luckwill, L.C. 1952. Growth-inhibiting and growth-promoting substances in relation to the dormancy and after-ripening of apple seeds. J. Hort. Sci. 27:53-65.

5. Timson, I. 1965. New method of recording germination data. Nature 207:216-217.

6. Tissaoui, T. and D. Come. 1973. Levee de dormance de l'embryon de pommier (Pirus malus L.) en l'absence d'oxygene et de froid. Planta 111:315-322.

Table 1. The effects of stratification time and subsequent exposure to high temperature and/or low oxygen tension (< 0.16%) on germination of apple seeds and embryos (Experiment 1); see text for sequence of treatments.

Strat. (wks at 5°C)	Exposure to 30°C	Sum 12					
		Seeds			Embryos		
		Air	Low O_2	mean	Air	Low O_2	mean
0[z]	-	0d	0d		150g	309de	
3	-	21cd[y]	27cd		568c	514c	
	+	0d	0d		527c	185fg	
	mean	11j	13j	12t	548i	350j	449s
6	-	157b	92bc		730b	721b	
	+	7d	0d		273ef	98g	
	mean	82i	46ij	64s	502i	410j	456s
9	-	450a	58cd		937a	806b	
	+	2d	0d		406d	308de	
	mean	226h	29ij	128r	671h	557i	614r
Means for atmosphere:		106m	30n		574m	439n	
Means for exposure to 30°	-	134p			713p		
	+	1q			300q		

[z] Data excluded from main effect means

[y] Within seeds or embryos, means followed by the same letter are not significantly different from one another within sets (a,b,c,d,e,f for all treatments; m,n for atmosphere; p,q for 30° treatment; r,s,t for stratification time; and h,i,j for atmosphere within stratification time) by DMRT, $P < 0.05$.

Table 2. The effects of stratification time and subsequent exposure to high temperature and/or low oxygen tension (0.3-0.5%) on germination of apple seeds and embryos (Experiment 2); see text for sequence of treatments.

Strat. (wks at 5°C)	Exposure to 30°C	Sum 12					
		Seeds			Embryos		
		Air	Low O_2	mean	Air	Low O_2	mean
0	-	4e	0e		483e	450e	
3	-	31e[y]	19e		663cd	737c	
	+	0e	0e		548de	533de	
	mean	15k	9k	12t	605k	635k	620s
6	-	459b	252c		936b	975b	
	+	70e	0e		944b	774c	
	mean	264i	126j	195s	940ij	875j	907r
9	-	923a	165d		1113a	1028ab	
	+	51e	46e		943b	771c	
	mean	487h	105j	296r	1028i	900j	964r
Means for atmosphere:		256m	80n		858m	803n	
Means for exposure to 30°	-	308p			909p		
	+	28q			752q		

[z] Data excluded from main effect means.

[y] Within seeds or embryos, means followed by the same letter are not significantly different from one another within sets (a,b,c,d,e,f for all treatments; m,n for atmosphere; p,q for 30° treatment; r,s,t for stratification time; and h,i for atmosphere within stratification time) by DMRT, $P< 0.05$.

Figure 1. Germination response of dried and non-dried apple seeds to temperature and oxygen level. Seeds dried prior to stratification at 5°C (Dried), or held in fruits at 5°C for 23 weeks prior to treatment (Non-dried).

Figure 1

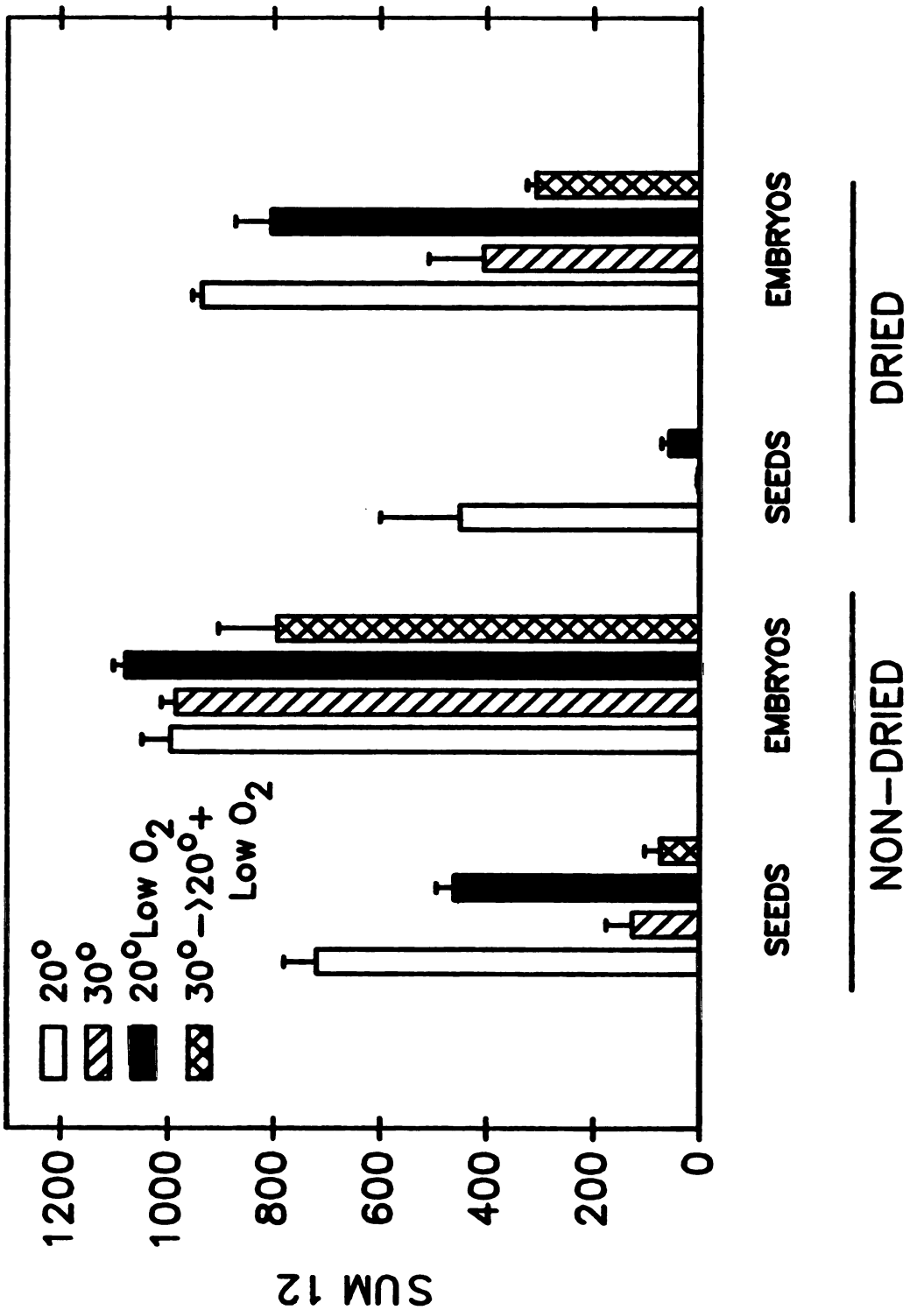

SUM 12
1200
1000
800
600
400
200
0
20°
30°
20°Low O2
30°->20°+
Low O2
SEEDS
EMBRYOS
SEEDS
EMBRYOS
NON-DRIED
DRIED

Figure 2. The effect of stratification time and subsequent exposure to 30°C and low oxygen tension (< 0.16%) on germination (Sum 12) of apple seeds and embryos.

FIGURE 2

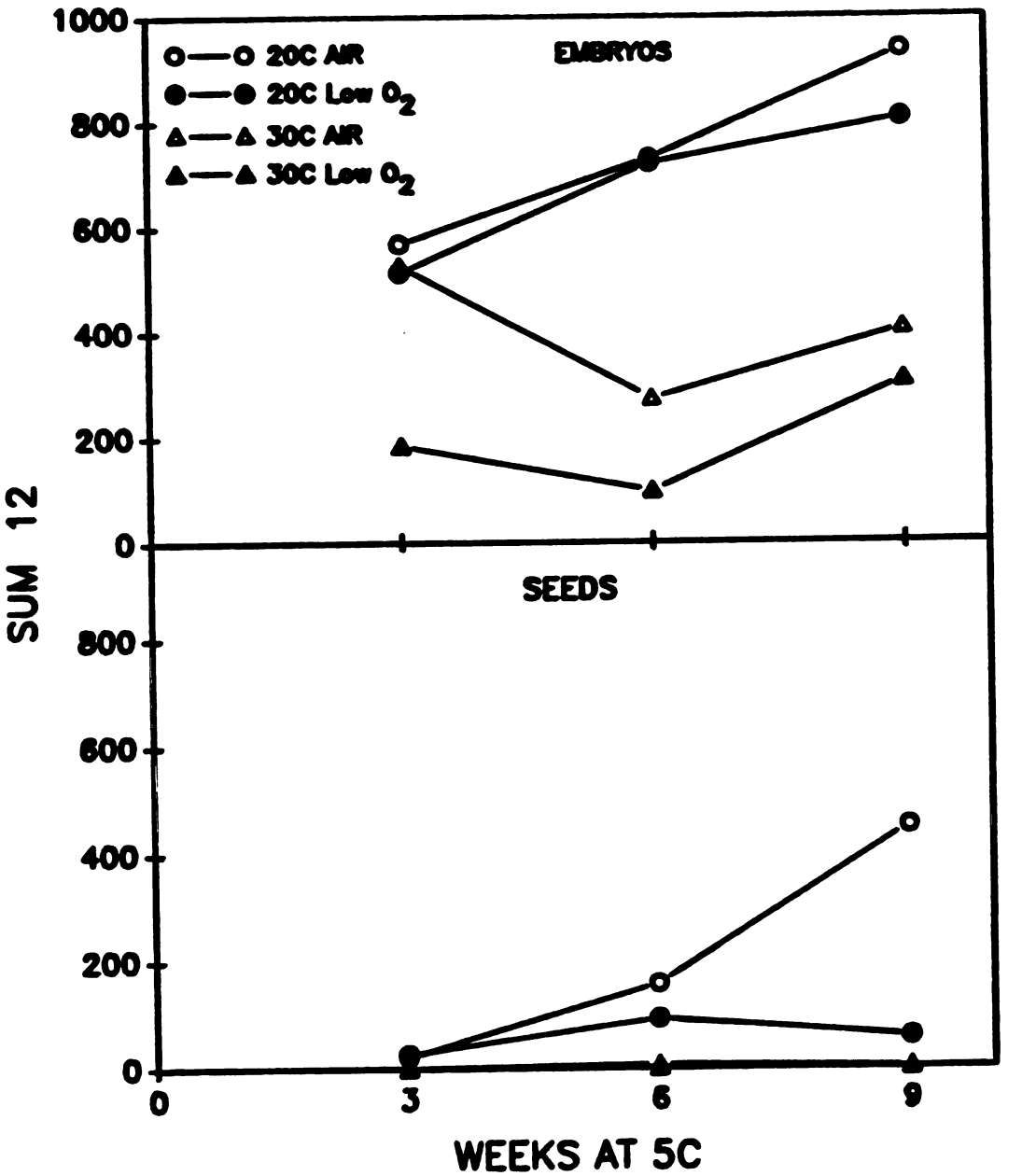
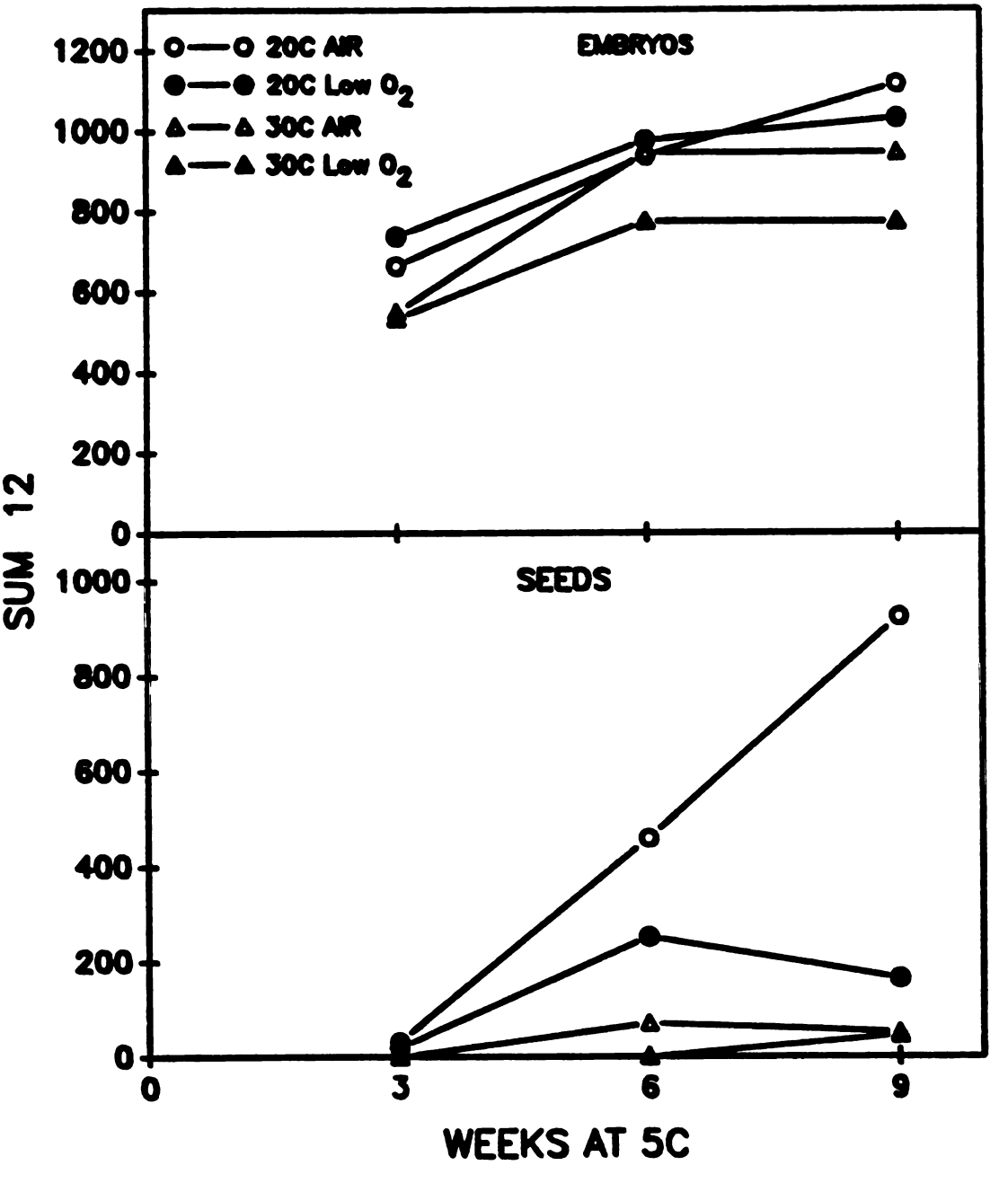

1000
800
600
400
200
0
20C AIR
20C Low O_2
30C AIR
30C Low O_2
EMBRYOS
SEEDS
SUM 12
800
600
400
200
0
0
3
6
9
WEEKS AT 5C

Figure 3. The effect of stratification time and subsequent exposure to 30°C and low oxygen tension (0.3-0.5%) on germination (Sum 12) of apple seeds and embryos.

FIGURE 3


20C AIR
20C Low O_2
30C AIR
30C Low O_2
EMBRYOS
SEEDS
SUM 12
1200
1000
800
600
400
200
0
0
3
6
9
WEEKS AT 5C

SECTION III

The Role of Abscisic Acid in Heat Stress Induced Secondary Dormancy in Apple Seeds

The Role of Abscisic Acid in Heat Stress Induced Secondary Dormancy in Apple Seeds

Abstract. Exposure of stratified apple seeds to a temperature of 30°C induces secondary dormancy. To determine if a rise in abscisic acid (ABA) content was associated with the loss in germination capacity, stratified seeds (3, 6, or 9 weeks at 5°) were held at 30° for 0, 3, or 6 days. Seeds were dissected into seed coat, cotyledons, and embryonic axis and analyzed for ABA content using HPLC and capillary column electron capture gas chromatography (EC-GC). Stratification at 5° either had no effect or increased ABA content in embryonic axes, cotyledons and seed coats. Exposure to 30° after stratification either did not affect or decreased ABA content in embryonic axes and seed coats; in contrast, cotyledonary ABA was increased. Seed coats, cotyledons, and embryonic axes stratified for 3, 6, or 9 weeks at 20° contained the same or higher levels of ABA in comparison with non-stratified seeds or seeds stratified at 5°. ABA changes were not correlated with germination capacity during stratification or after exposure to 30°. These data suggest that changes in ABA are unrelated to changes in dormancy status.

Since its chemical identification in 1965 (Ohkuma, et al.), abscisic acid has become the primary growth inhibitor studied in plants. In dormant seeds of apple, Pieniazek and Rudnicki (1967) first detected the presence of an ABA-like inhibitor, which Rudnicki (1969) later confirmed as ABA. Attempts to correlate the levels of ABA with the induction of dormancy in seeds have resulted in conflicting evidence. The data of Karssen et al. (1983) provide very strong evidence that embryonic ABA is associated with primary dormancy in Arabidopsis thaliana. In contrast, Balboa-Zavala and Dennis (1977) measured ABA levels in stratified apple seeds during the induction of secondary dormancy. They found that ABA levels in whole seeds decreased during the period of high temperature; however, levels in the embryonic axes, cotyledons, and seed coats were not separately measured. In order to clarify the role of ABA in the process of secondary dormancy induction, we have measured the levels of ABA in seed coat, cotyledons, and embryonic axes of seeds statified at 5°C, then induced into secondary dormancy by high temperature.

Materials and Methods

Seed source and methods of stratification. Seeds were removed from Frazier Spur 'Golden Delicious' fruit at harvest, dried, and stored at 5°C. Subsequently, seeds were soaked overnight in water, rinsed, placed in 150 X 15 mm Petri plates (400 seeds/plate) on filter paper wetted with 0.5% Captan solution, and held at 5° or 20° in the dark for

0, 3, 6, or 9 weeks.

Experimental conditions. After stratification, seeds (15 per replication, 3 replications per treatment) were rinsed and placed in 100 X 15 mm Petri plates containing the same solution used for stratification, and held at 30°C for 0,3, and 6 days. Seeds were then dissected into embryonic axes (125; 54 mg), cotyledons (100; 2.1 g) and testa plus endosperm (seed coat) (100; 1.4 g) and analyzed for ABA content (3 replications per treatment). Seeds held at 20° were not exposed to 30°.

Evaluation of germination. The results are presented as the sum of the mean daily percentage germination after 10 days (Sum 10; 1000 indicates 100% germination on the first day), according to the method of Timson (1965).

Extraction, purification, and quantification procedures. All seed tissues were lyophilized, ground in liquid N_2, then extracted with 80 and 100% acetone [acetone containing 1% acetic acid and 10 mg/l butylated hydroxy-toluene (BHT)]. $\pm^3$H-ABA (approximately 5000 cpm) was added to each sample for determination of recovery. The tissues were extracted by shaking gently in darkness at 4°C for 12-16 hours. The supernatant solution was drawn off with a Pasteur pipette and more solvent added; this was repeated twice. The extracts were dried and redissolved in aqueous 1% acetic acid. All samples were filtered, then purified by reverse phase HPLC on a uBondapack C_{18} (10um particle size), 10 X 8 cm cartridge column (Waters Associates). The samples were

eluted by means of a convex gradient (curve 5 on the Waters Associates Model 600 Solvent Programmer) from 0 to 50% ethanol in aqueous 1% acetic acid. The retention time of ABA was 18.5 min. at a flow rate of 2 ml/min, determined by UV absorption of authentic ABA at 254 nm. The fraction containing ABA was dried and methylated with ethereal diazomethane. Quantification of the methyl ester of ABA (Me-ABA) was performed with a Varian 3700 gas chromatograph equipped with a ^{63}Ni-electron capture detector. Samples were dissolved in ethyl acetate containing an internal standard (dieldrin), and analyzed on a Durabond DB-1 (J&W Scientific, Inc) gas capillary column (30m X 0.32mm X 0.25um). Injections (1 ul) were splitless. Temperatures of the injector and detector were 250^{o} and 290^{o}C, respectively. The column temperature was increased from 60 to 165^{o} in approx. 2.2 min., then increased to a final temperature of 240^{o} at a rate of 5^{o}/min. Carrier gas (He) linear velocity was 32 cm/sec., and N_2 was used as the detector make-up gas with a flow rate at the detector of 30 ml/min.

Identification of ABA by GC-MS. GC-MS verification of ABA in each seed tissue sample was carried out using a Hewlett-Packard 5890 Gas Chromatograph coupled to a Hewlett-Packard 5970 Mass Selective Detector. Samples were purified by HPLC as described above, methylated, then dissolved in ethyl acetate and injected onto a ChromPAK (12.5m X 0.25mm X 0.19 um) capillary column, for GC-MS analysis. Injections (1 ul) were on-column, with the injector port maintained at 250^{o}C,

and the column carrier gas (He) flowing at a rate of 1 ml/min. Initial column temperature was 80°, immediately increasing 20°/min to a final temperature of 230°. The MS was operated at an ionization potential of 70eV with a source pressure of 6-7 X 10^{-5} Torr.

Statistical analysis. The experiment was arranged in a 2-factor (treatment X time) factorial design. An analysis of variance was performed and Duncan's Multiple Range Test (DMRT) was used to determine mean separation. Simple correlations were run to measure the strength of the relationship between endogenous ABA content and germination capacity during stratification at 5°C or after exposure to 3 and 6 days at 30°.

Results

Control seeds and those stratified at 20°C germinated very poorly or not at all; stratification at 5° greatly increased Sum 10 values, although no difference was evident between 3 and 6 weeks (Table 1). As expected, holding chilled seeds at 30° for 3 or 6 days consistently reduced germination, the effect increasing with time at 30°.

GC-MS of the extracts of seed coats, cotyledons, and embryonic axes indicated the presence of ABA. Major fragments and intensities were in close agreement with those of synthetic cis,trans-ABA (Fig. 1).

The concentration of ABA in the embryonic axis was similar to that in non-stratified seeds, regardless of

treatment, with three exceptions (Table 2). The concentration was considerably higher after 6 weeks at either 5° or 20°, and subsequent exposure to 30° decreased ABA levels. This resulted in a significant temperature X time interaction. Concentrations in other samples, although consistently lower than those in control (non-stratified) axes, did not differ significantly.

In the cotyledons, ABA concentration was significantly higher in seeds held at 20°C than in all other treatments (Table 2). The concentration at 5°, was not significantly different from control values. Holding seeds at 30° affected ABA content significantly only in seeds stratified for 6 weeks. Significant temperature X time interaction reflected the change in ABA content at 20° vs. lack of change in other treatments.

In seed coats, ABA concentration was consistently higher at 20°C than in non-stratified seeds or in seeds held at 5° for 3 or 6 weeks. The concentration also appeared to rise in seeds held at 5°, but the rise became significant only after 9 weeks (Table 2). Transfer of stratified seeds to 30° reduced ABA content after 3 and 9 weeks of stratification at 5°. Interaction was again significant.

ABA changes were not correlated with germination capacity during stratification or after exposure to 30°C (Table 1 and 2).

Discussion

Stratification at 5°C or subsequent exposure to 30° did not greatly affect ABA content of embryonic axes (70-180 ng/g dw) or cotyledons (13-26 ng/g dw). Subbaiah (1987), using GC-MS, also reported that ABA levels remained fairly constant during stratification at 5° in embryonic axes (50-100 ng/g dw) and cotyledons (50-70 ng/g dw) of 'Northern Spy' apple seeds. Balboa-Zavala and Dennis (1977) measured ABA levels (fresh weight basis) during stratification at 5° in seed coats, cotyledons, and embryonic axes of 'Delicious' and 'McIntosh' apple cultivars. Since the dry weight of the embryos is approximately 33% of its wet weight (Harrington, 1923), levels in the cotyledons (44-108 ng/g fw) reported by Balboa-Zavala and Dennis were approximately 5-6 fold higher than those observed by Subbaiah; however, ABA levels for embryonic axes (9032-1582 ng/g fw) were approximately 100-200 times greater than levels observed in this study or by Subbaiah (1987). The very high values for embryonic axes values reported by Balboa-Zavala and Dennis are probably inaccurate due to the very small sample assayed (approximately 37 mg fresh weight), minimal sample purification, and reduced sensitivity in quantification due to the use of a packed GC column with an extremely short retention time for ABA (1.5 minutes). Artifacts could easily have affected the values obtained. Much larger samples of cotyledons (320 mg) and seed coats (245 mg) were extracted and the ABA values reported for these tissues are

in the approximate range reported in this study and by Subbaiah (1987).

ABA levels were not affected by 3 or 6 weeks stratification at 5°C; however, a large increase occurred in seed coats from seeds stratified for 9 weeks. This was unexpected and its significance is not known. Subbaiah (1987), using 'Northern Spy' and 'Lande' apple cultivars, found high levels of ABA in the testa and endosperm (equivalent to seeds coat in this paper) of non-stratified seeds. Stratification at 5oC decreased ABA levels substantially; this trend was also reported by Balboa-Zavala and Dennis (1977) in 'Delicious' and 'McIntosh' cultivars.

The same or higher levels of ABA were found in seed coats, cotyledons, and embryonic axes after 3, 6, and 9 weeks at 20° than in non-stratififed seeds or seeds stratified at 5°. In contrast, Subbaiah (1987) and Balboa-Zavala and Dennis (1977) reported that ABA levels dropped significantly in seeds held at both 20° and 5°. The reason for this contradiction is not known.

There was no consistent change in ABA levels in any of the seed tissues during exposure to 30°C after stratification at 5°. However, because of the higher metabolic rate at 30°, a study of turnover rate of ABA and its metabolites would be required to fully evaluate the role of ABA in this heat-induced stress process.

In conclusion, our data do not support the hypotheses that changes in ABA content are responsible for the breaking

of dormancy by chilling or for the induction of secondary dormancy by high temperature.

Literature Cited

Balboa-Zavala, O. and F.G. Dennis, Jr. 1977. Abscisic acid and apple seed dormancy. J. Amer. Soc. Hort. Sci. 102:633-637.

Harrington, G.T. 1923. Respiration of apple seeds. J. Agr. Res. 23:117-131.

Karssen, C.M., Brinkhorst-van der Swan, D.L.C., Breekland, A.E., and M. Koornneef. 1983. Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of Arabidopsis thaliana (L.) Heynh. Planta 157:158-165.

Ohkuma, K., Addicott, F.T., Smith, O.E., and W.E. Thiessen. 1965. The structure of abscisin II. Tetrahedron Lett. 29:2529-2535.

Pieniazek, J. and R. Rudnicki. 1967. The presence of abscisin-II in apple leaves and apple fruit juice. Bull. Acad. Pol. Sci. 15:251-254.

Rudnicki, R. 1969. Studies on abscisic acid in apple seeds. Planta 86:63-68.

Subbaiah, T. 1987. Abscisic acid relationships in apple seed dormancy. Ph.D. Thesis, Cornell University.

Timson, I. 1965. New method of recording germination data. Nature 207:216-217.

Table 1 Germination (Sum 10) of seeds stratified at 5° or 20°C and after exposure to 30°.

	Seed germination (Sum 10)				
		Time (weeks)			
Temperature	0	3	6	9	Mean
20°[z]	150e[y]	47	0	0	
5°		574b	591b	742a	636r
5°,3 days 30°	--	308d	373cd	461bc	381s
5°,6 days 30°	--	38e	88e	78e	68t
Mean		307m	351mn	427n	
Temperature X Time		n.s.	n.s.	n.s.	

[z] Data from the 20° treatment were not included in the factorial analysis

[y] Means followed by the same letter are not significantly different from one another within sets (a,b,c,d,e for all treatments; m,n for time; and r,s,t for temperature) by DMRT, $P < 0.05$.

n.s. = not significant

Table 2. Effects of stratification temperature and subsequent exposure to 30°C on ABA concentration in embryonic axes, cotyledons, and seed coats of apple seeds.

Stratification temperature (°C)	ABA content (ng/g dw) Time (weeks) 0	3	6	9	Mean
Embryonic axis					
20°	} 106c	62c	401a	88c	184h
5°		82c	179b	70c	110i
5°, then 3 days 30°		89c	102c	83c	91ij
5°, then 6 days 30°		56c	88c	68c	70j
Mean		72s	192r	77s	
Temperature X time		**	**	**	
Cotyledons					
20°	} 26cdef	58b	106a	54b	73h
5°		21def	13f	15ef	16j
5°, then 3 days 30°		25cdef	40c	29cde	31i
5°, then 6 days 30°		36cd	31cd	26cdef	31i
Mean		35s	47r	31s	
Temperature X Time		**	**	**	
Seed Coats					
20°	} 115cde	344b	528a	449ab	440h
5°		188cd	204c	436ab	276i
5°, then 3 days 30°		55e	210c	113cde	126j
5°, then 6 days 30°		75de	149cde	160cde	128j
Mean		165s	273r	290r	
Temperature X Time		**	**	**	

z Within seed tissues, means followed by the same letter are not significantly different from one another within sets (a,b,c,d,e,f for all treatments; r,s for time; and h,i,j for stratification temperature) by DMRT, $P < 0.05$.

** Significant at the 1% level.

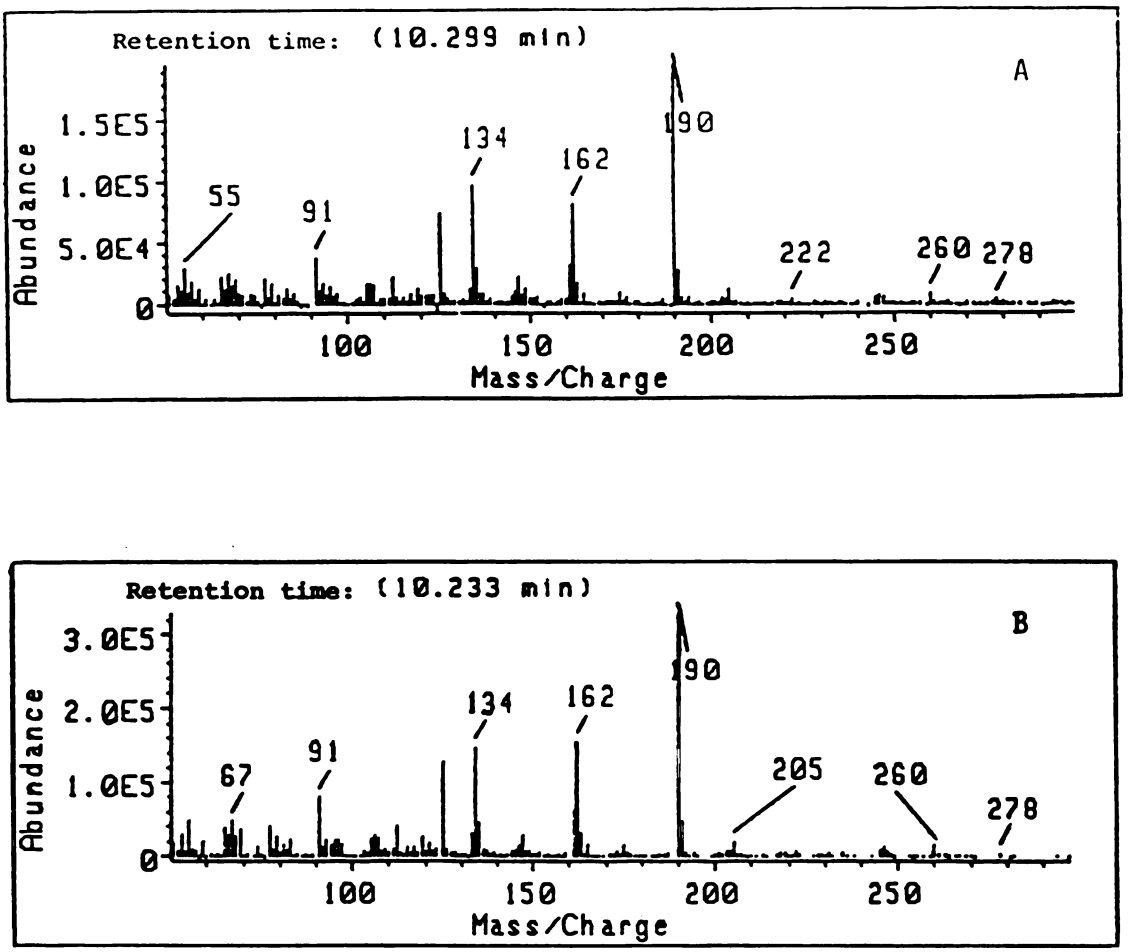


Figure 1. Mass spectrum of Me-ABA in a methylated extract of seed coats of apple (A), and of authentic Me-ABA (B).

SECTION IV

Is Ethylene required for Apple Seed or Embryo Germination ?

Is Ethylene Required for Apple Seed or Embryo Germination ?

Abstract

The observations made by others that ethylene (C_2H_4) is required for germination of stratified embryos of apple (*Malus domestica* Borkh.) was not confirmed. In addition, silver thiosulfate and norbornadiene (1000 ul·liter^{-1}), which inhibit C_2H_4 action, either slightly promoted or did not affect seed or embryo germination. The addition of 166 or 332 ul·liter^{-1} C_2H_4 significantly inhibited rate and final percent germination of fully stratified seeds. Removal of atmospheric C_2H_4 or addition of C_2H_4 (up to 332 ul·liter^{-1}) did not reduce the inhibitory effect of high temperature (30°C) on seed or embryo germination. We conclude that the presence of atmospheric C_2H_4 at concentrations greater than 20 nl·liter^{-1} is not essential for germination of apple seeds or embryos.

Ethylene has been implicated in germination of certain seeds. As little as 3.5 ul·liter^{-1} can stimulate germination in dormant Virginia-type peanut seeds (3). C_2H_4 at 100 and 1000 ul·liter^{-1} hastened apple embryo germination (2), but did not affect final germination when used at 100 to 50,000 ul·liter^{-1} (1). Sinska and Gladon (4) reported that the presence of mercuric perchlorate ($Hg(ClO_4)_2$) completely inhibited germination of partially and fully stratified apple embryos, and that addition of ethephon during stratification stimulated germination of partially stratified (30 days at 4^{o}C) embryos.

We tested the effects of removal of atmospheric C_2H_4, addition of C_2H_4, and inhibition of C_2H_4 action on apple seed and embryo germination at both 20^{o} and 30^{o}C.

Materials and Methods

Partially stratified seeds and embryos were obtained by removing seeds from 'Golden Delicious' fruits that had been stored at 5^{o}C for 1 year. The seeds were leached under running tap water (12^{o}) for 1 day, then stratified in the dark for 1 month at 5^{o} in petri dishes on filter paper moistened with about 0.5% Captan fungicide.

Fully stratified seeds and embryos were obtained by removing seeds from 'Golden Delicious' fruits at harvest, drying them, and storing at 5^{o}C. Subsequently, the seeds were soaked overnight in water, rinsed, placed in 100 X 15 mm Petri plates (120 seeds per plate) provided with two

filter papers wetted with 0.5% Captan solution, and stratified at 5^{o} in the dark for 52 days.

Seeds or embryos, 15 per replicate dish, six replications per treatment (4 replications in norbornadiene treatment), were placed in 60 X 15 mm Petri dishes containing two filter papers moistened with Captan solution. All seeds and embryos except those used for one treatment (Table 1), were germinated in 483 ml glass jars with air-tight lids.

Partially stratified seeds were germinated in the jars for ten days at 20^{o}C under a 16 hr photoperiod of 150 umols$\cdot s^{-1} \cdot m^{-2}$. Fully stratified seeds were held in the jars in darkness for 3 days (at 20^{o} or 30^{o}), then removed and germinated at 20^{o} in the dark.

Purafil (permanganate-alumina) or solutions of $Hg(ClO_4)_2$ in 2M $HClO_4$ were contained in 20 and 10 ml beakers, respectively, within the jars. Norbornadiene (NB), as a liquid, was placed onto a filter paper wick attached to the jar inner wall and the jars were quickly sealed. The quantity of NB used was calculated to yield upon volatilization concentrations of 1000 and 3000 ul$\cdot$liter^{-1}. C_2H_4 was injected into specified jars and the concentrations within the jars verified by gas chromatography.

Partially stratified seeds were soaked in silver thiosulfate (STS) solutions for 1 hr to inhibit ethylene action, then rinsed with water, transferred to Petri dishes containing Captan solution and placed inside jars.

Alternatively, STS solution was added directly to the filter papers in dishes containing fully stratified seeds or embryos, then replaced with Captan solution after 3 days (Table 3).

In separate experiments, seeds were held in jars at 20° or 30°C, and levels of CO_2 and O_2 were monitored (six replications of 15 seeds each per temperature). CO_2 levels did not exceed 0.5 ± 0.1% at 20° or 30° and 1.3 ± 0.4% at 20° after 3 and 10 days, respectively. O_2 levels did not fall below 17.2 ± 5.2% at 20 or 30° and 19.7± 1% at 20° after 3 and 10 days, respectively. Therefore, CO_2 and O_2 levels should not have affected C_2H_4 action or synthesis by these seeds.

Gases were sampled through a rubber septum in the jar lid using a 1 ml syringe. Quantification of C_2H_4 was performed with a Varian series 1400 gas chromatograph equipped with a flame ionization detector and a 60/80 mesh activated alumina column (100 cm), using N_2 (30 $ml \cdot min^{-1}$) as the carrier gas. Temperatures of the injector, column, and detector were 130, 100, and 150°C, respectively. Identity of C_2H_4 was confirmed by comparison of retention time of sample with that of an ethylene standard.

The results are presented both as final percentage germination and as the sum of the mean daily percentage germination after 10 days (Sum 10; 1000 indicates 100% germination on the first day), according to the method of Timson (5).

Most experiments were arranged in a completely random design. A randomized complete block design was used with fully stratified seeds in Table 1 and embryos in Table 2, using seed size as blocks. An analysis of variance was performed and Duncan's Multiple Range Test (DMRT) was used to determine mean separation. Only main effect means are presented for experiments in which interaction was not significant at $P < 0.05$. Regression analysis was also performed where appropriate,and standard deviations from the mean were calculated for partially stratified embryo treatments in Table 1.

Results

Removal of ambient C_2H_4 with $Hg(ClO_4)_2$ or Purafil did not significantly ($P < 0.05$) affect percent germination or Sum 10 of partially or fully stratified apple seeds or embryos at 20°C (Table 1). Germination was significantly lower at 30° than at 20°, and the removal of atmospheric C_2H_4 with Purafil did not affect the response (Table 1). Therefore only main effects are presented.

The addition of up to 72 ul·liter^{-1} C_2H_4 to the atmosphere did not significantly ($P < 0.05$) affect the rate or final percentage germination of partially stratified seeds (Table 2) using ANOVA analysis; however, regression analysis of Sum 10 values showed a significant positive effect of C_2H_4 on germination. C_2H_4 (166 and 332 ul·liter^{-1}) significantly inhibited final germination and Sum 10 of

fully stratified seeds using either method of analysis (Table 2). The inhibitory effect was not as prominent in fully stratified embryos, in which only the Sum 10 value was affected (Table 2). Germination of seeds was consistently lower at 30°C than at 20°, regardless of treatment (Tables 1,2).

Ethylene concentration in jars containing fully stratified control seeds was not significantly affected by temperature (Table 3). However, control embryos held at 30°C for three days evolved significantly more C_2H_4 than did those held at 20°. Embryos evolved more ethylene than did intact seeds.

Inhibition of C_2H_4 action by exposure to silver thiosulfate (STS) at 1, 0.1, or 0.01 mM for one hr did not significantly ($P < 0.05$) affect percentage germination or Sum 10 at 20°C of partially stratified seeds relative to controls using ANOVA (Table 4). Although a significant increase in germination capacity with increasing STS levels was detected using regression analysis ($P < 0.01$), only seeds exposed to the highest concentration of STS (1 mM) germinated better than the controls. Exposure of fully stratified seeds for 3 days to STS solutions (0.1 mM) did not affect final germination or Sum 10 of seeds or final germination of embryos; however, a small but significant reduction of Sum 10 was evident in embryos (Table 4).

Inhibition of C_2H_4 action by exposure to norbornadiene at 1000 and 3000 $ul \cdot liter^{-1}$ did not significantly ($P < 0.05$)

affect percentage germination or Sum 10 of fully stratified embryos or percent germination of seeds using ANOVA or regression analysis (Table 5). Although both ANOVA and regression analysis indicated a significant reduction in Sum 10 of seeds at 20°C, only seeds exposed to 3000 ul·liter^{-1} were inhibited compared to the control. The reduction in germination capacity at 3000 ul·liter^{-1} was probably due to NB's toxic effect (5). The lack of effect at 30°, which resulted in significant temperature x norbornadiene interaction, may be a reflection of the lower germination capacity at this temperature.

Discussion

In summary, neither removal of ethylene from the atmosphere nor inhibition of ethylene action by NB or STS appreciably affected germination of fully stratified seeds or embryos at 20 or 30°C. The addition of ethylene either had no effect or reduced germination of fully stratified seeds and embryos. Using regression analysis, a significant positive effect of C_2H_4 on germination on partially stratified seeds was detected. However, STS, an inhibitor of ethylene action, also significantly increased both percent germination and Sum 10 in these seeds.

If C_2H_4 does indeed promote the germination of apple embryos, treatments which stimulate C_2H_4 evolution in embryos should stimulate germination; however, we observed the opposite. Control embryos evolved significantly more

C_2H_4 at 30° than at 20°C, yet Sum 10 values were either not affected or were significantly lower at 30° (Table 3). In no case did any of the chemical treatments reduce the inhibitory effect of high temperature (30°) on germination of apple seeds or embryos. Therefore we conclude that the presence of C_2H_4 at concentrations greater than 20 $nl \cdot liter^{-1}$ (detection limit with our equipment) is not essential for germination of partially or fully stratified apple seeds or embryos. In addition, C_2H_4 concentrations equal to or greater than 166 $ul \cdot liter^{-1}$ may reduce germination of seeds and embryos.

Literature Cited

1. Kepczynski, J. and R.M. Rudnicki. 1975. Studies on ethylene in dormancy of seeds. I. Effect of exogenous ethylene on the after-ripening and germination of apple seeds. Fruit Sci. Rpts. 2:25-41.

2. Kepczynski, J., R.M. Rudnicki and A. A. Khan. 1977. Ethylene requirement for germination of partly after-ripened apple embryo. Physiol. Plant. 40:292-295.

3. Ketring, D.L. and P.W. Morgan. 1969. Ethylene as a component of the emanations from germinating peanut seeds and its effect on dormant Virginia-type peanut seeds. Plant Physiol. 44:326-330.

4. Sinska, I. and R.J. Gladon. 1984. Ethylene and the removal of embryonal apple seed dormancy. HortScience 19:73-75.

5. Sisler, E.C. and S.F. Yang. 1984. Anti-ethylene effects of cis-2-butene and cyclic olefins. Phytochemistry 23:2765-2768.

6. Timson, I. 1965. New method of recording germination data. Nature 207:216-217.

Table 1. Effects of temperature and removal of C_2H_4 from the atmosphere on germination of partially and fully stratified apple seeds and embryos.

Treatment	Seeds		Embryos	
	% Germ.[z]	Sum 10	% Germ.[z]	Sum 10
Partially stratified				
Control				
in jar	42a[y]	251a	97a[x]	788a[x]
no jar	41a	217a	--	--
Hg (ClO4)2	49a	266a	100a[x]	883b[x]
Purafil	52a	250a	--	--
Fully stratified				
Temperature - Main effects				
20°C	97m	729m	99m	708m
30°C	75n	361n	85n	486n
Purafil - Main Effects				
Control	86r	531r	94r	606r
Purafil	86r	559r	90r	589r

[z]After 10 days at 20°C.

[y]Mean separation within columns among treatments (ab), temperatures (mn), and Purafil vs. none (rs) by DMRT, $P < 0.05$.

[x]Only 10 embryos/replication, 6 replications; otherwise 15 embryos/replication.

Table 2. Effects of temperature and addition of C_2H_4 to the atmosphere on germination of partially and fully stratified apple seeds and embryos.

C_2H_4 applied ($ul \cdot liter^{-1}$)	Temp. (^oC)	Seeds % Germ.[z]	Seeds Sum 10	Embryos % Germ.[z]	Embryos Sum 10
		Partially stratified			
0	20	42a[y]	251a	--	--
7.4		45a	246a	--	--
72		51a	351a	--	--
		Fully stratified			
Temperature - Main effects					
	20	89m	636m	100m	815m
	30	59n	273n	99m	786m
C_2H_4 - Main effects					
0		86r	532r	99r	848r
166		70s	430s	--	
332		66s	402s	99r	752s

[z] After 10 days at 20^oC.

[y] Mean separation within columns among treatments (ab), temperatures (mn), and ethylene concentrations (rs) by DMRT, $P < 0.05$.

Table 3. Effect of temperature on C_2H_4 levels in air within jars containing fully stratified apple seeds or embryos. Gas samples removed after 3 days at 20° or 30°C.

Temperature (°C)	C_2H_4 ($nl \cdot liter^{-1}$)	
	Seeds[z]	Embryos[z]
20°	30 ± 20[y]	95 ± 25
30°	20 ± 10	450 ± 110

[z] Fifteen seeds or embryos per 483 ml jar; 12 jars (replications) per treatment. Approximate weight = 60 mg/seed, 30 mg/embryo.

[y] Standard error.

Table 4. Effects of temperature and inhibition of C_2H_4 action by silver thiosulfate (STS) on germination of partially and fully stratified apple seeds and embryos.

STS (mM)	Temperature (°C)	Seeds % Germ.[z]	Seeds Sum 10	Embryos % Germ.[z]	Embryos Sum 10
		Partially stratified			
0	20	42ab[y]	251ab	--	--
0.01		34b	184b	--	--
0.1		49ab	238ab	--	--
1		60a	349a	--	--
		Fully stratified			
Temperature - Main effects					
	20	96m	688m	99m	796m
	30	71n	342n	92n	654n
STS - Main effects					
0		86r	530r	96r	764r
0.1		81r	500r	96r	686s

[z] After 10 days at 20°C.

[y] Mean separation within columns among treatments (ab), temperatures (mn), and STS concentrations (rs) by DMRT, $P < 0.05$.

Table 5. Effects of temperature and norbornadiene (NB) on germination of fully stratified apple seeds and embryos.

Temp. (°C)	NB (ul·liter^{-1})	Seeds % Germ.[z]	Seeds Sum 10	Embryos % Germ.[z]	Embryos Sum 10
20	0	80a[y]	494a	98a	924a
	1000	87a	517a	100a	906a
	3000	73a	375b	97a	854a
	Mean	80m	462m	98m	895m
30	0	29b	77c	96a	884a
	1000	38b	118c	95a	766a
	3000	33b	109c	95a	896a
	Mean	33n	101n	95m	848m
	Means for NB				
	0	54r	286rs	97r	904r
	1000	62r	317r	98r	836r
	3000	53r	242s	96r	875r
Temperature x NB		n.s.	*	n.s.	n.s.

[z] After 10 days at 20°.

[y] Mean separation within columns among all treatments (abc), temperatures (mn), or norbornadiene concentrations (rs) by DMRT, $P < 0.05$.

* Significant at 5%.

APPENDIX

Appendix

The Effect of ABA on Apple Seed Germination

Introduction

Exogenous abscisic acid inhibits germination of non-dormant apple seeds (Rudnicki and Pieniazek, 1973) and embryos (Rudnicki, 1969). High temperature also inhibits germination of these seeds (Abbott, 1955; Visser, 1956). In order to understand the relationship between the inhibitory effect of ABA and that of high temperature on germination, apple seeds were exposed to ABA either during or after induction of secondary dormancy by high temperature.

Materials and Methods

Apple (Malus domestica cv. Golden Delicious) seeds were removed from the fruit at harvest, dried, and stored at 5°C. Subsequently, the seeds were soaked overnight in water, rinsed, placed in 100 X 15 mm petri plates containing filter paper wetted with 10 ml 0.5% Captan solution, and stratified at 5°.

In experiment 1, seeds stratified for 9 weeks were transferred to plates containing 0.15 or 0.3 X 10 mM ABA in Captan solution and placed at either 20° or 30°C for 3 days (30 seeds per plate; 3 replications). Seeds were then rinsed with water and the embryos dissected from half of the seeds; both were placed in 0.5% Captan solution and

germinated at 20°C in the dark.

In experiment 2, seeds stratified for 0, 3, or 6 weeks were exposed to 30°C for 0, 3, or 6 days (15 seeds per plate; 4 replications). Seeds, and embryos dissected from similarly treated seeds, were subsequently transferred to plates containing 0, 10^{-6}, 10^{-5}, 10^{-4}, or 10^{-3}M ABA and placed at 20°C for 10 days for germination. Stratification, exposure to high temperature and germination were carried out in darkness in all cases.

The results are presented as the sum of the mean daily percentage germination after 10 or 14 days (Sum 10 or Sum 14; 1000 and 1400, respectively, indicates 100% germination on the first day), according to the method of Timson (1965).

Standard deviations (SD) from the mean were calculated for experiment 1. Experiment 2 was arranged factorially for embryos, and seeds were in a completely random design. An analysis of variance was performed and Duncan's Multiple Range Test (DMRT) used to determine mean separation.

Results

In experiment 1, exposure to 30°C for 3 days significantly inhibited subsequent germination at 20° of stratified seeds and embryos (Fig. 1 and 2). ABA consistently inhibited germination of both seeds and embryos regardless of prior temperature treatment. Seed germination was completely inhibited at 0.15 mM, but some germination occurred in embryos even at the high ABA concentration. The

concentration of ABA required to equal the effect of high temperature was 0.15 mM in embryos but less than this in seeds.

In experiment 2, germination of seeds increased with stratification time, reaching a Sum 10 value of 275 after 6 weeks at 5°C (Table 1). ABA at concentrations of 10^{-5}M or higher inhibited germination of seeds regardless of stratification time. When stratified seeds were exposed to 30°C for 3 or 6 days, germination was almost completely inhibited, exogenous ABA having little effect.

The germination capacity of embryos was much greater than that of seeds after the same stratification period (Sum 10=827) (Table 2). Exposure to 30°C for 6 days promoted germination in non-stratified embryos, but inhibited it in stratified embryos (Table 2). Exposure to ABA subsequent to heat treatment further reduced embryo germination. ABA consistently inhibited germination regardless of stratification time, but the inhibitory effect increased with length of stratification. ABA at 10^{-5}, 10^{-4} and 10^{-3} M totally inhibited germination, except for slight germination (Sum 10 varied for 0 to 44) of embryos from seeds stratified for 6 weeks at 5° (data not shown).

Interaction between stratification time and exogenous ABA concentration was significant at $P < 0.01$, for ABA at 10^{-6}M was inhibitory only in stratified embryos (3 and 6 weeks at 5°C). Time of exposure to 30° X duration of stratification was significant at $P < 0.01$; in this case

chilling consistently increased Sum 10 in seeds exposed to 30° for 0 or 3 days, but had little or no effect on those exposed for 6 days.

Discussion

Exposure to 10^{-6} to 10^{-3} M ABA or to 30°C inhibited germination of stratified seeds and embryos, and ABA application during or after exposure to 30° further inhibited germination. Because the induction of secondary dormancy by high temperatures was not correlated with an increase in endogenous ABA levels in the seed coat, cotyledons, or embryonic axes of apple seeds (chapter 3 of this thesis), the mechanism of action of high temperature appears to differ from that of ABA.

Literature Cited

Abbott, D.L. 1955. Temperature and the dormancy of apple seeds. Rept. XIV Intern. Hort. Congr., Netherlands. 2A:746-753.

Timson, I. 1965. New method of recording germination data. Nature 207:216-217.

Rudnicki, R. 1969. Studies on abscisic acid in apple seeds. Planta 86:63-68.

Rudnicki, R. and J. Pieniazek. 1973. The effect of abscisic acid on stratification of apple seeds. Bull. Acad. Pol. Sci. 21:149-154.

Visser, T. 1956. Some observations on respiration and secondary dormancy in apple seeds. Proc. K. Ned. Akad. Wet. 59:314-324.

Table 1. The effect of stratification at 5°C and subsequent exposure to 30° and/or exogenous ABA on germination of apple seeds.

Weeks at 5°C	Days at 30°C	Sum 10 ABA (M) 0	10^{-6}	10^{-5}	10^{-4}
0	0	7c[z]	--	--	--
3	0	174b	127b	142b	--
	3	22c	34c	10c	--
	6	15c	13c	0c	--
6	0	275a	318a	193b	14c
	3	39c	31c	49c	9c
	6	0c	0c	0c	0c

[z] Means followed by the same letter are not significantly different from one another by DMRT, $P < 0.05$.

Table 2. The effect of stratification at 5°C and subsequent exposure to 30° and/or exogenous ABA on germination (Sum 10) of apple embryos at 20°.

Weeks at 5°C	Days at 30°C	Sum 10		
		ABA (M)		
		0	10^{-6}	mean
0	0	195[z]	154	175l
	3	207	174	190l
	6	315	307	311k
	mean	239p	212pq	225c
3	0	530	161	345jk
	3	603	253	428j
	6	376	47	211l
	mean	503n	153q	328b
6	0	827	579	703h
	3	725	379	552i
	6	427	113	270kl
	mean	660m	357o	508a
Means for ABA		467f	241g	
Means for days at 30°C	0	517r	298t	408d
	3	512r	268t	390d
	6	372s	156u	264e

[z] Means followed by the same letter are not significantly different from one another within sets (a-c for weeks at 5°; d,e for days at 30°; f-g for ABA; h-l for weeks at 5° X days at 30°; m-q for weeks at 5° X ABA; and r-u for days at 30° X ABA) by DMRT, $P < 0.05$.

Figure 1. The effect of ABA on germination (Sum 14) of seeds at 20° and 30°C following 9 weeks of stratification at 5°.

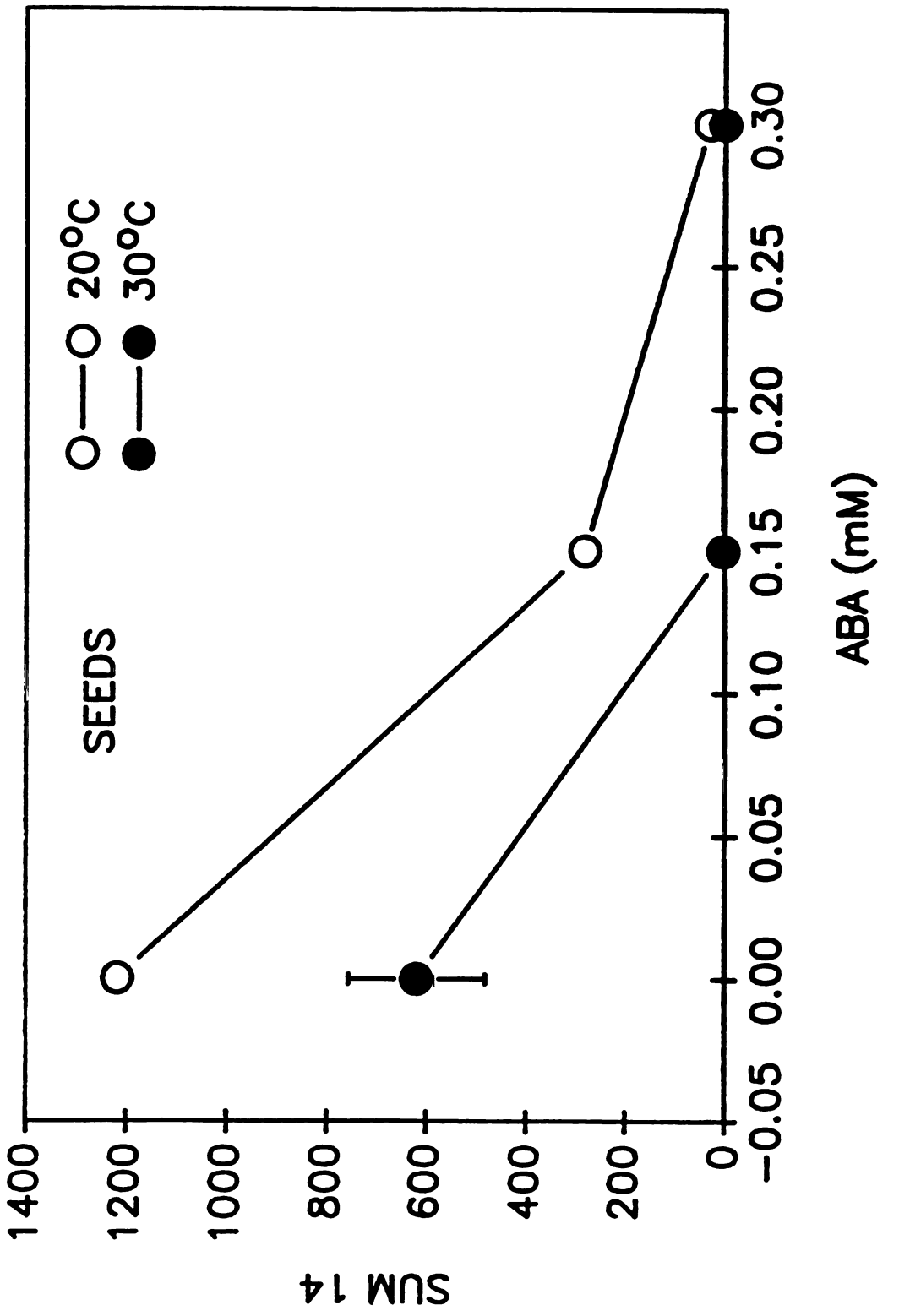

SUM 14
1400
1200
1000
800
600
400
200
0
−0.05
0.00
0.05
0.10
0.15
0.20
0.25
0.30
ABA (mM)
SEEDS
20°C
30°C

FIGURE 1

Figure 2. The effect of ABA on germination (Sum 14) of embryos at 20° and 30°C following 9 weeks of stratification at 5°.

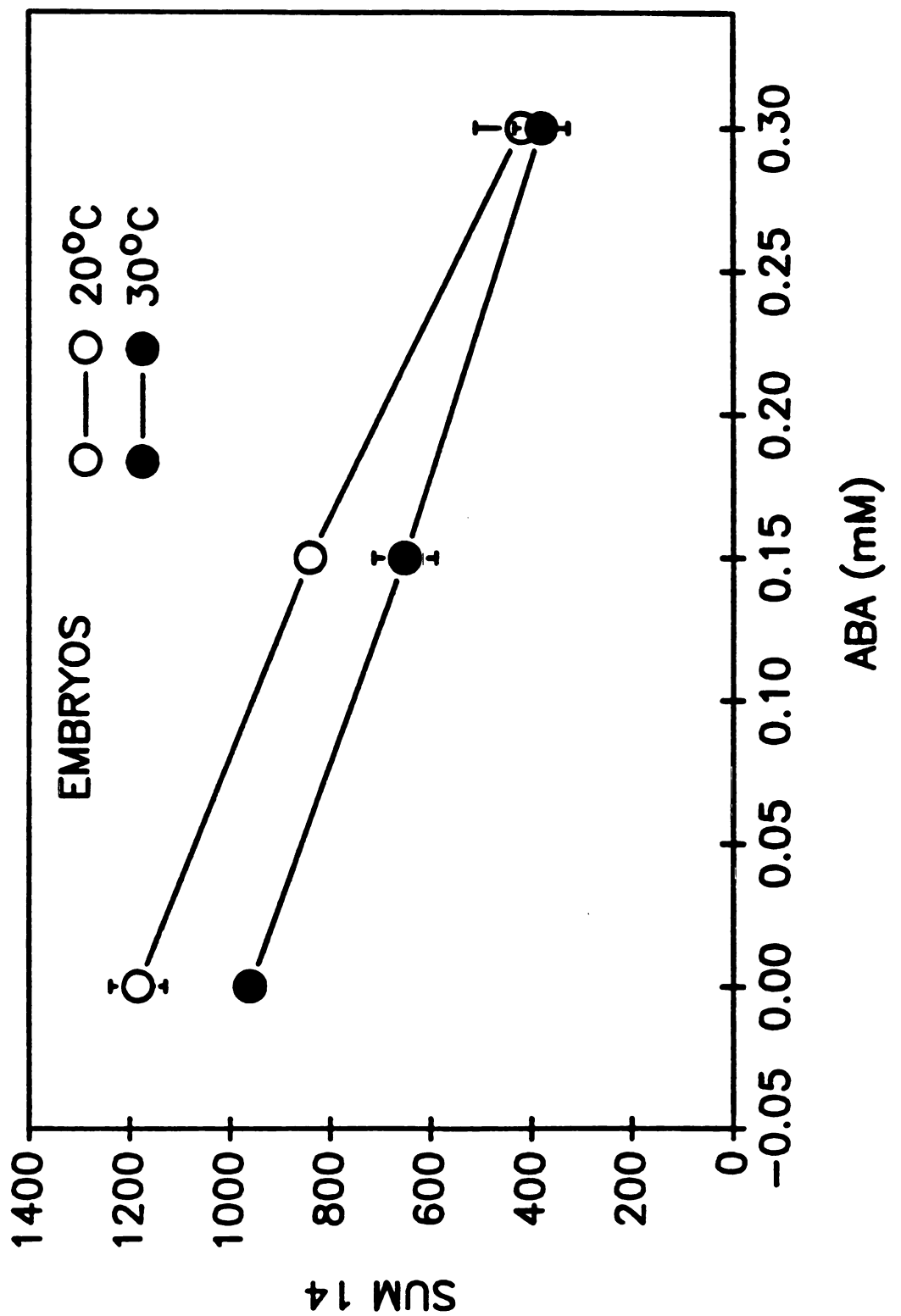

EMBRYOS
20°C
30°C
SUM 14
ABA (mM)
1400
1200
1000
800
600
400
200
0
-0.05
0.00
0.05
0.10
0.15
0.20
0.25
0.30

FIGURE 2

BIBLIOGRAPHY

BIBLIOGRAPHY

Abbott, D.L. 1955. Temperature and the dormancy of apple seeds. Rept. XIV Intern. Hort. Congr., Netherlands. 2A:746-753.

Balboa-Zavala, O. and F.G. Dennis, Jr. 1977. Abscisic acid and apple seed dormancy. J. Amer. Soc. Hort. Sci. 102:633-637.

Berrie, A.M.M., Buller, D., Don, R. and W. Parker. 1979. Possible role of volatile fatty acids and abscisic acid in the dormancy of oats. Plant Physiol. 63:758-764.

Bewley, J.D. 1980. Secondary dormancy (skotodormancy) in seeds of lettuce (Lactuca sativa cv. Grand Rapids) and its release by light, gibberellic acid and benzyladenine. Physiol. Plant. 49:277-280.

Bewley, J.D. and M. Black. 1982. Physiology and Biochemistry of Seeds, Vol.2. Springer-Verlag, New York. pp.207-212.

Bulard, C. 1986. Conditions of induction of secondary dormancy in apple after breaking of primary dormancy by anaerobiosis and low temperature. Physiol. Plant. 67:279-284.

Chen, S.S.C., and J.E. Varner. 1970. Respiration and protein synthesis in dormant and after-ripened seeds of Avena fatua. Plant Physiol. 46:108-112.

Come, D. and T. Tissaoui. 1968. Induction d'une dormance embryonnaire secondaire chez le pommier (Pirus malus L.) par des atmospheres tres appauvries en oxygene. C.R. Acad. Sci. Paris 266:477-479.

Come, D. and T. Tissaoui. 1972. Interrelated effects of imbibition temperature and oxygen on seed germination. p.157-168. In: W. Heydecker (ed) Seed Ecology. Pennsylvania State University Press, College Park, PA.

Come, D. and J. Ralambosoa. 1979. Influence simultanee de la lumiere et de l'oxygene sur la germination de l'embryon de pommier non-dormant. C.R. Acad. Sc. Paris 289:869-871.

Corbineau, F., Rudnicki, R.M., and D. Come. 1988. Induction of secondary dormancy in sunflower seeds by high temperature. Possible involvement of ethylene biosynthesis. Physiol. Plant. 73:368-373.

Crocker, W. 1906. Role of seed coats in delayed germination. Bot. Gaz. 42:265-291.

Cuming, A.C. and D.J. Osborne. 1978a. Membrane turnover in imbibed and dormant embryos of the wild oat (Avena fatua L.) I. Protein turnover and membrane replacement. Planta 139:209-217.

Cuming, A.C. and D.J. Osborne. 1978b. Membrane turnover in imbibed and dormant embryos of the wild oat (Avena fatua L.) II. Phospholipid turnover and membrane replacement. Planta 139:219-226.

Davis, W.E. 1930. Development of dormancy in seeds of cockelbur (Xanthium). Amer. J. Bot. 17:77-87.

Dennis, F.G. Jr., Martin, G.C., Gaskin, P. and J. MacMillan. 1978. Hormones in pear seeds. II. Levels of abscisic acid, dihydrophaseic acid, and their metabolites in relation to seed dormancy in several Pyrus species. J. Amer. Soc. Hort. Sci. 103:314-317.

Durand, M., Thevenot, C. and D. Come. 1973. Influences de l'acide abscissique sur la germination et la levee de dormance des embryons de Pommier (Pirus Malus L.). C. R. Acad. Sci. Paris D 277:53-55.

Esashi, Y., Okazaki, M., Yanai, N., and K. Hishinuma. 1978. Control of the germination of secondary dormant cocklebur seeds by various germination stimulants. Plant and Cell Physiol. 19:1497-1506.

Galli, M.G., Miracca, P., and E. Sparvoli. 1979. Interaction between abscisic acid and fusicoccin during germination and post germination growth in Haplopappus gracilis. Plant Sci. Lett. 14:105-111.

Galli, M.G., Miracca, P., and E. Sparvoli. 1980. Lack of inhibiting effects of abscisic acid on seeds of Haplopappus gracilis (Nutt.) Gray pregerminated in water for short times. J. Exp. Bot. 31:763-770.

Harrington, G.T. 1923. Respiration of apple seeds. J. Agr. Res. 23:117-131.

Ho, T.D. 1988. Regulation of gene expression by ABA in barley aleurone layers. 13th International Conf. Plant Growth Substances. p.23 (Abstract)

Jacobsen, J.V. and L.R. Beach. 1985. Evidence for control of transcription of α-amylase and ribosomal RNA genes in barley aleurone protoplasts by gibberellic acid and abscisic acid. Nature 316:275-277.

Kahn,A. 1960. An analysis of "dark-osmotic inhibition" of germination of lettuce seeds. Plant Physiol. 35:1-7.

Karssen, C.M. 1976. Uptake and effect of abscisic acid during induction and progress of radicle growth in seeds of Chenopodium album. Physiol. Plant. 36:259-263.

Karssen, C.M. 1980/81a. Environmental conditions and endogenous mechanisms involved in secondary dormancy of seeds. Israel J. Bot. 29:45-64.

Karssen, C.M. 1980/1981b. Patterns of change in dormancy during burial of seeds in soil. Israel J. Bot. 29:65-73.

Karssen, C.M., Brinkhorst-van der Swan, D.L.C., Breekland, A.E., and M. Koornneef. 1983. Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of Arabidopsis thaliana (L.) Heynh. Planta 157:158-165.

Kepczynski, J. and R.M. Rudnicki. 1975. Studies on ethylene in dormancy of seeds. I. Effect of exogenous ethylene on the after-ripening and germination of apple seeds. Fruit Sci. Rpts. 2:25-41.

Kepczynski, J., R.M. Rudnicki and A. A. Khan. 1977. Ethylene requirement for germination of partly after-ripened apple embryo. Physiol. Plant. 40:292-295.

Ketring, D.L. and P.W. Morgan. 1969. Ethylene as a component of the emanations from germinating peanut seeds and its effect on dormant Virginia-type peanut seeds. Plant Physiol. 44:326-330.

Khan, A.A. 1980/1981. Hormonal regulation of primary and secondary seed dormancy. Israel J. Bot. 29:207-224.

Khan, A.A. and C.M. Karssen. 1980. Induction of secondary dormancy in Chenopodium bonus-henricus L. seeds by osmotic and high temperature treatments and its prevention by light and growth regulators. Plant Physiol. 66:175-181.

Khan, A.A. and G. Zeng. 1985. Dual action of respiratory inhibitors: inhibition of germination and prevention of dormancy induction in lettuce seeds. Plant Physiol. 77:817-823.

Koornneeff, M., Jorna, M.L., Brinkhorst-van der Swan, D.L.C., and C.M. Karssen. 1982. The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of Arabidopsis thaliana (L.) Heynh. Theor. Appl. Genet. 61:385-393.

Le Deunf, Y. 1973. Interactions entre l'oxygene et la lumiere dans la germination et l'induction d'une dormance secondaire chez les semences de Rumex crispus L. Note C.R.Acad. Sci. Serie D 276:2381-2384.

Luckwill, L.C. 1952. Growth-inhibiting and growth-promoting substances in relation to the dormancy and after-ripening of apple seeds. J. Hort. Sci. 27:53-65.

Mumford, P.M. 1988. Alleviation and induction of dormancy by temperature in Impatiens glandulifera Royle. New Phytol. 109:107-110.

Nikolaeva, M.G. 1969. Physiology of deep dormancy in seeds. pp.227 IPST Press, Jerusalem.

Ohkuma, K., Addicott, F.T., Smith, O.E., and W.E. Thiessen. 1965. The structure of abscisin II. Tetrahedron Lett. 29:2529-2535.

Perino, C. and D. Come. 1977. Influence de la temperature sur les phases de la germination de l'embryon de Pommier (Pirus malus L.). Physiol. Veg. 15:469-474.

Pieniazek, J. and R. Rudnicki. 1967. The presence of abscisin-II in apple leaves and apple fruit juice. Bull. Acad. Pol. Sci. 15:251-254.

Rudnicki, R. 1969. Studies on abscisic acid in apple seeds. Planta 86:63-68.

Rudnicki, R. and J. Pieniazek. 1973. The effect of abscisic acid on stratification of apple seeds. Bull. Acad. Pol. Sci. 21:149-154.

Schopfer, P., Bajracharya, D., and D. Plachy. 1979. Control of seed germination by abscisic acid. I. Time course of action in Sinapis alba L. Plant Physiol. 64:822-827.

Sinska, I. and R.J. Gladon. 1984. Ethylene and the removal of embryonal apple seed dormancy. HortScience 19:73-75.

Sisler, E.C. and S.F. Yang. 1984. Anti-ethylene effects of cis-2-butene and cyclic olefins. Phytochemistry 23:2765-2768.

Sondheimer, E., Tzou, D.S., and E.C. Galson. 1968. Abscisic acid levels and seed dormancy. Plant Physiol. 43:1443-1447.

Stokes, P. 1964. Temperature and seed dormancy, In: Handbuch der Pflanzenphysiologie, Volume 15. Ruhland, W. (Ed.), Springer, Berlin, pp746-803.

Subbaiah, T. 1987. Abscisic acid relationships in apple seed dormancy. Ph.D. Thesis, Cornell University.

Timson, I. 1965. New method of recording germination data. Nature 207:216-217.

Tissaoui, T. and D. Come. 1973. Levee de dormance de l'embryon de pommier (Pirus malus L.) en l'absence d'oxygene et de froid. Planta 111:315-322.

Thornton, N. 1936. Carbon dioxide storage. IX. Germination of lettuce seeds at high temperatures in both light and darkness. Contrib. Boyce Thompson Inst. Plant Res. 8:25-40.

Thornton, N. 1945. Importance of oxygen supply in secondary dormancy and its relation to the inhibiting mechanism regulating dormancy. Contr. Boyce Thompson Inst. Pl. Res. 13:487-500.

Visser, T. 1956a. The role of seed coats and temperature in after-ripening, germination and respiration of apple seeds. Proc. K. ned. Akad. Wet. 59:211-222.

Visser, T. 1956b. Some observations on respiration and secondary dormancy in apple seeds. Proc. K. ned. Akad. Wet. 59:314-324.

Williams, P.M., Ross, J.D., and J.W. Bradbeer. 1973. Studies in seed dormancy. VII. The abscisic acid content of the seeds and fruits of Corylus avellana L. Planta 110:303-310.