This is to certify that the

thesis entitled

Fruit Induced Dormancy in Apple
(Malus Domestica Borkh.) Seed

presented by

Chee-Keong Wan

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Horticulture

Frank G. Dennis Jr.

Major professor

Date February 25, 1980

O-7639

FRUIT INDUCED DORMANCY IN APPLE (*MALUS DOMESTICA* BORKH.) SEED

By

Chee-Keong Wan

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Horticulture

1980

ABSTRACT

FRUIT INDUCED DORMANCY IN APPLE (*MALUS DOMESTICA* BORKH.) SEED

By

Chee-Keong Wan

Apple (*Malus domestica* Borkh.) seeds after-ripened (stratified) in the fruit germinated poorly in comparison with those stratified on moist filter paper. Experiments were designed to test if the inhibitory effects of the fruit were caused by restricted water uptake, the presence of germination inhibitors either in the seed coat or the locules (endocarp), or by production of volatiles.

After-ripening seeds in intact fruits strongly inhibited germination on either moist paper or on a wire mesh screen. Embryos excised from seeds after-ripened in the fruits germinated almost as well as those from seeds stratified outside the fruits on moist paper, but their germination was markedly reduced on screen. The moisture content of seeds stratified on screen was only slightly higher than that of seeds after-ripened in the fruit, yet the former germinated much more readily on both screen and moist paper. Also, soaking seeds prior to after-ripening and germination in locules did not permit germination. Furthermore, the germination of stratified seeds was reduced when placed in direct contact with the endocarp. Thus an inhibitor in the endocarp interferes

with the germination of seeds after-ripened in the fruit.

The inhibitor is unlikely to be abscisic acid (ABA) because the final ABA content of the testa from seeds after-ripened in the fruit, on screen, or on moist paper was not correlated with germination capacity. Furthermore, the level of ABA in the endocarp was too low to inhibit the germination of fully stratified seeds.

After-ripening or stratifying seeds in the presence of fruit-produced volatiles did not inhibit germination to any appreciable extent. By contrast, germination was inhibited when seeds were after-ripened in intact fruits. Therefore volatiles produced by the fruit are not a major factor responsible for fruit induced dormancy of apple seeds.

Germination capacity of seeds or embryos is independent of the rate of ethylene evolution. Stratifying seeds or treating embryos with ethephon promoted ethylene production but had no consistent effect on germination. Inhibition of ethylene biosynthesis by non-toxic levels of 8-hydroxyquinoline sulfate, aminoethoxyvinyl glycine, or silver nitrate was not accompanied by a reduction in germination. The results do not support a role for ethylene in apple seed germination or in the breaking of rest in embryos.

Dedicated to
my wife, Mary, and children

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. F. G. Dennis, Jr., my major professor, for his friendship, counsel, guidance, and willingness to assist during my program of research and preparation of this thesis. I am also grateful for the advice and encouragement offered by members of my Guidance Committee: Drs. Robert Carlson, Harold Davidson, Lawrence Copeland, and William Tai.

The author gratefully acknowledges the support of the Agriculture University, Malaysia for granting him study leave and financial assistance which made this study possible.

Finally, I thank my wife, Mary, and children for their patience, understanding, and encouragement during my graduate study at Michigan State.

TABLE OF CONTENTS

Page

SECTION II
FRUIT INDUCED DORMANCY IN APPLE (*MALUS DOMESTICA* BORKH.) SEED.
II. ROLE OF VOLATILES

LIST OF TABLES

Table Page

SECTION II

LIST OF FIGURES

Guidance Committee:

The thesis is organized in journal-style in accordance with departmental and university requirements. Two sections were prepared following the journal article format of the Journal of the American Society for Horticultural Science.

INTRODUCTION

The phenomenon of seed dormancy has long concerned nurserymen and intrigued plant physiologists. Extensive studies have been devoted to gaining a better understanding of the mechanisms involved in the after-ripening process. Growth inhibiting and promoting substances appear to be involved, but no single hormone seems to control the germination process. Rather, the interaction or intricate balance between growth substances probably regulates the process (2, 62).

Most of the studies of rest and germination of apple seeds have involved seeds removed from the fruits. The inability of seeds to germinate within the fruits despite prolonged storage at temperatures optimal for after-ripening (5, 47, 65) has not been adequately explained. Various factors have been suggested as the cause of inhibition of germination in studies made by several workers (5, 11, 12, 45, 51).

The main goal of this study was to determine the role of (a) moisture, (b) seed coat and/or locule inhibitors, and (c) volatiles (chiefly ethylene), in the inhibition of germination of apple seeds after-ripened in the fruit. An understanding of the controlling factor(s) might provide nurserymen with a practical tool for storing stratified seeds for extended periods of time without danger of their germinating prematurely.

LITERATURE REVIEW

This literature review deals primarily with the phenomenon of rest in apple (Malus domestica Borkh.) seed, although examples will be drawn from other species where appropriate.

The seeds of many species of temperate-zone fruits exhibit some form of dormancy at maturity, and will not germinate when planted immediately after harvest even though conditions are favorable for growth. Germination is prompt and uniform once the seeds have undergone a period of moist after-ripening (stratification) at low temperature. However, apple seeds retained within fruits stored at temperatures optimal for after-ripening do not germinate within the fruit despite extended periods of storage, and germinate very poorly on subsequent removal from the fruit (5, 47).

Terms used in dormancy studies

Dormancy is broadly defined as a temporary suspension or arrest of active growth in plant organs under conditions otherwise suitable for growth (39, 62). The types of dormancy include: (a) quiescence (external, exogenous, imposed, temporary, false, or summer dormancy), which results from unfavorable external conditions; and (b) rest (internal, endogenous, innate, true, primary, or constitutive dormancy) due to internal conditions. Secondary dormancy refers to the

reimposition of dormancy in partially or fully after-ripened seeds under conditions unfavorable for germination (14).

The terms after-ripening and stratification are not synonymous, although they have been used interchangeably in the literature. After-ripening refers to changes that occur within the seed during storage as a result of which germination can take place or is improved (37, 40). Stratification, on the other hand, is the holding of seeds under moist conditions at any temperature (40, 44). In thisthesis, it will be used to mean holding moist seeds at cold temperatures, except when otherwise noted.

Germination is defined as those processes which begin with water uptake, and which terminate in the emergence of the radicle or hypocotyl through the testa (7). In many seeds the radicle is the part of the embryo which first penetrates the seed coat. Therefore, germination is often equated with radicle protrusion.

Role of external factors in apple seed dormancy

Fruit. Apple seeds extracted from the fruits at the time of harvest and then stratified for 8 to 12 weeks under moist conditions at temperatures of 0^{o} to $10^{o}C$ will germinate on raising the temperature to $20^{o}C$. On the contrary, seeds removed from fruits which have been stored for similar periods of time at temperatures close to the optimum for after-ripening germinate very poorly (5, 47, 65). Various reasons have been suggested for the poor germinability

of seeds after-ripened in the fruit. Bartlett (5) found that germination percentage of apple seeds after-ripened in the fruit for 24 weeks at 4°C was less than that of seeds which had been after-ripened outside the fruit. This difference was ascribed to the fact that in the former case, the seeds were not fully imbibed. Visser (65) also identified moisture as a factor responsible for the poor germinability of seeds after-ripened in the fruits, but he did not exclude other factors. Abscisic acid (ABA) which has been identified in apple juice (45), seeds (4) and other tissues (45) is considered by many to be involved in after-ripening. Volatiles produced by the fruits reportedly inhibit germination of embryos (17, 43) and stratified seeds (51), and may prevent germination of seeds in apples stored at low temperatures.

Seed coat. Seed coverings also restrict embryo germination in apple. Removal of the testa permits the germination of non- or partially after-ripened seeds (18, 60). Visser (65) attributed the retarding effect of the seed coat to mechanical resistance, as its removal did not accelerate the after-ripening process. Other researchers (42, 64, 65) have suggested that the impermeability of the testa to water and gases, in particular, may delay or inhibit germination of insufficiently after-ripened seeds. However, the testa is permeable to water. The moisture content of intact seeds soaked in water immediately after

removal from the fruits rose from 14 to 24% of the fresh weight within 24 hours (47), and air-dried seeds regained 80 to 90% of their moisture content within 2 days of soaking (64). The testa restricts the passage of oxygen to the embryo (42, 66, 67) by as much as 90% (42), and upon its removal the respiratory activity of the embryo increased three-fold (66, 67). However, lack of oxygen is probably not a factor in delaying or impairing germination, as rest of the embryo can be broken by exposure to pure nitrogen (64).

Come (12, 13) found that seeds taken out of fruits which had been stored for 3 months at 0° to $4^{\circ}C$ germinated very poorly unless the seed coats were removed. He concluded that chlorogenic acid present in the seed coat, rather than a deficiency of moisture, limited germination. Others (36, 49) suggest that germination inhibitors are metabolized during moist after-ripening.

Stratification may alter the physical properties of the testa, which restrains both embryo expansion and radicle protrusion. Moist chilling may reduce the resistance of the testa to radicle emergence, although no data are known which support this concept. Once the seeds are completely after-ripened, the seed coat is no longer an effective barrier to germination. This could reflect either a change in seed coat resistance, or a change in embryo vigor.

Gases. The testa restricts the diffusion of oxygen, thereby limiting respiration and reducing or preventing seed germination (14). Visser (65, 66, 67) ascribed the

inhibition of germination of partially after-ripened apple seeds at relatively high temperatures to obstruction of respiratory activity by the seed coats. However, Tissaoui and Come (64) succeeded in breaking the rest of embryos without cold treatment by exposure to pure nitrogen.

Volatile compounds produced by apple fruits may be involved in the control of seed germination, for germination of seeds and/or embryos is inhibited by the presence of fruit tissues (43, 51, 53) or by the volatiles they produce (17). Furthermore, enriching the atmosphere with ethylene and other gases released from the fruits reportedly delays the after-ripening process (30). These investigators (30, 51, 53) concluded that fruit-produced volatiles inhibit the after-ripening process.

The germination of stratified seeds was reduced under sub-atmospheric pressure (0.1 atm.), leading Kepczynski and Rudnicki (30) to conclude that the internal ethylene concentration of apple plays a regulatory role in the breaking of their rest. Although ethylene is reportedly required for the termination of rest and germination of embryos (31), stratifying seeds in an atmosphere enriched with ethylene (0.1 to 5%) had no effect on their germination or on that of excised embryos (29). The conflicting results obtained with ethylene raise doubts as to its participation in the rest of apple seeds.

Moisture. Hydrated seeds subjected to low temperatures for a duration of 8 to 12 weeks usually germinate promptly

and uniformly. Drying during stratification delays, stops, or may cancel the effects of after-ripening (23). As noted above, Bartlett (6) observed that the germination of apple seeds after-ripened in the fruit was less than 50% of those stratified on moist cotton wool outside the fruit, and considered this to be the result of incomplete imbibition. Visser (65) reported similar results but did not exclude factors other than moisture content as causal factors.

Temperature. The most effective temperatures for after-ripening apple seeds range from 0° to 10°C with an optimum at 3° to 5° (1). Abbott (1) found that secondary dormancy in apple seeds is dependent upon temperature. Germination of partially stratified seeds was promoted at 5° to 13°C but was decreased at 18° to 28°C. A temperature of 17° was defined as the "compensation temperature" at which the germinability of partially stratified seeds remained unchanged.

Harrington and Hite (22) reported that apple seeds do not after-ripen in dry storage or when kept moist at temperatures of 20°C or above. Visser (67) observed that non-after-ripened seeds soaked in water and then stored under moist conditions at 25°C were unable to germinate however long the storage period.

Light. Light promotes or is required for germination in a number of species (8). In apple light partially overcame dwarfism in seedlings obtained from non-after-ripened seeds (68), and promoted germination of isolated embryos of dormant seeds (60). In spite of these reports, the role of

light as a factor actively involved in the termination of rest has not been positively established. Furthermore, light has not been shown to be required for after-ripening, and fully after-ripened apple seeds germinate equally well in light or darkness.

Growth regulators. Evidence for the role of hormones in the regulation of seed rest comes in part from studies with exogenous growth substances. The dwarfed condition of seedlings developing from non-after-ripened seeds (18) is relieved by gibberellic acid (GA_3) (6) which suggests that gibberellins may be involved in rest. Gibberellins also stimulate the germination of apple embryos excised from non-stratified seeds (19, 27). Furthermore, Westwood and Bjornstad (69), noted that seeds from GA_3-treated apple trees germinated 28% after partial chilling compared to only 8% for control seeds. However, Kopecky et al. (32) found that soaking apple seeds in GA_3 solutions prior to stratification did not influence rest. This lack of response could be due to the failure of the applied GA_3 to penetrate the seed coat, since excised embryos respond (19).

Cytokinins also can stimulate germination in dormant seeds. Badizadegan and Carlson (3) demonstrated that presoaking embryos excised from mature apple seeds in N^6benzyladenine (BA) solutions significantly increased their germination, but the resulting seedlings were dwarfed, with abnormal leaves and short internodes. A similar stimulatory effect of BA and/or kinetin has been noted by other

investigators (19, 27, 34), as well as synergism with GA in germination of excised embryos (27).

Ethephon (2-chloroethyl phosphonic acid), an ethylene releasing compound, reportedly stimulates the germination of apple seeds (29) and embryos (31). However, Halinska et al. (20) found that adding ethephon (1.5×10^{-6}M) to the stratification medium inhibited apple seed germination approximately 17 to 25% until the 70th day of stratification. Similarly, Sinha and co-workers (56) reported that 250 ppm ethephon was not effective in stimulating germination until the seeds had been stratified for 60 days. This observation was supported by Kepczynski and Rudnicki (29) who found that stratifying intact seeds in the presence of 0.1 to 5% ethylene promoted their germination only after 70 days of stratification. These findings imply that ethylene only stimulates germination of fully stratified seeds, and is ineffective in breaking rest in apple seeds.

Role of internal factors in apple seed dormancy

Endogenous growth inhibitors. The endogenous inhibitor most often presumed to control apple seed germination is abscisic acid (ABA), which has been identified in extracts prepared from resting apple seeds (4, 48). The compound strongly inhibits wheat coleoptile section growth as well as germination of stratified apple seeds and embryos (26, 27, 46, 47, 50, 52). The ABA level declines rapidly during

stratification (4, 48) and this is accompanied by an increase in the ability of the seeds to germinate. However, Balboa-Zavala and Dennis (4) observed that the decline in ABA was independent of temperature, and concluded that factors other than ABA content control rest. The inability of seeds to germinate in stored fruit has been ascribed to the ABA content of the fruit tissue (45).

Little is known as to what happens to the ABA during stratification. The rapid decline in ABA during stratification could be due to leaching (36, 48) and/or inactivation (46). Decarboxylation of ^{14}C-ABA in apple seeds undergoing low temperature stratification partially accounted for its disappearance (49). However, decarboxylation was later attributed to microbial contamination (38). Sondheimer et al. (61) reported that dormant ash seeds metabolized ABA to phaseic acid, dihydrophaseic acid, and an unidentified polar metabolite, and that resting and non-resting seeds metabolized ABA at the same rate.

Phenolic compounds such as phloridzin, phloretin, and chlorogenic acid occur in apple tissues, including seeds (13, 24, 46, 70), and pholoridzin retards the growth of apple seedlings at a concentration of 10^{-4}M (10). Come (11, 12, 13) postulated that the phenolic compounds found in the integuments of apple seeds regulate rest. He suggested that the phenols absorb some of the oxygen passing through the integuments and impede its penetration to the embryo. Hence, the embryo is deprived of the oxygen required for

germination. But Dziewanowska et al. (16) and Pieniazek and Grochowska (56) found that the amount of both phloridzin and chlorogenic acid in apple seeds decreased or disappeared completely at maturity (16, 70). Phloridzin by itself had no effect on germination of embryos but strongly inhibited the promotive effects of GA and/or BA (27).

Endogenous growth promoters. Extracts of apple seeds contain auxin-like growth promoters (28, 36, 63), and activity occurs in the seed coat, endosperm, and embryo during after-ripening (28). Activity was very low during the first 5 weeks of low temperature stratification, then rose to a maximum in the 7th week (32). However, no correlation was obtained between the level of auxin and the termination of rest.

The rest breaking effect of chilling on apple seeds has been attributed to the synthesis of gibberellins (GAs) (57). GAs (A_4, A_7, and A_9) were identified in extracts of dormant and non-dormant apple seeds (59), although the GAs identified may have been artifacts (15). The concentration of GA_4 rose very markedly in the 4th week of stratification at 2°C, then decreased to the initial level between the 50th and 60th day, although the GA_7 content remained at a more or less constant level during the entire period of stratification (57, 58, 59). Kopecky et al. (32) observed similar changes in GA content. The above observations do not establish a close association between the level of GA_4 and the termination of rest in seeds, for germination capacity did not

increase until several weeks after the GA_4 content had declined (57). Halinska and Lewak (21) observed an increase in the free GA_9 level at the end of the after-ripening period. They suggested that GA_9, unlike GA_4 and GA_7, may be involved in the final phase of after-ripening including germination.

Letham and Williams (33) identified 3 cytokinins (zeatin, zeatin ribotide, and zeatin riboside) in extracts of young apple fruits, while Zwar and co-workers (71) found 4 cytokinin-like components. Both bound and free cytokinin-like compounds also occur in mature apple seeds (9). Cytokinin-like activity in apple seeds rose during stratification, reaching a maximum in the 5th week, but the increased level of cytokinins was not directly correlated with the ability of the seeds to germinate (9, 32).

It has recently been suggested that ethylene is one of the hormones regulating rest in apple seeds (30, 31). Kepczynski and Rudnicki (30) found that germination of stratified seeds was reduced under sub-atmospheric pressure (0.1 atm.), and this was attributed to a decrease in the internal ethylene concentration of apple fruits. Endogenous ethylene production by excised embryos increased during after-ripening, and this was accompanied by an increase in their ability to germinate (31). Kepczynski et al. (31) concluded that endogenous ethylene is required for the termination of rest and germination of embryos.

Enzymes. Nikolaeva and Yankelevich (40) suggested that the breaking of dormancy and the ability of embryos to overcome the inhibiting influence of seed coats was caused by an increase in enzyme activity. The activity of enzymes (peroxidase, succinic dehydrogenase, lipases, and proteases) in seeds and embryos increased seven-fold during chilling, and any interruption of the cold stratification caused a sharp decline in enzyme activity (41). Lewak et al. (35) identified 3 phases in after-ripening of apple seed: (i) removal of the primary cause of rest; (ii) high metabolic activity; and (iii) initiation of germination. They postulated that enzymes are involved in controlling the second phase, and proposed that a gradual build-up of hydrolytic enzymes could be responsible for breaking of rest. The biosynthesis or release of hydrolytic enzymes is presumably under hormonal control (2), and treatment of dormant seeds with GA_4 or benzyladenine increases the activity of lipase, phosphatase, and peroxidases (54, 55). However, changes in enzyme activity during stratification seem to be the result, rather than the cause, of breaking rest in apple seed.

Summary

Mature apple seeds are in a state of physiological rest which is terminated by a period of moist chilling (stratification). Early studies emphasized the importance of external factors, particularly the seed coat, in inhibiting

the germination of the embryo. More recent work has assumed rest to be under hormonal control, and to be broken by changes in levels of inhibitors (such as ABA) and promoters (GAs and cytokinin). The effects of exogenous growth substances support this hypothesis.

Seeds extracted from fruits held in storage at an optimal temperature for after-ripening exhibit a very low ability to germinate. Various reasons have been suggested for this effect of fruits, including: (i) limited seed water content, (ii) inhibitory effect of volatiles, and (iii) inhibitory levels of ABA in fruit tissue. The purpose of this research was to further examine these and other factors in an attempt to explain fruit induced dormancy in apple seed.

SECTION I

FRUIT INDUCED DORMANCY IN APPLE (*MALUS DOMESTICA* BORKH.) SEED. ROLE OF WATER AND INHIBITORS

FRUIT INDUCED DORMANCY IN APPLE (*MALUS DOMESTICA* BORKH.) SEED

I. ROLE OF WATER AND INHIBITORS

Abstract. The roles of inhibitors, especially abscisic acid (ABA), in the seed coat and locules, and of seed water content in fruit-induced dormancy of apple (*Malus domestica* Borkh.) seeds were investigated. After-ripening seeds in the fruit inhibited their subsequent germination on either moist paper or on a wire mesh screen, whereas fruit volatiles has little if any effect on seeds stratified on screen. Soaking seeds either prior to or after stratification promoted germination of seeds held in the fruit or on screen, but did not overcome the effect of the fruit. Germination capacity of seeds after-ripened on screen increased with water content, although large increases in the former were sometimes associated with small increases in the latter. Seeds stratified on screen, then germinated on moist paper, germinated better than seeds stratified on moist paper, then germinated on screen, indicating that water content was more crucial during germination than during after-ripening. Germination capacity of embryos excised from seeds stratified in the fruit was considerably lower than that of embryos from seeds stratified on paper or screen, particularly when germinated on screen; thus fruit-induced dormancy is not simply a seed coat effect. The effect of the locule (endocarp) in inhibiting germination was blocked by aluminum foil, indicating the involvement of a diffusible, water-soluble

inhibitor. However, the ABA contents of the endocarp and of the testa were too low to account for the inhibition observed. ABA content of the testa declined regardless of whether seeds were held in the fruit, on screen, or on moist paper. Soaking seeds after-ripened in the fruit in ABA concentrations higher than that measured in the testa did not reduce the promotive effect of soaking, indicating that leaching of ABA was not responsible for this effect. Finally, partial or complete removal of the testa indicated that the effect of soaking was primarily mechanical, rather than chemical. It is concluded that an inhibitor(s) in the locule is the primary factor responsible for fruit induced dormancy.

Apple seeds will not germinate until they have been subjected to a period of after-ripening under moist conditions at 0° to $10^{\circ}C$. However, seeds held in fruits at temperatures close to the optimum for after-ripening germinate very poorly on removal from the fruit even after extended periods of storage (1, 14, 19).

The low germination of seeds after-ripened in apple fruits has been attributed to: (a) insufficient moisture content for after-ripening (1), (b) absence of free water to leach out seed inhibitors (9), (c) fruit-produced volatiles, including ethylene (17). A diffusible inhibitor in locules (endocarp) could also prevent germination.

The content of abscisic acid (ABA) or an ABA-like inhibitor declines rapidly during stratification of apple seeds (2, 13, 15) and other (3, 7, 8) seeds which require moist chilling to terminate rest. Application of exogenous ABA prevents germination of seeds and embryos in which rest has been broken (6, 13, 16, 17). Thus, the breaking of rest has been ascribed to the decline in ABA content (9, 15, 20). However, Balboa-Zavala and Dennis (2) found the decline in ABA in apple seeds to be independent of temperature and suggested that factors other than ABA control rest. Rudnicki and Czapski (18) found that decarboxylation of (1-^{14}C)ABA in apple seeds undergoing low temperature stratification partially accounted for its disappearance. However, Milborrow and Vaughan (11) reported that the amount of $^{14}CO_2$ evolved from sterile apple seeds during stratification amounted to less than 0.01% of the radioactivity supplied as (2-^{14}C)ABA. They concluded that the degradation observed by Rudnicki and Czapski (18) was probably brought about by microflora in the medium.

Inhibitors, including ABA, occur in the juice of a number of fleshy fruits (4, 10). ABA has also been identified in apple juice (6, 12), and Pieniazek and Rudnicki (14) suggested that it prevents the germination of seeds after-ripened in the fruit.

The testa also restricts germination, for its removal permits germination of partially after-ripened embryos (5).

Visser (19) attributed the retarding effect of the seed coat to mechanical resistance, as its removal did not accelerate the after-ripening process. The objective of this study was to determine the roles of water versus inhibitors in the after-ripening and germination of apple seeds held in the fruits during low temperature storage.

Materials and Methods

Plant material. Mature apple fruits of several cultivars were collected from experimental or commerical orchards. In some cases the fruits had fallen from the trees and were collected from the ground. Seeds were either removed from the fruits immediately or were removed after storage of the fruits for varying periods of time at $5^{\circ} \pm 1^{\circ}C$. None of the seeds was dried before use.

Methods of after-ripening and germination. Several methods of after-ripening and germination were used with these seeds. Seeds held in the fruit were exposed to fruit volatiles, but not to free water. Therefore leaching of chemical inhibitors from the seeds was prevented, but inhibitors in the lining of the locule (endocarp) might prevent germination. Seeds were held on wire mesh over moist paper or glass wool in petri dishes to simulate moisture conditions in the locule, yet eliminate the possible effects of volatiles and endocarp inhibitors. Finally, holding seeds on moist filter paper in petri dishes also eliminated

the effects of volatiles and endocarp inhibitors, but permitted both water uptake and leaching of inhibitors. In some experiments, seeds were soaked before or after stratification to test the effects of leaching and/or increased water content, or embryos were excised to determine the role of the testa in restricting germination.

Germination tests. Unless otherwise stated, germination tests employed 3 replicates of 20 seeds or embryos in 9-cm petri dishes lined with Whatman no. 1 filter paper, which was moistened with distilled water. The petri dishes were randomly placed in a growth chamber at $20^{\circ} \pm 1^{\circ}C$ in darkness. Seeds with visible radicle protrusion, and embryos whose radicles showed geotropic curvature were considered to be germinated. Germination was recorded for a period of 10 days, and the results are expressed as mean percentages.

Measurement of seed moisture content. Seeds were blotted with paper towels, weighed, held at $75^{\circ}C$ for 72 hours, then reweighed. Water content is expressed as a percentage of initial fresh weight of the seeds.

Statistical analysis. The data were subjected to analysis of variance, and the mean separation procedure of Duncan's Multiple Range Test was used where appropriate.

Exp. 1. Effects of the locule, of water content, and of fruit volatiles on after-ripening and germination of apple seeds. If volatiles limit after-ripening and/or germination of seeds held in fruits, seeds stratified on screen in the same containers with fruits should germinate poorly in comparison with similar seeds stratified in the absence of fruits. On the other hand, if chemicals in the locules inhibit stratification, seeds held on screen should germinate better in the presence or absence of fruits than those in the fruits. If water content during or after stratification in the fruits limits germination, soaking seeds prior to after-ripening should negate the effects.

Apple seeds, cv. 'Paulared', were collected from the University Research Farm, East Lansing, in 1978 and from a commercial orchard near Grand Rapids in 1979. To distinguish between the effects of (a) inhibitors in the locules, (b) water content, and (c) fruit volatiles, seeds were extracted from the fruits immediately after collection, and after-ripened at $5^{\circ} \pm 1^{\circ}C$: (a) in the locules of half-fruits in loosely covered plastic containers, (b) on a wire mesh screen over moist paper in the same containers, and (c) on wire screen over moist paper in a similar container without fruits. The relative humidity of the containers was maintained near 100% by lining the bottoms with a layer of moistened glass wool. Half of the seeds in each treatment were pre-soaked in distilled water for 24 or 48 hours, the

remainder were not soaked. Samples were transferred to a growth chamber at $20^{o} \pm 1^{o}C$ at 3 week intervals to evaluate germination in darkness on the same medium used for after-ripening (locules or screen). To assure that moisture was not limiting germination in 1979, droplets of water were placed on seeds held on screen for germination.

Exp. 2. Effects of after-ripening seeds in the fruit, on screen, and on moist paper upon seed water content and upon subsequent germination of seeds and embryos on moist paper or screen. If water content during after-ripening limits subsequent germination, after-ripening on screen should be equivalent to after-ripening in the fruit, and germination capacity should parallel water content. Previous workers had reported that holding seeds in the fruit does not affect the ability of the embryo to germinate, the inhibitory effect being confined to the seed (4). This concept was also tested in this experiment. Apple seeds used in the 1978 experiment were extracted from 'Paulared' fruits obtained from the University Research Farm, East Lansing. The study was repeated in 1979 using fruits of the same cultivar collected from a commercial source at Grand Rapids. The seeds were after-ripened at $5^{o} \pm 1^{o}C$: (a) in intact fruit, (b) on moist filter paper, and (c) on screen over moist paper. Samples were removed from storage at 3 week intervals for germination tests, on both moist paper and on screen, of both intact seeds and excised embryos.

Exp. 3. Separation of effects during after-ripening from effects during germination. Treatments were arranged in a factorial to attempt to separate the effects of moisture supply and/or inhibitors during stratification versus effects during germination. Seeds (cv. 'Paulared') were held for 12 weeks at 5° ± 1°C: (a) in intact fruits, (b) on moist paper, or (c) on screen over moist paper, then half the seeds in each treatment were soaked in distilled water for 24 hours at 20° ± 1°C. The seeds were test germinated: (a) in the locules of half-fruits, (b) on moist paper in a petri dish, and (c) on screen over moist paper in a petri dish.

Exp. 4. Effect of blocking diffusion of locule inhibitors on germination of seeds stratified on moist paper. If a water-soluble inhibitor(s) prevents germination in the fruit, blocking its diffusion to non-dormant seeds should prevent its effect. Seeds (cv. 'Jonathan') stratified on moist paper for 12 weeks were germinated on the endocarp of fruit sections placed horizontally on moist paper. Seeds were placed: (a) directly on the endocarp, (b) on moist paper in contact with the endocarp, (c) on moist paper backed by aluminum foil in contact with the endocarp, (d) on moist paper, and (e) on screen over moist paper in petri dish.

Exp. 5. Effect of method of after-ripening seeds on ABA content of the testa. If stratification in the fruit presents leaching and/or metabolism of ABA, or allows a continuous supply from the endocarp, ABA content of the seed coat should decline less rapidly in the fruit than outside the fruit. Holding on a screen should prevent leaching, but not metabolism, while holding on moist paper should prevent neither. Seeds were removed from 'Paulared' fruits immediately after collection from a commercial orchard at Grand Rapids in 1979. The seeds were then after-ripened: (a) in intact fruits, (b) on a screen over moist paper in petri dish, or (c) on moist paper in petri dish for 12 weeks. Three samples of 20 seeds from each treatment were removed after 12 weeks and germinated on moist filter paper. Two samples of 50 seeds each were removed for ABA determination at intervals of 3 weeks. The seed coat was separated from the embryo and shaken in 25 ml of distilled water at 5°C for 7 days. The water was changed daily and pooled, and this diffusate was processed (Fig. 1) to give an acidic fraction (free ABA) and a base hydrolyzable fraction (bound ABA).

The ABA content of the locules at the beginning of the experiment was also determined. Two replicates of 2 gram each of the locule (endocarp) tissue were obtained from a total of 10 fruits per replicate. All the flesh tissue was scraped off and each sample was shaken in 50 ml of distilled water for 24 hours at 5°C. The water diffusate was processed into free and bound ABA fractions as described for seed coat diffusates.

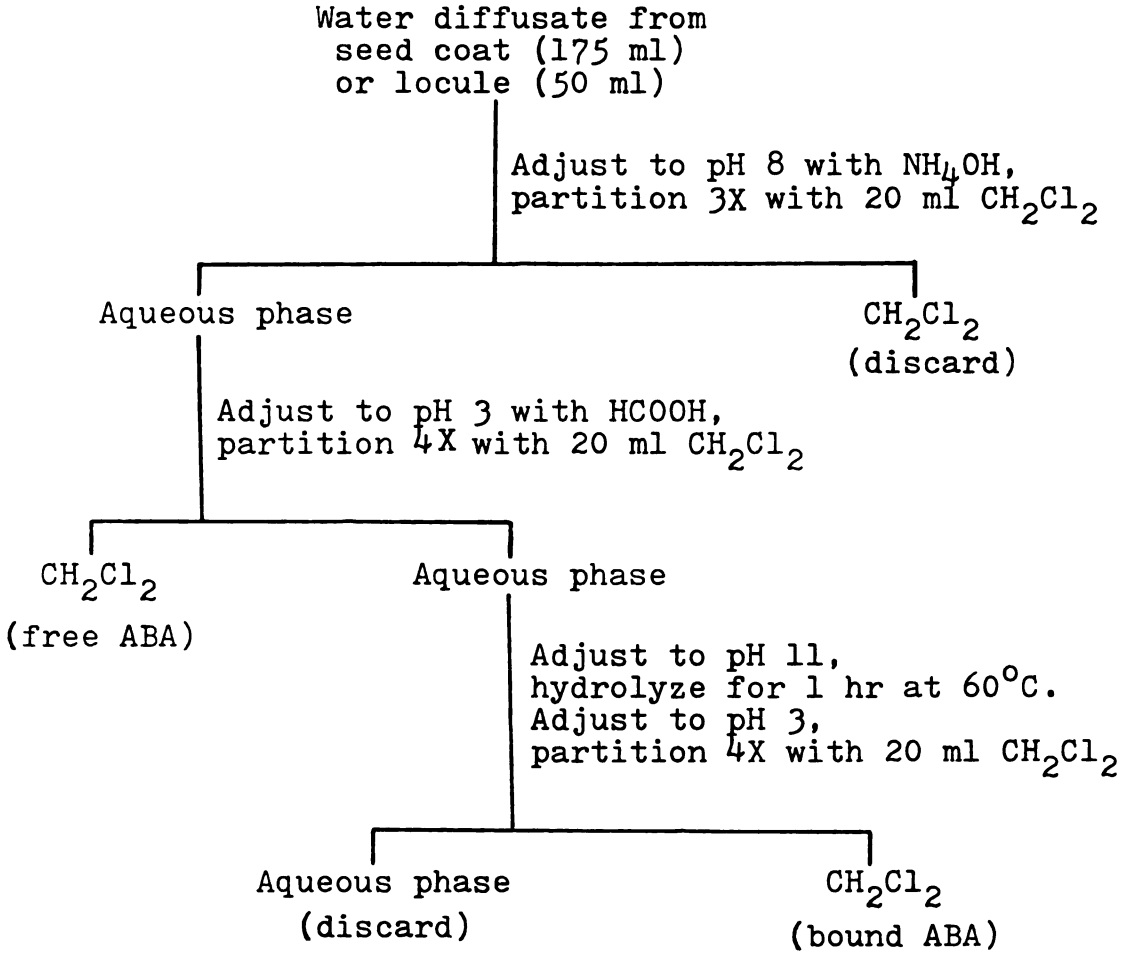


Figure 1. Fractionation procedure for free and bound ABA in water diffusates from seed coats and locules (endocarp)

The extracted free and bound fractions from both seed coats and locules were evaporated to dryness and resuspended in pure methanol. The fractions were methylated with diazomethane, the methanol-diazomethane solution was evaporated to dryness, and the residue was redissolved in ethyl acetate. Aliquots of the ethyl acetate solution were injected into a Packard 7300 gas liquid chromatograph equipped with an electron capture detector (^{63}Ni foil) operated at 5 volts. A glass column (152.4 x 0.35 cm i.d.) was packed with 1% XE-60 on 100/120 mesh Gas Chrom Q. The column temperature was 200°C, and inlet and detector temperature was 220°C. The carrier gas was nitrogen at a flow rate of 40 ml/min at 40 psig. Nitrogen scavenger gas was supplied to the detector at 90 ml/min. The retention time for authentic c,t-ABA was 1.5 min. A standard curve based upon peak height was used for quantitation of c,t-ABA in the diffusates.

Exp. 6. Effects of moisture level, seed coat resistance, and ABA upon germination of seeds. If leaching of ABA were responsible for the promotive effect of soaking seeds, inclusion of comparatively high concentrations of ABA in water would prevent the effect. Furthermore, if chemical inhibitors in the seed coat were responsible for limited germination, partial seed coat removal should be less effective than total removal. If mechanical properties of the seed coat limit germination, on the other hand, (a) removal of the testa in contact with radicle should be just as

effective as total removal, and (b) soaking should have little, if any, effect on germination of seeds from which the testa has been partially or totally removed. To determine the effects of soaking and partial or total testa removal in the presence and absence of ABA, seeds were extracted from 'Jonathan" apple fruits which had been harvested in September 1979, and held at $5^{\circ} \pm 1^{\circ}C$ for 12 weeks. Seeds were then soaked in distilled water for 0, 24, 48, or 72 hours with or without the addition of 10 ppm ABA. Three samples of 20 seeds per treatment were then test germinated with the seed coat: (a) intact, (b) removed over the radicle end, or (c) entirely removed (excised embryos). Seeds were germinated on a wire mesh screen over moist filter paper in petri dishes.

Results

Exp. 1. Effects of the locule, of water content, and of fruit volatiles on after-ripening and germination of apple seeds. All seeds left in the locules failed to germinate in both 1978 and 1979, whether they had been soaked in water or not (Table 1). Germination of seeds held on screen increased as after-ripening time increased from 6 (1979) or 9 (1978) to 12 weeks, and was promoted by soaking in water prior to after-ripening in most instances. Therefore, contact with the locules inhibited either after-ripening or germination or both. Seeds held in containers

Table 1. Effects of after-ripening and germination medium and of soaking in water prior to after-ripening on germination of apple, cv. 'Paulared', seeds.

After-ripening and germination in:	Soaking[y] in water	Percent germination[x]				
		1978		1979[z]		
		After-ripening (wk)		After-ripening (wk)		
		9	12	6	9	12
Locules of half-fruits	Yes	0 a	0 e	0 b	0 e	0 c
	No	0 a	0 e	0 b	0 e	0 c
Screen with half-fruits	Yes	2 a	82 b	3 b	33 b	70 b
	No	0 a	20 d	0 b	5 d	70 b
Screen without half-fruits	Yes	0 a	93 a	10 a	45 a	80 a
	No	0 a	38 c	3 b	12 c	70 b
After-ripening (AR):						
Locules of half-fruits		0 r	0 t	0 s	0 t	0 s
Screen with half-fruits		0 r	51 s	2 s	19 s	70 r
Screen without half-fruits		0 r	66 r	7 r	29 r	75 r
Soaked in water (S):						
Yes		0 m	58 m	4 m	29 m	50 m
No		0 m	19 n	1 m	6 n	47 m
Interaction (AR) x (S):		n.s.	*	n.s.	*	n.s.

[x]Mean separation within columns and sets by DMRT at 5% level. No germination occurred after 4 or 6 wks (1978) or 3 wks (1979) regardless of treatment.

[y]Seeds soaked in distilled water for 24 hrs in 1978, and 48 hrs in 1979.

[z]Moisture added to surface of seeds during germination test.

*Interaction significant at the 5% level.

without half-fruits germinated significantly better than those in containers with half-fruits in 6 of 7 comparisons in which germination was appreciable, indicating a small but measurable inhibition by volatiles. Interaction between soaking and method of after-ripening on germination was significant in 2 cases, indicating beneficial effects of soaking only when seeds were not held in locules. Moistening the seeds during germination appeared to favor germination (compare 1978 vs. 1979), but this was not critically tested.

Exp. 2. Effects of after-ripening seeds in the fruit, on screen, and on moist paper upon seed water content and upon subsequent germination of seeds and embryos on moist paper or screen. Seeds held on moist filter paper contained approximately 25% more water than did those held in intact fruits, whereas seeds held on screen contained at most 11% more (Table 2). After-ripening seeds in the fruit strongly inhibited subsequent germination in comparison with after-ripening on screen or on paper, and no germination occurred when seeds were held on screen after removal from the fruits (Table 3, Fig. 2A, C). Thus the fruit has an inhibitory effect over and above any effect on water content. Seeds held continuously on paper germinated only slightly better in most instances than those held on screen during after-ripening, then germinated on paper, and the latter germinated significantly better than did seeds after-ripened on paper, then germinated on screen. These results indicate that

Table 2. Moisture content of seeds, cv. 'Paulared', during after-ripening in intact fruit, on screen, or on moist paper at 5°C (1979)

After-ripening method	Moisture content (%)[y]				
	After-ripening (wk)				
	0	3	6	9	12
In intact fruit	47 a	46 c	44 c	44 c	47 b
On moist paper	47 a	57 a	57 a	57 a	58 a
On screen	47 a	49 b	49 b	49 b	48 b

[y]Mean separation within columns by DMRT at 5% level.

Table 3. Effects of after-ripening apple, cv. 'Paulared', seeds in fruit, on screen, and on moist paper upon subsequent germination on moist paper or screen.

After-ripening method	Germination on:	Percent germination[z] 1978 After-ripening (wk) 9	12	1979 After-ripening (wk) 6	9	12
In intact fruit	Moist paper	8 b	28 c	3 b	0 e	27 c
	Screen	0 d	0 d	0 b	0 e	0 d
On screen over moist paper	Moist paper	12 bc	85 a	27 a	72 b	98 a
	Screen	0 d	12 d	0 b	5 d	32 c
On moist paper	Moist paper	40 a	95 a	32 a	82 a	100 a
	Screen	2 cd	58 b	0 b	15 c	78 b
After-ripening (AR):						
In fruit		4 g	14 h	2 g	0 h	14 h
On screen		6 g	49 g	14 f	39 g	65 g
On moist paper		21 f	77 f	16 f	49 f	89 f
Germination (G):						
On moist paper		18 r	69 r	21 r	51 r	75 r
On screen		1 s	23 s	0 s	7 s	37 s
Interaction (AR) x (G):		*	*	*	*	*

[z]No germination occurred after 4 or 6 wks (1978) or 3 wks (1979) of after-ripening regardless of treatment. Mean separation within columns and sets by DMRT at 5% level.

*Interaction significant at the 5% level.

moisture content was much more critical during germination than during after-ripening. Interaction between method of after-ripening and method of germination was primarily due to quantitative, rather than qualitative, differences in response (Fig. 2A, C). Similar results were obtained in 1978 and in 1979.

The germination on moist paper of embryos excised from seeds held in the fruits was significantly lower than that of those from seeds stratified on screen or moist paper for the first 6 (1978) or 9 (1979) weeks of after-ripening, but no differences were evident after 12 weeks (Table 4, Fig. 2B, D). The difference was more pronounced when the embryos were germinated on screen (data for 1979 only). Differences between embryos after-ripened on screen versus moist paper were small but significant for the first 4 to 9 weeks, with the former being slightly inhibited in germination. Interaction in 1979 between after-ripening and germination methods again indicated quantitative, rather than qualitative, differences in response. Germination of embryos from seeds after-ripened in the fruit was considerably lower at 6 and 9 weeks in 1979 than in 1978.

About 5 additional weeks of chilling were necessary to induce 50% germination when embryos were germinated on screen rather than on paper, germination paralleling that of intact seeds held on moist paper (Table 4, Fig. 2C, D). Thus the restriction on germination imposed by lack of water and the true state of embryo dormancy are better revealed when

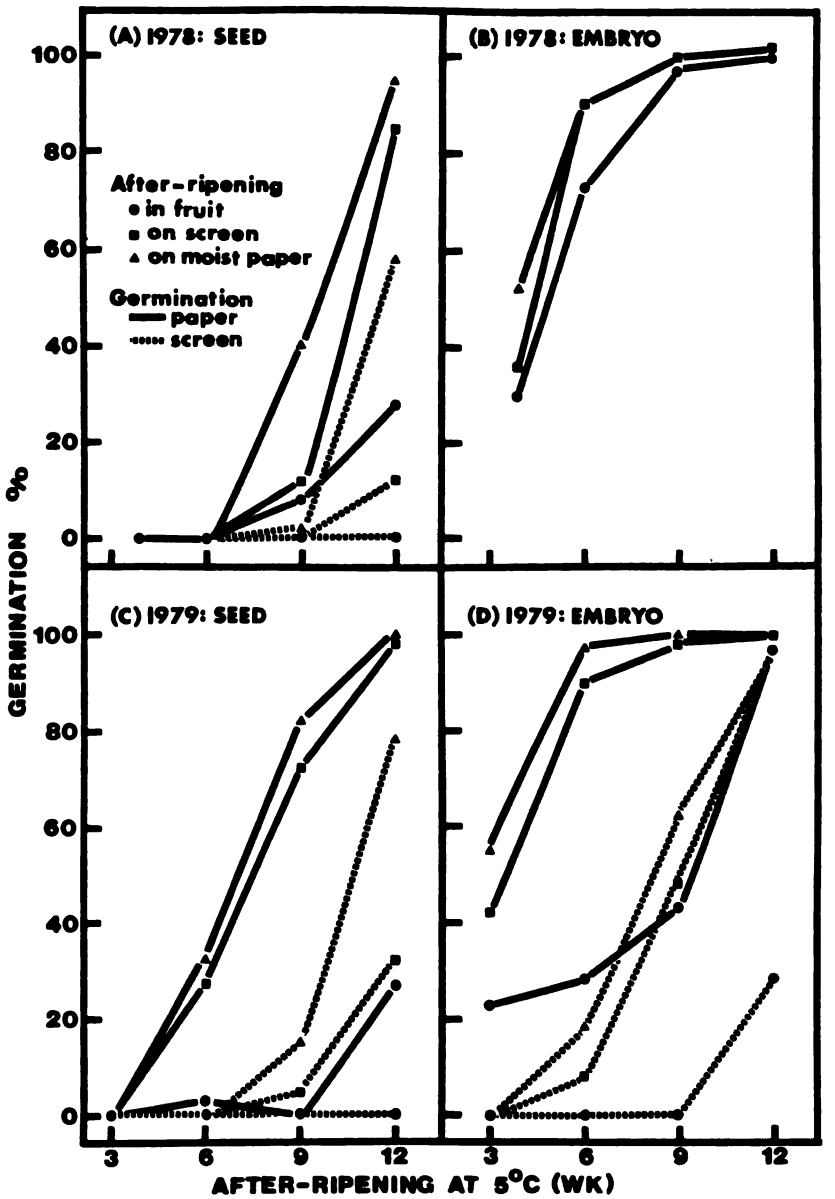
Table 4. Effects of after-ripening apple, cv. 'Paulared', seeds in fruit, on screen, and on moist paper upon subsequent embryo germination on moist paper or screen.

After-ripening method	Germination on:	Percent germination[z]							
		1978				1979			
		After-ripening (wk)				After-ripening (wk)			
		4	6	9	12	3	6	9	12
In intact fruit	Moist paper	30b	73b	97a	100a	23c	28b	43c	97a
	Screen	--	--	--	--	0d	0e	0d	28c
On screen over moist paper	Moist paper	35b	90a	100a	100a	42b	90a	98a	100a
	Screen	--	--	--	--	0d	8d	48c	95ab
On moist paper	Moist paper	52a	90a	100a	100a	55a	97a	100a	100a
	Screen	--	--	--	--	0d	18c	62b	95ab
After-ripening (AR):									
In fruit		--	--	--	--	12n	14n	22n	63n
On screen		--	--	--	--	21mn	49m	73m	98m
On moist paper		--	--	--	--	28m	58m	81m	98m
Germination (G):									
On moist paper		--	--	--	--	40r	72r	80r	99r
On screen		--	--	--	--	0s	9s	37s	73s
Interaction (AR) x (G):		--	--	--	--	*	*	*	*

[z]Mean separation within columns and sets by DMRT at 5% level.

*Interaction significant at the 5% level.

Figure 2. Effects of method and time of after-ripening and of germination medium on germination of apple, cv. 'Paulared', seeds and excised embryos.


(A) 1978: SEED
(B) 1978: EMBRYO
(C) 1979: SEED
(D) 1979: EMBRYO
After-ripening
in fruit
on screen
on moist paper
Germination
paper
screen
GERMINATION %
AFTER-RIPENING AT 5°C (WK)
0
20
40
60
80
100
3
6
9
12

water is limiting than when it is not. Taken as a whole, the data from this experiment indicate that holding seeds in the fruit does indeed affect embryo dormancy, contrary to previous reports (4).

Exp. 3. Separation of effects during after-ripening from effects during germination. Although all main effects (after-ripening treatment, germination medium, and water soak) were significant, interpretation was complicated by the fact that all 4 interactions were also significant (Table 5). Nevertheless, the data reveal a number of facts about the control of seed germination by the fruit.

Seeds after-ripened and/or germinated in the locules germinated poorly in comparison with those held outside the fruits, and no germination occurred in seeds both after-ripened and germinated in the locules (Table 5). Soaking following after-ripening stimulated germination only in seeds not stratified or germinated on moist paper. The promotive effect of soaking seeds could be due either to leaching of a water-soluble inhibitor, to increased water content, or to weakening of the seed coat; the data do not permit unequivocal resolution of this question. However, the better germination of non-soaked seeds which were stratified on screen, then germinated on paper (98%) in comparison with similar seeds stratified on paper, then germinated on screen (78%) suggests that water content is the crucial factor. The few days in contact with moisture in the former case should have had little effect on leaching

Table 5. Effects of after-ripening and germination medium and of soaking in water after 12 weeks of after-ripening on germination (%) of apple seeds, cv. 'Paulared'.

After-ripening treatment	Soaked (24 hrs)	Germination medium[z]		
		Locules	Paper	Screen
In intact fruit	Yes	0 b	42 b	27 c
	No	0 b	27 c	0 d
On moist paper	Yes	40 a	100 a	98 a
	No	38 a	100 a	78 b
On screen over moist paper	Yes	38 a	98 a	98 a
	No	2 b	98 a	32 c
After-ripening (AR):				
In intact fruits		18 g		
On moist paper		76 e		
On screen		61 f		
Germination medium (G):				
Locules		20 t		
Paper		77 r		
Screen		56 s		
Soaked (S):				
Soaked		60 m		
Not soaked		42 n		
Interactions: (AR) x (S)		*		
(AR) x (G)		*		
(G) x (S)		*		
(AR) x (S) x (G)		*		

[z]Mean separation within columns and sets by DMRT at 5% level.

*Interaction significant at the 5% level.

of an inhibitor in comparison with the 12 weeks of exposure in the latter treatment. The "locule effect" was clearly not merely a response to restricted water uptake, for germination in the locule was inhibited in both soaked and non-soaked seeds regardless of method of stratification. This strongly suggests that the locule contains a germination inhibitor.

Exp. 4. Effect of blocking diffusion of locule inhibitors on germination of seeds stratified on moist paper. When fully stratified seeds were allowed to germinate on the endocarp of fruit sections only 38% germinated in comparison with 98% on screen (Table 6). When moist paper was used between the endocarp and the seed, germination increased to 88%, possibly because of a dilution effect. This was significantly less than that observed on moist paper isolated from the endocarp by aluminum foil, indicating a small but definite inhibitory effect of material diffusing from the endocarp.

Exp. 5. Effect of method of after-ripening seeds on ABA content of the testa. Methylated samples of both the free and bound fractions contained a peak which had the same retention time as authentic c,t-ABA. When 'blank' samples (water only) were processed no peak was observed. Exposure of methylated samples of c,t-ABA and of diffusates to ultraviolet light (265 nm) for 16 hours greatly reduced peak heights. The initial concentration of ABA in the

Table 6. Effect of various treatments on germination of 'Jonathan' seed on surface of endocarp of stored fruit

Germination test	Germination (%)[z]
On locules	38 c
On locules + moist paper	88 b
On locules + foil + moist paper	97 a
On moist filter paper in petri dish	100 a
On screen in petri dish	98 a

[z]Mean separation within columns by DMRT at the 5% level

testa was approximately 9 µg/g (9 ppm) of free ABA and 0.8 µg/g (0.8 ppm) bound ABA.

The amount of ABA in the diffusates from the testas of seeds after-ripened in the fruit, on screen, and on moist filter paper declined considerably as after-ripening progressed (Table 7). After 3 weeks of after-ripening free ABA had declined to 20, 46, and 51% of the original level in seeds stratified on moist filter paper, on screen, and in intact fruits, respectively. The ABA level continued to decline thereafter. After 12 weeks the percentage of free ABA recovered was 0.3% for moist paper, 4% in fruit, and 16% on screen. The original content of bound ABA was one-tenth of that of free ABA, but this also declined, except in seeds after-ripened on screen. Only 27% of the seeds held in the fruit germinated on moist filter paper after 12 weeks of after-ripening as opposed to 100% and 98%, respectively, of those after-ripened on moist paper and on screen (Table 7). Thus, germination capacity was not correlated with ABA content of the seed coat diffusates.

The water diffusates obtained from locules, following the removal of all fleshy tissue, contained 2.5 µg of free ABA and 0.4 µg of bound ABA per gram fresh weight of tissue (2.5 and 0.4 ppm, respectively) approximately one-fourth of the concentration of free ABA and one-half of that of the bound ABA found in the seed coat. The free acidic fraction at a concentration equivalent to 0.25 ppm ABA did not inhibit

Table 7. Effects of after-ripening method on the amount of ABA in water diffusates from apple, cv.'Paulared', seed coats and on germination of seeds.

After-ripening (wk)	After-ripening method			Means
	In fruit	On screen	On paper	
	Free ABA (ng/seed coat)[z]			
0	178 a	178 a	178 a	178 r
3	90 b	82 b	35 b	69 s
6	30 c	80 b	18 b	43 s
9	11 c	33 b	10 b	18 s
12	8 c	28 b	0.5 b	15 s
Means....	63 m	81 m	48 m	
	Bound ABA (ng/seed coat)			
0	17 a	17 a	17 a	17 r
3	14 a	10 a	7 ab	10 r
6	20 a	21 a	4 ab	10 r
9	10 b	18 a	3 ab	8 r
12	5 b	17 a	0.2 b	7 r
Means....	11 m	17 m	7 m	
	Germination after 12 weeks (%)			
Means....	27 b	98 a	100 a	

[z]Mean separation within columns and sets by DMRT at 5% level
Means for 2 replicates of 50 seed coats each (ABA content) or 3 replicates of 20 seeds each (germination).

germination of fully stratified seeds, nor did 10 ppm of ABA (data not shown). A concentration of 100 ppm c,t-ABA resulted in 40% inhibition of germination of fully stratified seeds. Therefore, ABA alone cannot account for the inhibitory activity of the endocarp.

Exp. 6. Effects of moisture content, seed coat resistance, and ABA upon germination of seeds after-ripened in the fruit. Response to imbibition varied with seed coat treatment (Table 8). Germination of intact seeds increased with soaking time, whereas that of seeds from which the testa had been partially or wholly removed did not. Significant interaction reflected this difference. Although germination was higher following total removal of the testa than following partial removal, the difference was non-significant, suggesting that mechanical, rather than chemical, characteristics of the testa restricted germination. Inclusion of ABA (10 ppm) in the water used for soaking did not affect germination significantly (overall means for ABA at 0 and 10 ppm were 83 and 78%, respectively) regardless of seed coat removal. Thus leaching of ABA is probably not responsible for the effect of soaking.

Discussion

The data presented in this study confirm the observations of others (1, 14, 19) that seeds after-ripened in the fruit germinate poorly. Limited water content of seeds

Table 8. Effects of soaking time and seed coat removal on germination of seeds, cv. 'Jonathan', after-ripened in the fruit for 12 weeks at 5°C, then germinated on screen.

Soaking (hr)	Germination (%)[z]			Means
	Seed coat treatment			
	Intact	Removed over radicle end	Entirely removed	
0	32 c	83 a	95 a	70 s
24	75 b	90 a	97 a	87 rs
48	78 b	92 a	97 a	89 r
72	95 a	93 a	93 a	95 r
Means	70 n	90 m	97 m	
Interaction (Soaking x Seed Coat Treatment)				*

[z]Within sets means followed by same letter are not significantly different at 5% level by DMRT.

*Interaction significant at 5% level.

during after-ripening does not fully explain the effect of fruits on seed germination as has been suggested (1, 19). The moisture content of seeds after-ripened on screen was only slightly higher than that of seeds after-ripened in the fruit (Table 2), yet the former germinated much more readily than the latter on both screen and moist paper (Table 3). Furthermore, seeds soaked prior to after-ripening in the locules of half-fruits and germinated in situ failed to germinate (Table 1). On the other hand, seeds after-ripened on screen germinated moderately well without exposure to free water and readily when soaked or held on moist paper.

High water content is necessary for good germination of both seeds and embryos (Tables 3, 4). Contact with moist paper during germination appeared to reduce the chilling requirement of excised embryos in comparison with those held on screen. This difference probably indicates that testa removal permits germination before the after-ripening requirement is completely satisfied. When environmental conditions are less than optimum (e.g. water is limiting), germination is delayed. However, the considerably longer periods of after-ripening required by embryos held in the fruit are not easily explained on the basis of differences in water content, and may reflect the presence of higher levels of germination inhibitors in the embryo itself. Embryos germinated on screen required 5 weeks more of stratification to attain 50% germination than

did those germinated on paper. This response approximated that of intact seeds germinated on moist paper, and is probably a better indication of true embryo dormancy than is germination in the presence of free water.

Soaking seeds after-ripened in the fruits for 12 weeks promoted germination appreciably (Tables 5, 8). This partially supports Bartlett's (1) proposal that poor germination following after-ripening in the fruit resulted from incomplete imbibition. However, inadequate moisture cannot fully account for the inhibitory effect of the fruit. This is further substantiated by the observation that both soaking in water before or after stratification and exposure to moist filter paper stimulated germination except in seeds stratified and germinated in the locule (Table 1).

An alternative explanation for these effects is that germination inhibitors are leached from the seeds during soaking or contact with moist paper. However, the data in Tables 3 and 5 suggest that this is not the case. Seeds after-ripened on screen, then germinated on paper germinated significantly better than did those after-ripened on paper, then germinated on screen. A few days of contact with moist paper during germination in the former case were more effective than 12 weeks of contact during after-ripening in the latter. This suggests that water content, rather than inhibiting substances in the testa, are the crucial factors limiting germination.

The hypothesis that chemicals in the locules exert an inhibiting effect on germination of seeds after-ripened in the fruit is substantiated by the fact that germination of seeds after-ripened outside the fruit is inhibited by contact with the endocarp (Tables 5, 6). This inhibitory effect was not alleviated completely even when seeds were soaked in water for 24 hours. Furthermore, the germination of fully stratified seeds was reduced somewhat when germinated on a piece of moist filter paper in contact with the endocarp (Table 6). Therefore, the locules must contain a diffusible inhibitor which is responsible for the poor germinability of seeds after-ripened in the fruit.

Abscisic acid (ABA) does not appear to be responsible for inhibiting seed germination in the locules, as the concentration (2.5 μg/g fresh wt. tissue) found was much lower than the concentration of 20 to 40 ppm ABA needed in the stratification medium to inhibit seed germination (16). A concentration of 100 ppm c,t-ABA resulted in only 40% inhibition of germination of fully stratified seeds in my study. Therefore, other compounds are probably responsible for inhibition of seed germination by the endocarp.

Diffusates from the testas of apple seeds contained ABA as reported in previous studies (2, 15). Luckwill (9) found that levels of inhibiting substances in the testas of seeds stored dry at room temperature did not change with time, whereas levels declined in seeds kept in a moist

medium at 4°C. He suggested that moisture in the stratification medium was necessary to leach out the inhibitor. Results of this study (Table 7), on the contrary, show that ABA content of the testa diminished whether seeds were exposed to free water or not. Nevertheless, the most rapid loss of ABA from the testa occurred in seeds after-ripened on moist paper, indicating that leaching may have contributed to some extent to the decline. The depletion of ABA from the testas of seeds after-ripened in the absence of free water suggests that it was metabolized. However, bound ABA, a known metabolite, declined concomitantly with free ABA.

Rudnicki (15) correlated the decline in ABA in seeds during low temperature stratification with an increase in their germinability. No direct relationship between ABA content and germination was observed in this study (Table 7), confirming the work of Balboa-Zavala and Dennis (2). Although the level of ABA fell as rapidly in seed held in the fruit as in those held on screen or moist paper, germination was not correlated with ABA content. These observations suggest that germination of seeds after-ripened in the fruit is not inhibited by the ABA present in the seed coat.

Soaking of seeds following after-ripening in the fruit for 12 weeks improves germination only if the testa is intact (Table 8). Removal of the testa or a portion thereof eliminates the response. This suggest that the testa serves

as a mechanical barrier to germination, rather than as a source of germination inhibitor(s).

Literature Cited

1. Bartlett, C.E.C. 1961. The after-ripening of apple seeds in the fruit during cold storage. Annu. Rept. Long Ashton Agr. Hort. Res. Sta., Bristol. 1961: 66-67.

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

3. Bonamy, P. A., and F. G. Dennis, Jr. 1977. Abscisic acid levels in seeds of peach. II. Effects of stratification temperature. J. Amer. Soc. Hort. Sci. 102: 26-28.

4. Evenari, M. 1949. Germination inhibitors. Bot. Rev. 15: 153-194.

5. Flemion, F. 1934. Dwarf seedlings from non-after-ripened embryos of peach, apple, and hawthorn. Contr. Boyce Thompson Inst. 6: 205-210.

6. Kaminski, W. 1968. Inhibitory effect of apple juice on the germination of apple and cherry seeds and the growth of apple seedlings. Acta. Soc. Bot. Polon. 37: 173-178.

7. Lin, C. F., and A. A. Boe 1972. Effects of some endogenous and exogenous growth regulators on plum seed dormancy. J. Amer. Soc. Hort. Sci. 97: 41-44.

8. Lipe, W., and J. C. Crane. 1966. Dormancy regulation in peach seeds. Science 153: 541-542.

9. 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.

10. Milborrow, B. V. 1967. The identification of (+)-Abscisin II, (+)-Dormin, in plants and measurement of its concentrations. Planta 76: 93-113.

11. _____, and G. Vaughan, 1979. Long term metabolism of (+)-(2-14C) abscisic acid by apple seeds. J. Expt. Bot. 30: 983-995.

12. Pieniazek, J., and R. Rudnicki. 1967. The presence of abscisin II in apple leaves and apple fruit juice. Bull. Acad. Polon. Sci. 25: 251-254.

13. _____, and M. J. Grochowska. 1967. The role of the natural growth inhibitor (abscisin II) in apple seed germination and the changes in the content of phenolic substances during stratification. Acta. Soc. Bot. Polon. 36:579-587.

14. _____, and R. Rudnicki. 1970. The inhibitory effect of endogenous abscisic acid (ABA) on after-ripening of apple seeds in the fruit. Bull. Acad. Polon. Sci. 27: 707-711.

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

16. _____, and J. Pieniazek. 1973. The effect of abscisic acid on stratification of apple seeds. Bull. Acad. Polon. Sci. 21: 149-154.

17. _____, and _____. 1973. Apple fruit volatiles and inhibitors of apple seed germination. Bull. Acad. Polon. Sci. 21: 827-829.

18. _____, and J. Czapski. 1974. The uptake and degradation of 1-^{14}C-abscisic acid by apple seeds during stratification. Ann. Bot. 38: 189-192.

19. Visser, T. 1959. After-ripening and germination of apple seeds in relation to the seed coats. Proc. Kon. Ned. Wetensch. C57: 175-185.

20. Wareing, P. F., and P. F. Saunders. 1971. Hormones and dormancy. Annu. Rev. Plant Physiol. 22: 261-288.

SECTION II

FRUIT INDUCED DORMANCY IN APPLE (*MALUS DOMESTICA* BORKH.) SEED. ROLE OF VOLATILES

FRUIT INDUCED DORMANCY OF APPLE (MALUS DOMESTICA BORKH.) SEED
II. ROLE OF VOLATILES

Abstract. The role of fruit volatiles, chiefly ethylene, in preventing apple (Malus domestica Borkh.) seed germination within the fruit was investigated. After-ripening and germinating seeds in the presence of fruits did not delay after-ripening appreciably, whereas germination was markedly reduced when seeds were stratified in the fruits. Removal of the seed coat markedly promoted seed germination in the presence or absence of fruits. Germinability of seeds after-ripened in fruits was equally inhibited in regular, controlled atmosphere, and low pressure storage at 0°C, although three-to five-fold differences in internal ethylene concentration were observed. No correlation could be established between the rate of ethylene biosynthesis by seeds or excised embryos and their ability to germinate. Addition of ethephon (2-chloroethylphosphonic acid) to the stratification medium promoted ethylene production by seeds, but had no consistent effect on germination. The presumed inhibitors of ethylene action or synthesis 8-hydroxyquinoline sulfate, aminoethoxyvinyl glycine, and silver nitrate reduced ethylene production but did not inhibit germination at non-toxic concentrations. It is concluded that fruit induced dormancy of apple seeds is not controlled by an ethylene-induced response, nor is ethylene essential for breaking rest in apple embryos.

After-ripening apple (Malus domestica Borkh.) seeds in fruits inhibits their subsequent germination on removal from the fruit (1, 9). Ethylene is produced by apple fruits during low temperature storage and constitutes 70 to 80% of the total volatile substances emanated (7). The volatiles produced by the fruits (3, 10) or flesh (11) inhibit the germination of fully after-ripened seeds (10, 11) and embryos (8). Kepczynski and Rudnicki (5, 6) concluded that ethylene was responsible for the inhibition of germination of seeds after-ripened within apple fruits, although exogenous ethylene did not affect after-ripening. Paillard (8) showed that apple fruit emanations free of volatiles other than ethylene did not inhibit embryo germination. Kepczynski et al. (7) observed that the production of ethylene by excised embryos paralleled their germination capacity during after-ripening. Inhibition of ethylene production by 8-hydroxyquinoline sulfate and aminoethoxyvinyl glycine was accompanied by an inhibition of embryo germination. However, Halinska, et al. (4) observed that addition of ethephon to the stratification medium inhibited apple seed germination up to the 70th day, after which germination was stimulated. Similarly, Sinha, et al. (12) reported that ethephon (250 ppm) was not effective in stimulating germination until the seeds had been stratified for 60 days. The present study was undertaken to determine the role of fruit volatiles, chiefly ethylene, in preventing the germination of seeds after-ripened in the fruit.

Materials and Methods

Exp. 1. Effect of fruit tissue volatiles on seed and embryo germination. This experiment was designed to test whether after-ripening is delayed when apple seeds are stratified in the presence of fruit-produced volatiles. Apple fruits (cv. 'Paulared') were collected from a research orchard at East Lansing and a commercial orchard near Grand Rapids, in 1978 and 1979, respectively. Seeds were extracted from the fruits soon after harvest and placed in open petri dishes lined with moist filter paper. The seeds were then stratified at $5^{o} \pm 1^{o}C$ by placing them in loosely covered plastic containers with and without fruit tissue. Three samples of 20 seeds each were removed at intervals of 3 weeks for germination tests. Germination of both intact seeds and excised embryos on moist filter paper was recorded after 10 days at $20^{o} \pm 1^{o}C$ in the presence or absence of fruit tissues.

Exp. 2. Effect of gas mixture and pressure during low temperature storage on seed and embryo germination. If ethylene is involved in after-ripening, germination should be affected by low pressure and/or controlled atmosphere storage, which reduce ethylene levels in the fruits. Apple fruits (cv. 'McIntosh') were harvested on September 19, 1978, from a research orchard at East Lansing. Seeds were after-ripened either in the fruits or on moist sand in petri

dishes at 0°C under: (a) regular storage in ambient atmosphere, (b) controlled atmosphere in 3% O_2, 1% CO_2, 96% N_2 and (c) low pressure at 50 mm atmosphere. Samples were removed from storage after 2 and 5 months, and both intact seeds and excised embryos were germinated on moist filter paper at 20° ± 1°C for 10 days at ambient atmosphere.

The internal ethylene concentration of the fruits was determined prior to storage, and after 2 and 5 months of storage, using the method described by Burg and Burg (4). Gas samples were drawn from the fruits immediately after removal from the storage chambers. The gas samples were analyzed from 20 fruits per storage treatment, using a Varian Aerograph series 1700 gas chromatograph equipped with a flame ionization detector and a column (45 x 0.32 cm) of 60 to 80 mesh aluminum oxide operated at 60° or 80°C.

Exp. 3. Effect of treating seeds and embryos with ethephon and ethylene biosynthesis inhibitors on ethylene production and germination. If ethylene is required to break the rest of seeds, the addition to the stratification medium of chemical compounds which alter ethylene biosynthesis should affect germination. Seeds were removed in the spring of 1979 from 'Paulared' fruits which had been stored at 5°C ± 1°C for 16 weeks. Secondary dormancy was induced by holding the seeds at 32° ± 2°C for 3 weeks under moist conditions. At the end of this period the embryos were excised and test germinated to ensure they were in a state of rest. Seeds were then soaked for 24 hours

in: (a) distilled water (control), (b) 100 ppm ethephon, (c) 400 ppm aminoethoxyvinyl glycine, (d) 200 ppm 8-hydroxyquinoline sulfate, and (e) 50 ppm silver nitrate. The concentrations of the chemicals used were determined to be non-toxic in a preliminary study.

The experiment was repeated in the fall of 1979 with fresh seeds of the same cultivar obtained from a commercial orchard at Grand Rapids. Germination tests were conducted on filter paper moistened with distilled water rather than with the chemical solutions used previously.

Ethylene production was measured by placing 30 seeds or embryos in a 25 ml flask lined with filter paper. The paper was moistened with distilled water or the appropriate test solutions. The flasks were closed with rubber serum caps and incubated in the dark at $20^{\circ} \pm 1^{\circ}C$ for 24 hours. Five gas samples were drawn from each flask with a 1 cc syringe and ethylene content was determined by gas chromatography. One cc of ambient air was put back into the flask after each time a sample of gas was drawn. Gas samples were also taken from a flask lined with filter paper moistened with distilled water without seeds and used to correct for ambient levels of ethylene. All values were converted to $nl.g^{-1}$ of seeds hr^{-1}. Two replications of 30 seeds each were used per treatment.

Germination tests. Unless otherwise stated, germination tests were carried out with 3 replicates of 20 seeds or

excised embryos in 9-cm petri dishes lined with two layers of Whatman no. 1 filter paper which was moistened with distilled water. The petri dishes were randomly placed in a growth chamber at 20° ± 1°C in darkness. Seeds with visible radicle protrusion, and embryos whose radicles showed geotropic curvature were considered to be germinated. Germination was recorded for a period of 10 days, and the results expressed as mean percentages.

Statistical analysis. The data from the study were subjected to analysis of variance, and the mean separation procedure of Duncan's Multiple Range Test was used where appropriate.

Results

Exp. 1. Effect of fruit tissue volatiles on seed and embryo germination. Intact seeds began to acquire some ability to germinate after 6 weeks of stratification (Table 1, Fig. 1). At this time little or no inhibitory effect of fruit tissue volatiles on seed germination was evident, germination in the presence and absence of fruit tissues being 28% and 37%, respectively. The presence of fruit tissues significantly reduced germination of seeds stratified for 9 weeks, but no difference was evident after 12 weeks.

Embryo excision markedly stimulated germination after 3, 6, and 9 weeks of stratification (Table 1, Fig. 1).

Table 1. Effects of volatiles from apple, cv. 'Paulared', fruit tissues on germination of seed and excised embryos.

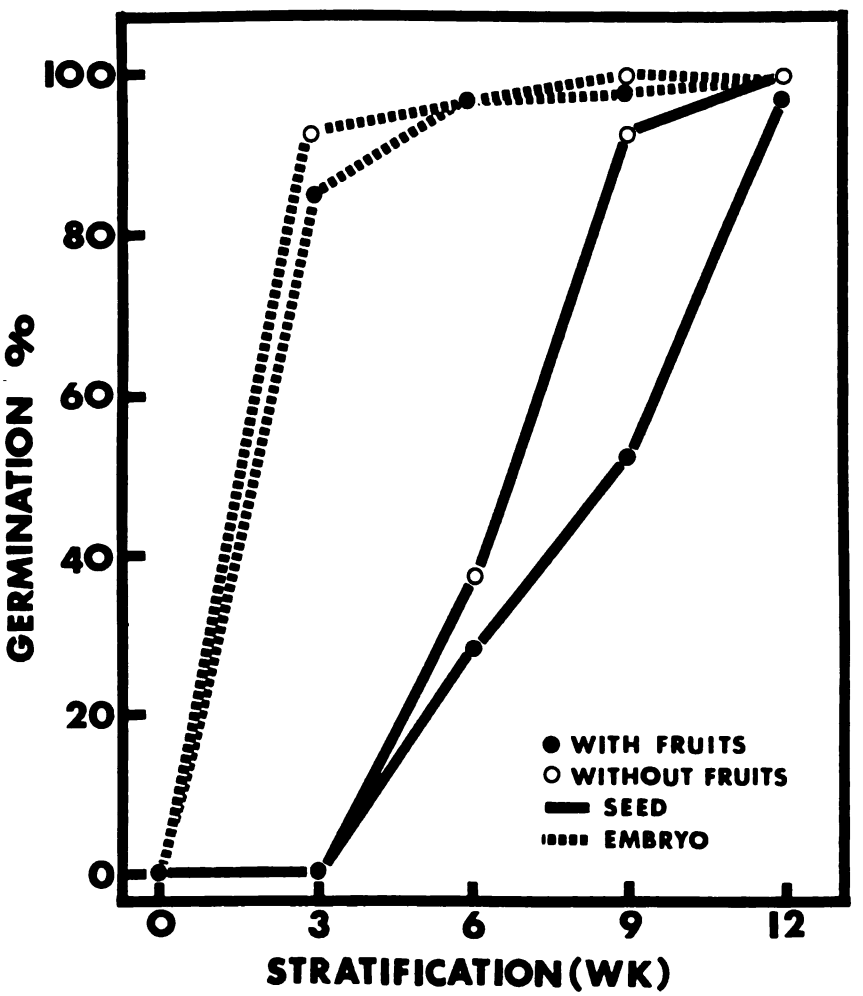
Stratification[y] and germination	Seed coat	Germination (%)[z] Stratification (wk) 3	6	9	12
Presence of fruit tissue	Intact	0 c	28 b	52 b	97 a
	Excised	85 b	97 a	98 a	100 a
Absence of fruit tissue	Intact	0 c	37 b	93 a	100 a
	Excised	93 a	97 a	100 a	100 a
Fruit tissue (F):					
Presence		43 m	63 m	75 n	99 m
Absence		47 m	67 m	97 m	100 m
Seed coat (C):					
Intact		0 s	33 s	73 s	99 r
Excised		89 r	97 r	99 r	100 r
Interaction (F) x (C):		*	n.s.	*	n.s.

[y]Seeds stratified in petri dishes at 5°C, seeds and embryos germinated at 20°C for 10 days.

[z]Within columns and sets means followed by same letter are not significantly different at the 5% level (DMRT).

*Interaction significant at the 5% level.

Figure 1. Effects of stratification time at 0°C with or without fruits on subsequent germination of intact apple seeds and excised embryos, cv. 'Paulared'.


100
80
60
40
20
0
GERMINATION %
0
3
6
9
12
STRATIFICATION (WK)
WITH FRUITS
WITHOUT FRUITS
SEED
EMBRYO

Germination was reduced 8% by the presence of fruit tissues after 3 weeks of stratification, but not thereafter. Significant interactions were evident between fruit tissue effects and seed coat effects at 3 and 9 weeks, for the former were evident only in embryos after 3 weeks of stratification, only in intact seeds after 9 weeks.

Exp. 2. Effect of gas mixture and pressure during low temperature storage on seed and embryo germination. Internal ethylene concentrations differed in fruits stored under the different conditions (Table 2). Low pressure storage was most effective in suppressing ethylene evolution, and controlled atmosphere intermediate.

All excised embryos germinated, regardless of treatment or sampling time. Therefore only data for intact seeds are presented (Table 3, Fig. 2). Seeds after-ripened in the fruits germinated poorly regardless of method of storage. Higher germination was associated with a higher internal ethylene concentration in the fruits (Table 2). However, there was no correlation when fruits were held for 5 months (Table 3). The percentage germination of seeds removed from fruits held in low pressure storage (internal ethylene concn 23 ppm) was almost twice that for seeds from fruits held in controlled atmosphere storage (ethylene concn 54 ppm). Germination of seeds from fruits held in regular storage was intermediate. Regardless of method of storage, the percent germination rose in response to an additional

Table 2. Internal ethylene concn (ppm) in 'McIntosh' fruits stored at 0°C under different storage atmospheres.

Storage condition	Ethylene concn (ppm)[z]		
	Storage (mo)		
	0	2	5
Regular	0.10 a	121 a	72 a
Controlled atmosphere	0.10 a	81 b	54 a
Low pressure	0.10 a	28 c	23 b

[z]Mean separation within columns by DMRT at 5% level.

Table 3. Effect of different low temperature (0°C) storage treatments and method of stratification on the germination of seeds and excised embryos after-ripened in 'McIntosh' fruits[z].

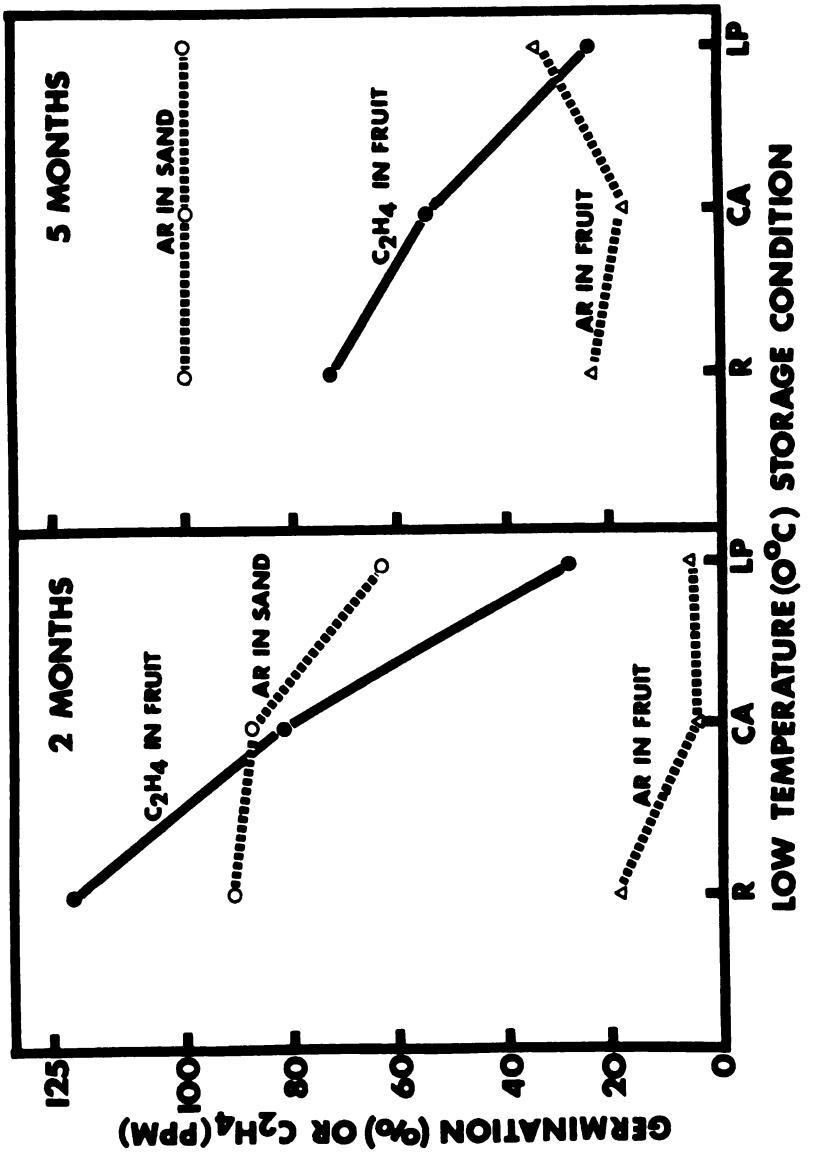
Storage condition	Seeds stratified in:	Germination (%)[y]	
		Storage (mo)	
		2	5
Regular	Fruit	18 c	23 bc
	Moist sand	90 a	100 a
Controlled atmosphere	Fruit	3 c	17 c
	Moist sand	87 ab	100 a
Low pressure	Fruit	5 c	33 b
	Moist sand	63 b	100 a
Storage condition (A):			
Regular		54 f	62 f
Controlled atm.		45 fg	59 f
Low pressure		34 g	67 f
Seeds stratified in (B):			
In fruit		9 s	24 s
On moist sand		80 r	100 r
Interaction (A) x (B):		*	n.s.

[y]Within columns and sets means followed by the same letter are not significantly different at the 5% level (DMRT).

*Interaction significant at the 5% level.

[z]All seeds were germinated on moist paper in petri dishes at 20°C.

Figure 2. Effects of method of low temperature storage (R=regular; CA=controlled atm; LP=low pressure) on ethylene content (●——●) of fruits and on germination of seeds on moist paper following after-ripening on sand (O·····O) or in fruits (Δ·····Δ).


2 MONTHS
5 MONTHS
C_2H_4 IN FRUIT
AR IN SAND
AR IN FRUIT
C_2H_4 IN FRUIT
AR IN SAND
AR IN FRUIT
0
20
40
60
80
100
125
R
CA
LP
R
CA
LP
GERMINATION (%) OR C_2H_4 (PPM)
LOW TEMPERATURE (0°C) STORAGE CONDITION

3 months of storage although the increase was small for regular storage.

The germination of seeds stratified in moist sand in the same storage chambers as the fruits was much greater than that of seeds after-ripened in the fruits regardless of method of storage or length of stratification period (Table 3, Fig. 2). Low pressure storage reduced germination after 2 months in sand, but all seeds germinated after 5 months regardless of storage method. Poor germination after 2 months was probably due to drying of the stratification medium, however, rather than to storage method *per se*. Germination of neither seeds held in the fruits nor seeds stratified in sand appeared to be correlated with ethylene content of the fruits.

Exp. 3. Effect of treating seeds and embryos with ethephon and inhibitors of ethylene synthesis on ethylene production and germination. Ethylene evolution from control seeds declined with time in the first experiment, but that from embryos remained relatively constant for the first 9 weeks, then rose 8-fold at 12 weeks (Table 4). Treatment with 100 ppm ethephon greatly stimulated ethylene evolution in both seeds and embryos. In seeds, 1000 ppm ethephon was less promotive than was 100 ppm, possibly because of supra-optimal concentration. All other treatments reduced ethylene evolution in both seeds and embryos, with greater percentage reduction in the former. None of

Table 4. Effects of treatment with ethephon and inhibitors of ethylene biosynthesis during moist stratification on ethylene production of 'Paulared' apple seeds and excised embryos during 24 hours period of incubation at 20°C. 1978[z].

Treatment	Concn (ppm)	C_2H_4 production ($nl.g^{-1}.hr^{-1}$) after stratification (wk)[y]							
		4	6	9	12	4	6	9	12
		Intact seeds				Excised embryos			
Water	-	2.84c	1.38c	0.66c	0.05c	0.08c	0.10b	0.10b	0.80b
Ethephon	100	33.29a	43.12a	47.58a	24.88a	49.92a	69.01a	49.86a	53.02a
	1000	6.86b	18.78b	38.93b	12.01b	-	-	-	-
8-HQS[x]	100	0.32c	0.28a	0.24c	0.04c	-	-	-	-
	200	0.20c	0.20c	0.10c	0.04c	0.02b	0.04b	0.03b	0.04b
AVG[w]	400	-	-	-	-	0.02b	0.01b	0.01b	0.01b
$AgNO_3$	50	0.02c	0.18c	0.12c	0.04c	0.06b	0.07b	0.05b	0.04b
	100	0.02c	0.08c	0.10c	0.04c	0.06b	0.07b	0.05b	0.04b

[z]Secondary dormancy was induced in seeds removed from fruits (1978 crop) stored at 5°C for 4 months prior to chemical treatment and stratification.

[y]Mean separation within columns by DMRT at 5% level.

[x]8-hydroxyquinoline sulfate.

[w]Aminoethoxyvinyl glycine.

these reductions was statistically significant. However, the reduction was found to be statistically significant when the ethephon treatment was omitted (data not shown).

None of the chemicals stimulated germination significantly. However, 8-HQS inhibited it in seeds, but not in excised embryos (Table 5). The higher concentration inhibited germination after 6 and 9 weeks of stratification but not after 12 weeks.

Only seeds were used in the second experiment. Ethylene production in control seeds was lower than in the first experiment, and the decline was rapid, rather than being gradual (Table 6). Ethephon again promoted ethylene evolution, although production was considerably lower than before (1978). All other chemicals again inhibited evolution of ethylene, but differences were non-significant at 5%. The reduction in ethylene production, however, was significantly different from control when the ethephon was omitted in the analysis (data not shown).

In this experiment, ethephon inhibited germination at one or both concentrations after 6 and/or 9 weeks, the higher concentration being less effective then the lower. This differed from the response in the first experiment, in which ethephon did not affect germination even at 1000 ppm. Response to 8-HQS and $AgNO_3$ also differed, with no significant inhibition being observed with the former, but the latter being inhibitory at both concentrations after

Table 5. Effects of treatment with ethephon and inhibitors of ethylene biosynthesis during moist stratification on germination of seeds and excised embryos. 1978[z].

Treatment	Concn (ppm)	Germination (%) weeks after stratification[y]				
		4	6	9	12	4
		Intact seeds				Excised embryos[x]
Water	-	15 a	47 a	93 ab	100 a	95 a
Ethephon	100	13 ab	42 ab	90 ab	100 a	93 a
	1000	18 a	53 a	97 a	100 a	—
8-HQS[w]	100	17 a	37 ab	85 b	100 a	—
	200	5 b	23 b	58 c	98 a	87 a
AVG[v]	400	—	—	—	—	90 a
$AgNO_3$	50	13 ab	48 a	98 a	100 a	100 a
	100	22 a	45 ab	98 a	100 a	—

[z]Secondary dormancy was induced in seeds removed from fruits (1978 crop) stored at 5°C for 4 months prior to chemical treatment and stratification.

[y]Mean separation within columns by DMRT at the 5% level.

[x]All embryos from seeds stratified for 6 to 12 weeks germinated, regardless of treatment.

[w]8-hydroxyquinoline sulfate

[v]Aminoethoxyvinyl glycine.

Table 6. Effects of treatment with ethephon and inhibitors of ethylene biosynthesis during moist stratification on ethylene production and germination of 'Paulared' apple seeds. 1979[z].

Treatment	Concn (ppm)	Stratification (wk)								
		0	4	6	9	12	4	6	9	12
		C_2H_4 production ($nl.g^{-1}.hr^{-1}$)[y]					Germination (%)[z]			
Water	-	3.42	0.07c	0.17c	0.07c	1.26c	5 a	20 ab	87 ab	98 a
Ethephon	250	-	3.97b	2.95b	14.60b	15.28a	0 a	0 d	17 c	100 a
	500	-	4.66a	3.75a	28.14a	7.14b	0 a	7 cd	88 ab	100 a
8-HQS[x]	100	-	0.02d	0.09b	0.02c	0.09c	3 a	18 ab	93 a	98 a
	200	-	0.02d	0.09b	0.02c	0.06c	2 a	10 bc	67 b	98 a
$AgNO_3$	50	-	0.04cd	0.09b	0.04c	0.08c	0 a	5 d	83 ab	100 a
	100	-	0.04cd	0.09b	0.03c	0.08c	2 a	3 d	78 ab	100 a
AVG[w]	200	-	0.02d	0.13b	0.02c	0.10c	5 a	15 abc	97 a	100 a
	400	-	0.02d	0.13b	0.02c	0.10c	0 a	5 d	90 a	100 a

[z]Seeds were removed from fruits shortly after harvest in 1979.

[y]Mean separation within columns by DMRT at 5% level.

[x]8-hydroxyquinoline sulfate.

[w]Aminoethoxyvinyl glycine.

6 weeks of stratification, but not thereafter. AVG at 400 ppm also inhibited germination at 6 weeks, but not at 9 or 12 weeks.

Correlation coefficients revealed no consistent relationship between ethylene evolution and germination in either seeds or excised embryos. Values ranged from -0.50 (seeds, 4 wk) to +0.32 (seeds, 12 wk).

Discussion

Apple seeds removed from fruits held at low temperature germinated poorly in comparison with those stratified in moist sand under the same conditions (Table 3), yet embryos excised from the former germinated readily on moist paper. Several methods were tested to determine if ethylene or other volatiles were responsible for inhibiting germination.

If volatiles inhibited germination, seeds stratified in the presence of fruits should germinate poorly in comparison with those removed from the fruits. The data in Table 1 indicate little, if any, effect of the presence of the fruits on germination.

If ethylene itself limits germination of seeds held in the fruit, treatments which stimulate or inhibit ethylene synthesis in fruits and/or seeds should inhibit germination. Storage methods which inhibited ethylene synthesis of fruits did not promote germination, and sometimes inhibited it (Tables 2, 3). Although ethephon

greatly stimulated the rate of evolution of ethylene in seeds and embryos (Tables 4, 6), it had no consistent effect on their germination. Similarly, chemicals which inhibited ethylene evolution, and therefore should have stimulated germination, did not hasten germination, and in fact reduced it in some cases in partially stratified seeds (Tables 4, 5, 6).

These facts, taken together, do not support the hypothesis that ethylene or other volatiles are controlling factors in fruit-induced dormancy of apple seeds.

Paillard (8) inhibited the germination of apple embryos by circulating emanations from 30 to 35 climacteric apple fruits held in a closed container at 20°C through the germination chamber. Passing the air through activated charcoal, which presumably adsorbed volatiles other than ethylene, prevented the inhibition. Paillard (8) probably used excessive numbers of fruits in a closed system, resulting in much higher concentrations of volatiles than would be found with a single fruit under conditions of low temperature storage.

Literature Cited

1. Bartlett, C. E. C. 1961. The after-ripening of apple seeds in the fruit during cold storage. Annu. Rept. Long Ashton Agr. Hort. Res. Sta., Bristol. 1961: 66-67.

2. Burg, S. P. and E. A. Burg. 1962. Role of ethylene in fruit ripening. Plant Physiol. 37: 179-189.

3. Fidler, J. C. 1955. Volatile organic products of metabolism of fruits, J. Sci. Food Agr. 6: 293-295.

4. Halinska, A., B. Zarska-Maciejewska, and S. Lewak. 1975. Inhibitors as factors causing the uniform germination of apple seeds. Fruit Sci. Rept. Skierniewice, Poland 2:23-49.

5. Kepczynski, J. and R. Rudnicki. 1975. Studies on ethylene in dormancy of seeds. I. Effects of exogenous ethylene on the after-ripening and germination of apple seeds. Fruit Sci. Rept. Skierniewice, Poland 2:25-41.

6. _____ and _____. 1976. Studies on ethylene in dormancy of seeds. II. The effect of endogenous ethylene on after-ripening of apple seeds in fruit stored at low temperature. Fruit Sci. Rept. Skierniewice, Poland 3:27-33.

7. _____, _____, and A. A. Khan. 1977. Ethylene requirement for germination of partly after-ripened apple embryos. Physiol. Plantarum 90: 293-295.

8. Paillard, N. 1974. Influence des produits volatils emis par les pommes sur la germination des embryons de pommier. Physiol. Veg. 12: 739-749.

9. Pieniazek, J. and R. Rudnicki. 1967. The inhibitory effect of endogenous abscisic acid (ABA) on after-ripening of apple seeds in the fruit. Bull. Acad. Polon. Sci. 27: 707-711.

10. Rudnicki, R. and J. Pieniazek. 1973. Apple fruit volatiles as inhibitors of apple seed germination. Bull. Acad. Polon. Sci. 23: 548-553.

11. _____, and _____. 1975. The effect of apple flesh on dormancy release and germination of seeds. Bull. Acad. Polon. Sci. 23: 548-553.

12. Sinha, M. M., R. S. Pal, and D. N. Aswathi. 1977. Effect of stratification and plant growth regulating substances on seed germination and seedling growth in apples. Progressive Hort. 9: 27-30.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Apple (Malus domestica Borkh.) seeds removed from fruits held at low temperature (5°C) germinated poorly on moist paper or on screen unless the embryos were excised. Limited moisture content of seeds does not explain all of the inhibitory effect of the fruit, for the moisture content of seeds stratified on screen was only slightly higher than that of seeds after-ripened in the fruits, yet the former germinated much more readily than the latter on both screen and moist paper. Furthermore, seeds after-ripened and germinated in locules failed to germinate regardless of water content. Similarly, germination of seeds which were after-ripened outside the fruits (moist paper) was inhibited by placing them on the surface of locules (endocarp). These results indicate that a chemical(s) diffusing from the endocarp prevents germination.

Water content was more critical during germination then during after-ripening for both seeds and embryos. This is not due to leaching out of the inhibitors, as seeds stratified on screen, then germinated on paper, germinated as well as those stratified on moist paper, then germinated on screen, in spite of the former having been subjected to only a short period (several days) of leaching. Imbibing seeds in water prior to stratification increased germination. However, imbibition improved germination only when the testa was left intact. Removal of the testa or a

portion thereof eliminated the promotive effect of soaking in water. The results suggest that seed moisture content is only one of the several factors accounting for the effect of soaking. The testa probably serves as a physical barrier to germination, rather than as a source of germination inhibitor(s).

Both free and bound ABA were found in diffusates from the seed coat and locule. The ABA content of the testa from seeds after-ripened in the fruits declined with time, but seed germination was still inhibited. Also, the amount of ABA contained in the diffusates from the endocarp was insufficient to account for the inhibition of germination. Therefore, the inhibitory effect of the fruit cannot be attributed entirely to ABA.

Seed germination capacity was not appreciably reduced by stratification in the presence of fruits or fruit tissues. No correlation could be established between ethylene evolution and germination in either seeds or excised embryos. Stratifying seeds in the presence of chemical compounds which either promoted or inhibited ethylene biosynthesis did not affect germination provided the chemicals were used at non-toxic levels. Thus fruit-produced volatiles do not appear to play a major role in preventing germination in the fruit.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Abbott, D. L. 1956. Temperature and the dormancy of apple seeds. Rept. XVI Intern. Hort. Congr., Netherlands. Sec. 24: 746-753.

2. Amen, R. D. 1968. A model of seed dormancy. Bot. Rev. 34: 1-34.

3. Badizadegan, M. and R. F. Carlson. 1967. Effects of N^6benzyladenine on seed germination and seedling growth of apple (Malus sylvestris Mill.). Proc. Amer. Soc. Hort. Sci. 91: 1-8.

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

5. Bartlett, C. E. C. 1961. The after-ripening of apple seeds in the fruit during cold storage. Annu. Rept. Long Ashton Agr. Hort. Res. Sta., Bristol. 1961: 66-67.

6. Barton, L. V. 1956. Growth response of physiological dwarfs of Malus arnoldiana Sarg. Contr. Boyce Thompson Inst. 18: 308-311.

7. Bewley, J. D. and M. Black. 1978. Biochemistry of seeds in relation to germination. Springer-Verlag, Berlin, New York. 306 pp.

8. Black, M. 1969. Light-controlled germination of seeds. p. 193-217 In: Dormancy and survival. Academic Press Inc., New York.

9. Borkowska, B. and R. Rudnicki. 1974. Changes in the cytokinin level in stratified apple seeds. Proc. XIX Intern. Hort. Congr., Warsaw. Vol. 1B: p. 496.

10. Borner, H. 1959. The apple replant problem. I. The excretion of phloridzin from apple root residue. Contr. Boyce Thompson Inst. 20: 39-56.

11. Come, D. 1965. Influence de la temperature sur les taux et la vitesse de germination des graines de Pommier (Pirus malus L.). Comptes Rendus Acad. Sci. Paris 260: 1725-1728.

12. _____. 1965. Influence de la temperature sur la germination des graines de pommier ayant subi leur post-maturation au sein des fruits. Bull. Soc. France Physiol. Vegetale 11: 73-80.

13. _____. 1967. L'inhibition de germination des graines de pommier (Pirus malus L.) non dormentes. Role possible des phenols tegumentaires. Comptes Rendus Acad. Agr. France 53: 1359-1362.

14. Crocker, W. 1916. Mechanisms of dormancy in seeds. Amer. J. Bot. 3: 99-120.

15. Dennis, F. G. Jr., G. C. Martin, P. Gaskin, and J. MacMillan. 1980. Gibberellins in mature apple seeds: Contaminants? Planta 147: 376-377.

16. Dziewanowska, K., M. J. Grochowska, and S. Lewak. 1974. Changes in phloridzin and chlorogenic acid content and in indolylacetic acid oxidase activity during development of apple seeds. Fruit. Sci. Rept. Skierniewice, Poland 1: 3-9.

17. Fidler, J. C., and C. J. North. 1969. Production of volatile organic compounds by apples. J. Sci. Food Agr. 20: 521-526.

18. Flemion, F. 1934. Dwarf seedlings from non-after-ripened embryos of peach, apple and hawthorn. Contr. Boyce Thompson Inst. 6: 205-210.

19. Frankland, B. 1961. Effects of gibberellic acid, kinetin, and other substances on seed dormancy. Nature 192: 678-679.

20. Halinska, A., B. Zarska-Maciejewska, and S. Lewak. 1975. Inhibitors as factors causing the uniform germination of apple seeds. Fruit Sci. Rept. 2: 23-29.

21. _____, and S. Lewak. 1978. The presence of bound gibberellins in apple seeds. Bull. Acad. Polon. Sci. 26: 119-122.

22. Harrington, G. T., and B. C. Hite. 1923. After-ripening and germination of apple seeds. J. Agr. Res. 23: 153-161.

23. Haut, I. C. 1932. The influence of drying on after-ripening and germination of fruit tree seeds. Proc. Amer. Soc. Hort. Sci. 29: 371-374.

24. Hutchinson, A., C. D. Taper, and G. H. P. Towers, 1959. Studies of phloridzin in Malus. Can. J. Biochem. Physiol. 37: 901-910.

25. Isaia, A., and C. Bulard. 1978. Relative levels of some bound and free gibberellins in dormant and after-ripened embryos of Pyrus malus cv.'Golden Delicious'. Z. Pflanzenphysiol. 90: 409-414.

26. Kaminski, W. 1968. Inhibitory effect of apple juice on the germination of apple and cherry seeds and the growth of apple seedlings. Acta Soc. Bot. Polon. 37: 173-177.

27. _____, and J. Pieniazek. 1968. The effect of different growth regulators on the germination of apple cultivar 'Antonovka' dehusked seeds. Bull. Acad. Polon. Sci. 16: 719-723.

28. Kawase, M. 1958. The after-ripening of dormant apple seeds in relation to auxin content. J. Hort. Assoc. Japan. 27: 256-264.

29. Kepczynski, J., and R. Rudnicki. 1975. Studies on ethylene in dormancy of seeds. I. Effects of exogenous ethylene on the after-ripening and germination of apple seeds. Fruit Sci. Rpt. Skierniewice Poland 2: 25-41.

30. _____, and _____. 1976. Studies on ethylene in dormancy of seeds. II. The effect of endogenous ethylene on after-ripening of apple seeds in fruit stored at low temperature. Fruit Sci. Rept. Skierniewice, Poland 3: 27-33.

31. _____, _____, and A. A. Khan. 1977. Ethylene requirement for germination of partly after-ripened apple embryo. Physiol. Plantarum 40: 293-295.

32. Kopecky, F., J. Sebanek, and J. Blazkova. 1975. Time course of the changes in the level of endogenous growth regulators during the stratification of the seeds of "Paneske-ceske" apple. Biol. Plant. 17: 81-87.

33. Letham, D. S., and M. W. Williams. 1969. Regulators of cell division in plant tissues. VIII. The cytokinins of the apple fruit. Physiol. Plantarum 22: 925-936.

34. Lewak, S., and B. Bryzek. 1974. The influence of cytokinins on the embryo photosensitivity and acid phosphatase activity during stratification. Biol. Plantarum 16: 334-340.

35. _____, A. Rychter, and B. Zarska-Maciejewska. 1975. Metabolic aspects of embryonal dormancy in apple seeds. Physiol. Veg. 13: 13-22.

36. 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.

37. Mayer, A. M., and A. Poljakoff-Mayber, 1963. The germination of seeds. MacMillan Co., New York. 236 pp.

38. Milborrow, B. V., and G. Vaughan. 1979. The long term metabolism of (+)-(2-14C) abscisic acid by apple seeds. J. Expt. Bot. 30: 983-995.

39. Moore, T. C. 1979. Biochemistry and physiology of plant hormones. Springer-Verlag, New York. 274 pp.

40. Nikolaeva, M.G. 1969. Physiology of deep dormancy in seeds. Nat. Sci. Foundation, Washington, D.C. 219 pp.

41. _____, and B. B. Yankelevich. 1974. The changes in enzyme activity in apple seeds during stratification and induction of secondary dormancy. Proc. XIX Intern. Hort. Congress, Warsaw, Vol. 1B: 502.

42. _____, and D. A. Knape. 1974. Germination and gas exchange of apple seeds depending on stratification and degree of maturation. Proc. XIX Intern. Hort. Congr., Warsaw. Vol. 1B: 497.

43. Paillard, N. 1974. Influence des produits volatils emis par les pommes sur la germination des embryons de pommier. Physiol. Veg. 12: 739-749.

44. Pellet, H. 1973. Seed stratification. Proc. Intern. Plant Prop. Soc. 23: 266-275.

45. Pieniazek, J., and R. Rudnicki. 1967. The presence of abscisin II in apple leaves and apple fruit juice. Bull. Acad. Polon. Sci. 25: 251-254.

46. _____, and M. J. Grochowska. 1967. The role of the natural growth inhibitor (abscisin II) in apple seed germination and the changes in the content of phenolic substances during stratification. Acta Soc. Bot. Polon. 36: 579-587.

47. _____, and R. Rudnicki. 1970. The inhibitory effect of endogenous abscisic acid (ABA) on after-ripening of apple seeds in the fruit. Bull. Acad. Polon. Sci. 27: 707-711.

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

49. _____, and J. Czapski. 1974. The uptake and degradation of 1-^{14}C-abscisic acid by apple seeds during stratification. Ann. Bot. 38: 189-192.

50. _____, W. Kaminska, and J. Pieniazek. 1971. The interaction of abscisic acid with growth stimulators in germination of partially after-ripened apple embryos. Biol. Plantarum. 13: 122-127.

51. _____, and J. Pieniazek. 1973. Apple fruit volatiles as inhibitors of apple seed germination. Bull. Acad. Polon. Sci. 21: 827-829.

52. _____, and _____. 1973. The effect of abscisic acid on stratification of apple seeds. Bull. Acad. Polon. Sci. 21: 149-154.

53. _____, and _____. 1975. The effect of apple flesh on dormancy release and germination of seeds. Bull. Acad. Polon. Sci. 23: 547-553.

54. Rychter, A., and S. Lewak. 1971. Apple embryos peroxidases. Phytochemistry 10: 2609-2613.

55. _____, R. Rudnicki, and S. Lewak. 1971. Regulation of acid phosphatase activity by abscisic acid and gibberellin in apple seeds during stratification. Bull. Acad. Polon. Sci. 19: 211-214.

56. Sinha, M. M., R. S. Pal, and D. N. Awasthi. 1977. Effects of stratification and plant growth regulating substances on seed germination and seedling growth in apples. Progressive Hort. 9: 27-30.

57. Sinska, I., and S. Lewak. 1970. Apple seeds gibberellins. Physiol. Veg. 8: 661-667.

58. _____, and _____. 1974. Gibberellins biosynthesis in apple seed dormancy. Proc. Intern. Hort. Congr. XIX, Warsaw. Vol. 1B: p. 493.

59. _____, _____, P. G. Gaskin, and J. MacMillan. 1973. Re-investigation of apple seed gibberellins. Planta 114: 359-364.

60. Smolenska, G. S., and S. Lewak. 1971. Gibberellins and photo-sensitivity of isolated embryos from non-stratified apple seeds. Planta 99: 144-153.

61. Sondheimer, E., E. C. Galson, E. Tinelli, and D. C. Walton. 1974. The metabolism of (S)-2-^{14}C-abscisic acid in ash seeds. Plant Physiol. 54: 803-808.

62. Taylorson, R. B., and S. B. Hendricks. 1977. Dormancy in seeds. Annu. Rev. Plant Physiol. 28: 331-354.

63. Teubner, F. G. 1953. Identification of the auxin present in apple endosperm. Science 118: 418.

64. Tissaoui, T., and D. Come. 1973. Levée de la dormance de l'embryons de pommier (Pirus malus L.) en l'absence d'oxygene et de froid. Planta 11: 315-322.

65. Visser, T. 1954. After-ripening and germination of apple seeds in relation to the seed coats. Proc. Kon. Ned. Wetensch C57: 175-185.

66. _____. 1956. The role of seed coat and temperature in after-ripening, germination and respiration of apple seed. Proc. Kon. Ned. Wetensch. C59:211-222.

67. _____. 1956. Some observations on respiration and secondary dormancy in apple seeds. Proc. Kon. Ned. Wetensch. C59: 314-324.

68. Wareing, P. F., and P. F. Saunders. 1971. Hormones and dormancy. Annu. Rev. Plant Physiol. 22:261-288.

69. Westwood, M. N., and H. O. Bjornstad. 1969. Effect of gibberellin A_3 on fruit shape and subsequent seed dormancy of apple. HortScience 3: 19-20.

70. Woodcock, D. 1947. Isolation of phloridzin from apple seeds. Nature 159: 100.

71. Zwar, J. A., W. Bottomley, and N. P. Kefford. 1963. Kinin activity from plant extracts. Aust. J. Biol. Sci. 16: 407-415.