RELATION OF PHENOLIC COMPOUNDS TO GERMINATION OF PEACH SEEDS

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INTRODUCTION

The phenomena of seed dormancy have interested researchers for many years. Little by little the details are being unfolded in various plant species. Seed dormancy may result from such sources as mechanical restriction, immature embryo, or chemical inhibition.

Various chemicals, e.g., thiourea, have been found which will terminate seed dormancy, but in many cases this results in the production of abnormal seedlings (36, 37, 76, 97). However, reports in the literature show that abnormalities may be due to temperature (80).

The breakdown of amygdalin in germinating peach seeds possibly presents a fruitful area for investigating seed dormancy. Amygdalin is hydrolyzed to mandelonitrile and glucose by prunsin (104). Mandelonitrile is further hydrolyzed by emulsin to cyanide and benzaldehyde (104). It has been shown previously that benzaldehyde strongly affects growth (46). Phenolic compounds have been isolated from many plant tissues (39, 82, 92, 93, 94) and their influence on certain biochemical systems within the plant has been investigated (38, 74, 78, 79, 83, 95, 105). Recent evidence would indicate that they are involved in plant growth and development (78, 105). Therefore, phenolic compounds could play a prominent role in controlling dormancy of peach seed.

With this knowledge at hand, research was undertaken to determine the role of phenolic compounds in peach seed germination. It was recognized that this role could be stimulatory, inhibitory, both, or neither.

Also various means of terminating dormancy were compared with regard to their influence upon certain of the phenolic compounds. In order to conduct this investigation, several new techniques were established for isolating and aiding in the identification of certain of the phenolic compounds.

REVIEW OF LITERATURE

In many plants the phenomena of seed dormancy, regardless of cause, have a survival benefit. The term "dormancy" as applied to viable seeds is generally restricted to those which fail to germinate in a reasonable length of time when subjected to an adequate moisture supply, a temperature within the range of 18-30° C and the normal gaseous composition of the atmosphere. Dormancy can be due to various causes. It may be due to the immaturity of the embryo, impermeability of the seed coat to water and/or gases, prevention of embryo growth by mechanical restrictions, special requirements for temperature or light, endogenous factors which inhibit germination, age of seed, and, in certain cases, immaturity of the embryo. These factors have been discussed in several classic reviews on seed germination (16, 17, 24) and in some excellent reviews in the last several years (64, 96, 102, 108). This review will be particularly concerned with endogenous factors which control seed dormancy since this is the type of dormancy we are dealing with in the case of peach seeds (11, 14, 36).

Viable seed that fail to germinate when exposed to conditions generally considered favorable for germination can be induced to germinate in most cases by the correct exposure to certain environmental factors. Very often the environmental cue for the resumption of growth is attained from climatic components, e.g., low temperature of a given duration, alternating periods of moisture stress, daylength, etc. These

environmental components precondition the seeds, so that germination occurs when they are supplied with adequate moisture, a warm temperature and atmospheric gases of the normal composition. After-ripening may be defined as physiological changes occurring in any part of the seed which enable the seed to germinate and the seedling to grow normally (64). The necessity for a period of after-ripening may be due to several factors. In the case of the immature embryo, further developmental changes may be required before germination (115). In other seeds, chemical changes must occur in the embryo before they germinate (64). In still others, chemical changes must occur in the integuments and/or other tissues associated with the embryo (96).

In contrast to seeds which will not germinate until subjected to certain environmental factors before being placed under favorable germination conditions of moisture, warm temperature and atmospheric gases, some seeds will germinate readily without preconditioning. However, it is interesting to note that the latter will also lose their readiness to germinate if subjected to stress conditions much the same as seeds that require factors to terminate dormancy (65, 98). This phenomenon is referred to as secondary dormancy. Secondary dormancy can be induced in certain seeds by subjection to high or low temperatures, high CO_2 levels or continuous light.

Germination Inhibitors

A large number of substances are capable of inhibiting germination. Those compounds which are generally toxic to living organisms will also, at toxic concentrations, prevent germination simply by killing the seed. However, these compounds have been of little value in determining the

underlying causes of dormancy. Compounds which prevent germination without killing the seeds are by far the more valuable in determining the mechanism of dormancy.

The simplest type of inhibition is caused by non-toxic chemicals in high concentration and this has been shown to be due to high osmotic pressures (16). These high osmotic conditions may be obtained by inorganic salts, sugars, or other substances. An example of such inhibition is the inability of seed within some mature fruit to germinate. The large quantities of soluble solids present in the flesh create high osmotic conditions around the seed and prevent germination. The threshold of osmotic pressure which prevents germination differs with the species. As soon as the seeds are removed from the high osmotic environment and placed in water, they will germinate (64).

A more complex type of inhibition is that caused by substances which are known to interfere with certain metabolic pathways. Since germination cannot occur without active metabolism, any substance that would alter normal metabolism, would probably alter the germination pattern of seed. Compounds such as cyanide (4), dinitrophenol (68), azide (68), fluoride (24), hydroxylamine (24), and others (4, 24) which are respiratory inhibitors, have inhibited germination at concentrations approximating those which inhibit metabolic processes. Therefore, it seems that inhibition of germination by this class of compounds is a result of their effect on metabolism (64), but only in the case of cyanide (4) have these chemicals been implicated in natural seed dormancy.

Another class of compounds that inhibited germination are auxins (54). An example of such a case would be the use of low concentrations

of 2,4-dichlorophenoxyacetic acid (2,4-D) to inhibit germination. Although auxins have been shown to be necessary for growth of isolated embryonic tissues and to increase at the time of germination or shortly before (32, 51); however, there has been no convincing evidence that they are directly involved in the dormancy mechanism (25, 42, 55). Only in a few instances (14, 25, 37) have auxins been shown to stimulate germination and these instances were cases where the dormant state of the seed was altered by pretreatments. This is in contrast to the influence of auxins on fruit growth and development (59, 60, 61).

On the other hand, growth inhibitors are of general occurrence in dormant seeds, and there is abundant evidence for their involvement in the physiological mechanisms of dormancy.

Evidence for the involvement of growth inhibitors in seed dormancy is the demonstration that they are often present in dormant seeds and that the application of such materials can impose dormancy on seeds in certain cases. Nutrile (70) was the first to show this. He applied coumarin to lettuce seeds and showed they required preconditioning again before they would germinate. These experiments were substantiated by Evenari (25).

Many phenolic compounds have been found to inhibit germination. These have a widespread occurrence and distribution in plants and fruits and thus it is thought that they may occur as natural germination inhibitors (92). It was suggested by van Sumere (92, 93) that the phenolic compounds may be classified along with coumarins as dormancy inducing agents. Coumarin, ferulic acid and other phenolic compounds have been found to occur in the skin as well as the cortical tissue of

potato and Hemberg (42, 43) suggested that the rest period of the potato may be due to an abundance of growth inhibiting substances in the periderm. Koves and Varga (53) surveyed the dry fruits of several species with reference to inhibitory substances. Inhibitors were found in all fruits and those that have been chemically identified were phenolic acids or their depsides and polydepsides. Numerous benzoic and cinnamic acid derivatives such as high molecular weight tannic acids, protocatechuic, caffeic and chlorogenic, ferulic, p-coumaric and p-oxybenzoic acids had a lesser activity (53). Salicylic acid, and in some cases unidentified cinnamic acid derivatives, had strong activity. Most of these inhibitors were washed out or destroyed as the fruit remained on the tree for a prolonged period of time.

Although the phenolic substances range in structure from simple phenols to complex compounds, such as lignin, it seems that the most important phenols, insofar as growth regulation is concerned, are the monocyclic aromatic compounds (82). In recent years attention has been given to the role of hydroxycinnamic and hydroxybenzoic acids in plant growth and development. The biosynthesis of these acids in higher plants has received renewed attention recently (22, 82, 94). The major pathway for the formation of these compounds undoubtedly involves phenylalanine via shikimic acid. The inter-conversion of the hydroxybenzoic acids gave rise to many derivatives (39, 45, 50). p-Hydroxybenzoic acid and caffeic acid have been isolated from plants and shown to be active as growth regulators (103, 105). Other phenolic compounds that have shown lesser activity include salicylic, gallic, ferulic, caffeic, vanillic, protocatechuic, chlorogenic, p-oxybenzoic, and p-coumaric acids (53, 64, 92).

Another possible function of the phenolic acids in seed germination may be their role in the synthesis and degradation of indoleacetic acid (IAA) (74, 83). Pilet (78, 79) reported that the mono-hydroxybenzoic acids increased the in vitro destruction of IAA. Of these, p-hydroxybenzoic acid had the greatest effects, causing stimulatory growth of stem sections at low concentrations and inhibiting elongation at higher concentrations. Many other naturally occurring phenolic acids were studied by Zenk and Muller (116) as to their influence on the destruction of exogenously applied IAA. By growth experiments with IAA-1-^{14}C and determination of the $^{14}CO_2$ evolved, it was shown that monophenols stimulate the decarboxylation of IAA under conditions where growth was suppressed (95). When Mn^{++} was present, this decarboxylation was enhanced. To add to the complexity of the relation of phenols to growth, Gordon and Paleg (38) have shown that phenols, under conditions leading to their oxidation, reacted with tryptophan to form IAA.

Probably the most active and most widely used germination inhibitor is coumarin. Coumarin is characterized by an aromatic ring and an unsaturated lactone structure. No single group in the coumarin molecule has been shown to be the cause of its inhibitory action. Reduction of the unsaturated lactone ring or substitution by hydroxyl, methyl, nitro, chloro and other groups in the ring system reduced the inhibitory activity (63, 70).

The flavonoid, naringenin, which has been isolated from peach buds by Hendershott and Walker (44), has an action similar to coumarin on lettuce seeds. Phillips (77) demonstrated that it will impose dormancy on lettuce seeds that can be reversed by light or by application of gibberellins.

Recently, several new compounds have been isolated which exhibited growth regulatory properties. One group of compounds which show a marked elongation effect on rice and lettuce is related to helminthosporol (84). 'Dormin', a terpenoid compound has shown a marked influence on the regulation of bud growth in some woody plants. It appears that the structure of 'dormin' and 'abscisin II' are the same (15, 71). Eagles and Wareing showed that an inhibitor ('dormin') concentrated from an extract of birch leaves could completely arrest apical growth when applied to the leaves of seedlings. Evidence was also found for high levels of 'dormin' in birch leaves under short days, with the emergence from dormancy presumably resulting from an interaction between 'dormin' and growth-promoting substances (20, 21). A recent finding in the study of dormancy regulation in peach seeds was that an inhibitor isolated from the seed integuments chromatographed identical to 'dormin' (57, 58). However, Daley (18) has shown that several inhibitors are present in peach seed cotyledons and that several chromatographed in the zone labeled 'dormin' by Lipe and Crane (58).

Bennet-Clark and Kefford (8) first described a complex of inhibitory substances that appeared on paper chromatograms of plant extracts running ahead of IAA when developed in a solvent of isopropanol/ammonia/water. This inhibitory area, possessing R_f values of 0.6 to 0.8, has been classified as the beta-inhibitor complex (48, 49). This inhibitory complex has been shown to be widespread in plants and has been related to both dormancy and correlative growth. For instance, Varga (100) has reported that the juice of lemons, strawberries and apricots contains inhibitors which appear to correspond to the beta-inhibitor complex. Lipe (57) found that the inhibitors in 'Lovell' peach seeds are similar to the

beta-inhibitor complex. Elution and rechromatography of the beta-inhibitor-complex has yielded both acidic and neutral substances (56). Recently, the beta-inhibitor-complex concentrated as acidic compounds from extracts of dormant maple buds was shown to be a complex of phenolic substances (86). It includes coumarin and salicylic, ferulic, p- and o-coumaric, m-oxybenzoic acid (93, 108) and 'dormin' (15).

Many of the previously mentioned phenolic compounds have been found to occur in various plant tissues, especially in fruits (64, 108). For example, Varga (100) and Koves and Varga (53) have shown that many phenolic compounds such as salicylic, ferulic, caffeic, chlorogenic, p-coumaric, protocatechuic and p-oxybenzoic acids are present in fruits. Along these same lines, it is interesting to note that peach juice is injurious to peach seed germination (85). It has been suggested that the inhibition of seed germination in fruit was generally not due to a single compound but was due to the synergistic action of several compounds that might be present within the fruit or the seed itself (108).

The activity of endogenous inhibitors may not be solely directed at the prevention of germination per se, but may also influence some of the other factors controlling dormancy. Black and Wareing (10) reported that the removal of the embryo from intact seed reduced the light requirement for germination of seed of the Betula spp. They also suggested that the inhibitor in the seed coat increased the oxygen requirement of the embryo. Wareing and Foda (109, 110) found that leaching the embryo of Xanthium seed removed the inhibitor and that maintaining the seed in a pure oxygen atmosphere caused a reduction in the inhibitor within 30 hours. Elliott and Leopold (23) showed that the inhibitors from Avena seeds inhibited alpha-amylase activity.

Villiers and Wareing (106, 107, 108) reported that chilling Fraxinus excelsior seeds had no effect on the activity of the inhibitor but that dormancy was overcome during chilling by production of a growth stimulator in the embryo tissues. Flemion and De Silva (31) also demonstrated with peach seeds that with the bioassay they were using they could find little correlation between growth inhibitors and the termination of dormancy.

The promotive effects of oxygen on germination of seeds and the parallel effects of light led Paech (73) to suggest that dormancy was regulated by phenolic substances in the seed coat. The oxidative activities of phenolic compounds could trap oxygen, preventing its entry into the seed. The action of the phenolics could be blocked by oxygen or light through the photooxidation of the phenolics themselves.

The effects of gibberellin in breaking the dormancy of many seeds indicated that it could possibly be the stimulator of growth if it were formed during the period in which dormancy was broken (35). Murakami (66) has shown gibberellin to be present in a wide diversity of seeds. As seeds of Avena fatua emerged from dormancy a growth-promoting substance suggestive of gibberellin was formed (67). These seeds were also brought out of dormancy if soaked in gibberellin solutions. Kahn (47) reported gibberellin overcame dormancy of lettuce seed regardless of whether it was imposed by high temperature, by far-red light, or by osmotic solutions.

Recently, a mode of action was suggested for gibberellic acid (99). It has been reported that gibberellic acid stimulated alpha-amylase production in the aleurone layer of the coat of cereals which in turn increased the rate of starch hydrolysis. The stimulation of alpha-

amylase was believed to be due to the direct influence of gibberellic acid on messenger RNA polymerase, an enzyme that is involved in producing the alpha-amylase enzyme (101).

Physiology of Seed Germination

The actual germination of a seed reflects the cumulative effect of interactions between many factors both external and internal. These factors range from hereditary traits to environmental influences during development and storage. For simplicity of this review, the influencing factors will be grouped into external and internal factors. Excellent reviews have been published on the physiology of seed germination (16, 17, 24, 64, 96, 102).

EXTERNAL FACTORS:

Among the external factors required for seed germination are an adequate supply of moisture, a suitable temperature range and composition of gases in the atmosphere, light, and sometimes certain chemicals. The requirement for these conditions varies according to the species and variety and is determined by hereditary factors and by the conditions which prevailed during seed formation. Frequently it appears there is a correlation between the environmental requirement for germination and the ecological conditions occurring in the habitat of the plant and the seeds (64).

Water: One of the first processes which must occur for germination of dry seeds is the uptake of water. The extent of this uptake is determined by (a) the composition of the seed coat, (b) the permeability of the seed coat to water, (c) the availability of water (liquid or gaseous) in the environment, and (d) soluble solids (64).

Gases: Germination, a process of living cells, requires an expenditure of energy. Energy requiring processes in living cells are usually supported by processes of oxidation, in the presence or absence of oxygen. These processes, respiration and fermentation, involve an exchange of gases, an output of carbon dioxide in both cases and the uptake of oxygen for respiration. Consequently, seed germination is markedly affected by the composition of the ambient atmosphere (64).

The partial pressure of oxygen in the atmosphere can be reduced considerably without greatly interfering with the rate of respiration. In fact, the seeds of some water plants germinate better under lower oxygen tensions than in air. Seeds of many terrestrial plants can germinate under water where the concentration of oxygen often corresponds to a partial pressure of oxygen very much less than that of the atmosphere (65).

In the early stages of germination of seeds of species such as Pisum sativum, respiration is largely or almost totally anaerobic because of the relative impermeability of even hydrated seeds of such species to oxygen. As soon as the seed coats are ruptured, aerobic respiration replaces the anaerobic oxidative processes (65).

The influence of carbon dioxide concentration is usually the reverse of that of oxygen. Many seeds fail to germinate when the carbon dioxide tension is high. There seems to be a minimal requirement for carbon dioxide in order for germination to occur in Atriplex halimus and Salsola as well as lettuce whereas some other species of Atriplex are resistant to high levels of carbon dioxide as long as the oxygen concentration is kept constant (7).

Temperature: Different kinds of seeds have specific ranges of temperature within which they germinate. Very low and very high temperatures tend to prevent the germination of all seeds. A rise in temperature does not necessarily cause an increase in either the rate or the percentage of germination. Therefore, germination is not characterized by a simple temperature coefficient (107).

Light: Among cultivated and non-cultivated plants there is considerable evidence for light as a factor influencing germination. For example, lettuce, tobacco and many crucifers require light to germinate (33, 75, 77, 96). Seeds may be divided into those which germinate only in the dark, those which germinate only in continuous light, those which germinate after being given a brief illumination and those which are indifferent to the presence or absence of light during germination (96).

Studies have shown that different spectral zones affected germination differently. Light of wavelength less than 2900 A^{o} has inhibited germination of all seeds tested (33). Between 2900 A^{o} and 4000 A^{o} the germination of some seeds is inhibited (33). In the visible range, 4000 A^{o} - 7000 A^{o}, it was shown that light in the range of 5600 A^{o} - 7000 A^{o} and especially red light, usually promoted germination (64, 75). If seeds exposed to red light were followed promptly by an exposure to far-red light (7350 A^{o}), germination was partially or totally inhibited (65). An excellent review of the phytochrome system and its relation to germination has been made by Siegelman and Butler (87).

INTERNAL FACTORS:

The changes which take place during the germination process are to a certain extent determined by the type of seed and its chemical composition. The composition is in turn influenced by environmental condi-

tions present during seed formation as well as the hereditary factors of the species involved.

Once the germination process is initiated, there is mobilization and translocation of compounds from storage organs to the actively growing meristematic tissues (64). Studies with tree peony embryo and endosperm tissues reveal that biochemical changes which take place with germination are different for tissue after-ripened at 5° C from those that are kept in the greenhouse at 21° - 30° C. The latter can be considered dormant tissue (5, 6, 27). Major biochemical changes in organic acids, amino acids and sugars were noted. These typify what has been found with many seeds. A good discussion of this aspect of seed germination can be found in the book by Mayer and Poljakoff-Mayber (64).

Since phosphates play an extremely important role in a variety of reactions of seeds, some discussion of the metabolism of phosphorus-containing compounds would be in order. The phosphates are required for the formation of nucleic acids which in turn are intimately concerned with protein synthesis and the hereditary constitution of plant cells. They are components of many other key compounds including phospholipids which function in controlling surface properties and permeability of cell membranes. Also, the various phosphorylated sugars and nucleotides are very closely linked with the energy-producing processes in the cell during germination (64).

Phosphorus primarily appears in seeds as organic phosphorus, with very little being present as inorganic orthophosphate. Phytin is frequently present and may constitute up to 80% of the total phosphorus content of the seed (64). Since most of the phosphate is present in the bound form, orthophosphate may be the limiting factor in certain of the

reactions of the germination process. With this in mind the large amount of phytin present may be considered as a reserve of inorganic phosphate which can be liberated as germination proceeds by phosphatase activity or more specifically phytase activity. Phytin is also present in the embryo, disappearing rapidly during germination. The phosphorus is replenished by transport from the endosperm to the embryo during germination (2). The rate of phytin hydrolysis and subsequent transport of phosphorus to the growing sites presents a possible limiting factor for the rate of germination and subsequent seedling development.

Recently, reports of myo-inositol acting as a growth factor in plant tissue have been made (3). This is of particular interest in regard to phytin since it is the salt of phytic acid or inositol hexaphosphate. The "neutral fraction" of coconut milk contains myo-inositol along with scyllo-inositol and sorbitol, but myo-inositol was regarded as the most important, as far as activity in growth-stimulation was concerned. Myo-inositol may stimulate the growth of seedlings and the germination of certain seeds. In addition, myo-inositol has stimulated growth of callus in cultures of elm (Ulmus campestris), Norway spruce (Picea abies), tobacco (Nicotiana tabacium), Vinca rosea, and carrot (normal and tumorous) tissues (3).

Studies on the nucleotide content of seeds during germination disclosed that the ATP content rose initially during imbibition and then decreased (34). The content of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) in seeds and seedlings rises in all cases during germination. During the early stages of germination there was a net increase in the RNA of peanut cotyledons (102). Some of this RNA synthesis was thought to be associ-

ated with increased numbers of mitochondria, or in mitochondrial function, and the ability of the cells to form chloroplasts. However, a part of the increase was postulated to be associated with the appearance of enzymes required for metabolism of the storage materials in the peanut. A peak was reached in about 8 days followed by a more or less parallel decline in RNA content and enzymic activities. These declines were concomitant with an increase in RNase activity.

As germination proceeded there was a sharp rise in carbon dioxide evolution and a gradual rise in oxygen uptake of pea seeds. However, after 24 hours there was a sharp decrease in the respiratory quotient (88). This same pattern was observed for wheat for both carbon dioxide evolution and oxygen uptake (67).

The energy pathways in seeds have been studied in some detail. Both glycolysis and the organic acid metabolism have been observed in germinating seeds (68, 91). Evidence for the presence of the pentose phosphate-shunt pattern of metabolism has been found in mung beans (13). In seeds containing large quantities of fats and oils, the tricarboxylic acid pathway of metabolism may be partially replaced by the glyoxylate pathway of metabolism which is a modified form of the tricarboxylic acid cycle (52, 62, 68, 114). The glyoxylate pathway functions in the conversion of fats to sugars.

Physiology of Peach Seed Germination

Peach seeds are characterized by a requirement for a period of low temperature for natural termination of dormancy. Chemicals have been found that will induce germination of dormant seed. These factors and others are discussed below.

ENVIRONMENTAL FACTORS:

The optimum temperature of 5° C with a range of 5-10° C for 60-90 days has been found best suited for the termination of dormancy of peach seeds (12, 16, 19, 29). The duration needed varies with varieties. Some varieties require fewer hours of chilling to break dormancy than do others (12). If a warm temperature treatment immediately follows exposure of seeds to low temperatures, the growth capacity of the seeds will be greatly reduced (14, 80). The reduction in growth capacity can subsequently be restored by subjecting the seeds to additional exposures to low temperatures.

Observations indicate that peach seeds are indifferent or day-neutral toward the influence of light on germination (R. H. Biggs, Unpublished data).

It has been observed that the amount of free water present during germination will influence the process. If seeds were allowed to be in contact with free water, as in a petri dish, they generally became bloated as a result of too rapid an uptake of water. However, if the seeds were placed in moist vermiculite, they were not bloated (36). This has been shown to occur with other types of seeds, particularly the legumes (64).

CHEMICAL FACTORS:

External: Tukey and Carlson (97) showed that applications of thiourea to dormant 'Lovell' peach seeds induced germination. Evidence obtained by Garrard (36) indicated that both the sulfhydryl and the imido group are requisite to the activity of thiourea. Mercaptoethanol, mercaptoethylamine, and urea were not effective either alone or in combination in promoting germination of 'Okinawa' peach seed (76). The

induction of germination of dormant peach seeds has resulted in the formation of abnormal seedlings when induction was by means of thiourea or seed coat excision (28, 36, 37, 97). However, it has been recently shown that the temperature during germination plays a major role in the development of abnormalities in the seedlings (80) and that warm temperatures during treatments with chemicals or by embryo excision was responsible for increasing the severity of abnormalities (9) and not the treatments. Thus, it is possible that two mechanisms are functioning within the embryo; one that breaks dormancy and initiates germination, and another which controls the development of the epicotyl.

Gibberellic acid has been found to induce the germination of dormant peach seeds but by a different mode of action than that of thiourea (76). Gibberellic acid can decrease, to some extent, the occurrence of leaf anomalies on peach seedlings and stimulate stem elongation (30). It is possible that gibberellic acid has a modifying influence on both germination and epicotyl development.

Internal: Pollock and Olney (72, 81) have studied extensively the rest period of seeds of sour cherry, Prunus cerasus, with respect to metabolic changes and growth. Their results showed that during low temperature treatment to terminate dormancy, nitrogen and phosphorus are translocated from the cotyledons to the embryonic axis of the embryo. The rate of translocation of nitrogen was equal to the rate of cell division; therefore, the nitrogen content per cell seemed to remain constant. The rate of translocation of phosphorus was in excess of cell division and the phosphorus concentration in the cells increased. The experiments indicated that the translocated phorphorus was incorporated into all phosphate compounds in the cells. In fully turgid seeds

kept at warm temperatures, phosphorus tended to accumulate as inorganic phosphate rather than in organic metabolites. These authors suggested that the rest period may be associated with a block in the phosphate metabolism of the cells. This has not been substantiated at the present time.

Pollock (80), using 'Elberta' peach seed, suggests that the causal agent of the dwarfing effect in seedlings is independent of the growth inhibitor content of the seed. The physiological and anatomical aspects of dwarfing suggested a control by a self-duplicating system localized in a limited region of the apical meristem and transmitted only by cell division. This system was temperature sensitive during the time between the first visible root growth and shoot elongation.

Investigations have been made into the effect of the degradation products of the glucoside amygdalin within the seed. Upon imbibition, mandelonitrile could be detected in the seeds (1); it was assumed to have arisen from the hydrolysis of amygdalin to mandelonitrile and glucose by prunsin. The mandelonitrile was further hydrolyzed to cyanide and benzaldehyde (104), presumably by emulsin.

The presence of benzaldehyde during imbibition, as a result of the degradation of amygdalin, suggested that possibly benzoic acid and some of its derivatives may be formed (26, 104). An alternative to this pathway is that in which mandelic acid, formed from mandelonitrile, undergoes enzymatic conversion to benzoic acids (40, 41, 89).

This study will be concerned with the changes in phenolic compounds during the breaking of dormancy and subsequent germination of the seed.

MATERIALS AND METHODS

All seeds were obtained from the 1965 and 1966 crops of Prunus persica cv. 'Okinawa'. This material was chosen for several reasons. Principally, the seeds are relatively homozygous in respect to the chilling requirement to terminate seed dormancy (9), the seeds require a relatively short period of low temperature stratification to overcome the dormant state (9), and when the embryos are excised they germinate readily without any apparent abnormalities if the temperature range during germination is 18-25° C (76).

The seeds were removed from the endocarp just prior to each experiment and allowed to imbibe water from moistened vermiculite. Depending upon the nature of the experiment, the time in moistened vermiculite varied. The seeds were planted in seed flats containing a 2:1 mixture of perlite: vermiculite. Techniques for each experiment will be discussed separately.

Tests for interaction of thiourea and seed coat excision on germination: In order to determine the most effective thiourea concentration to promote the greatest amount of germination with the least amount of anomolous growth, a range of concentrations was tested. This was as follows: 0.0, 1.0, 3 x 10^{-1}, 10^{-2} and 10^{-3} M thiourea. The seeds were kept in a moist medium for 42 hours and then followed by 6 hours' soaking in the respective thiourea concentrations. Before imbibition, the seeds were surface sterilized for 3 minutes with a 1,000 ppm merthiolate in

25% ethanol: water solution. After the soaking period, the seeds were blotted and planted in flats and kept in the dark at 20° C for 16 days before being placed in a greenhouse. Each treatment was replicated 3 times with 40 seeds per replication.

A second experiment was designed to determine if any interaction existed between thiourea and the seed coat on the degree of anomalous development of the subsequent seedlings. Thiourea concentrations of 0.0, 10^{-1}, and 3×10^{-1} M were applied to intact seeds and to excised embryos after 42 hours imbibition. After a 6 hour treatment period, the seeds were removed from the solutions, blotted, and planted in seed flats. At 3 time intervals of 24, 48 and 72 hours, seed coats were removed from samples of intact seeds treated with thiourea and the excised embryos replanted. All treatments were kept in the dark at 20° C for 10 days, except for brief period of examination. After 10 days the flats were moved to the greenhouse. Each treatment was replicated 3 times with 9 seeds per replication.

Testing chemicals for modification of germination of peach seeds: Benzaldehyde, benzoic acid, cyanide, p-hydroxybenzoic acid and mandelonitrile in a series of concentrations were tested on seed germination. Because of volatility and water solubility of the chemicals, methods of treatment varied. Each treatment was replicated 3 times with a randomized block design and observation on germination were taken at 7 and 12 days after the start of seed imbibition in all cases. Data was analyzed statistically using F test and Duncan's multiple range (90).

In the cyanide treatments, the concentrations used were 0.0, 1.0, 10^{-1}, 3×10^{-2}, and 10^{-2} M made with potassium cyanide. Seeds were allowed to imbibe for 42 hours, seed coat removed and the embryos placed

in an aqueous solution of the chemical for 6 hours. They were then planted in seed flats and placed in a growth chamber with a controlled temperature of $20^{o} \pm 2^{o}$ C and a 12-hour day of approximately 900 ft-C light intensity.

Benzoic acid and p-hydroxybenzoic acid were tested at concentrations of 0.0, 10^{-1}, 3×10^{-2}, 10^{-2}, and 10^{-3} M. To test the respective concentrations of each compound, approximately 5 g of dry perlite were placed in 100 ml beakers and the perlite saturated with the solution of chemical to be tested. After equilibration of the mixture, fully turgid seeds, attaining this condition in moist vermiculite in 48 hours at 20^{o} C, were placed in the perlite plus chemical media, and maintained under aerobic conditions. After 5 days in the media, the seeds were transferred to flats containing a 2:1 mixture of perlite: vermiculite. All phases of the experiments were conducted in growth chambers at $20^{o} \pm 2^{o}$ C with a 12-hour day of 900 ft-C. light intensity.

Since benzaldehyde and mandelonitrile are only slightly soluble in water, the method of treatment was modified. For these tests, a logarithmic range of quantities of the material per unit of perlite was used. A measured amount of the chemical was absorbed onto fine perlite and water added to the medium. The concentrations noted are based on the amount that was available to the water phase. Five grams of the mixture were used per container per treatment and care was taken to maintain aerobic conditions. For preparation of the seed before treatment, they were allowed to imbibe for 48 hours, embryos excised and placed in the perlite-chemical mixture. The embryos were left in the media for 5 days at 20^{o} C. Then they were removed from the chemical environments, planted in flats and placed in a greenhouse for the remainder of the observational period.

Extraction and preparation of fractions from seeds for gas chromatography: The isolation of the fractions was made from seeds that were fully turgid after 48 hours in moist vermiculite at 20° C. The seeds (10 g) were ground in a Servall Omni-mixer at 16,000 rpm for 3 minutes in 30 ml of 80% ethanol. The homogenate was filtered, the subsequent filtrate dried under vacuum and the residue dissolved in 0.1 M tartaric acid. This aqueous solution was partitioned against ethyl ether and the ether phase separated and partitioned against an aqueous solution of 0.1 M sodium bicarbonate. The aqueous bicarbonate phase was acidified with tartaric acid to pH 2.0 and then partitioned again with 100 ml ethyl ether. The resulting ether solution was concentrated under nitrogen gas. The ether-soluble acidic fraction was subjected to gas chromatography before and after treatment with acetylating agents.

Diazopropane was prepared with slight modification by the method of Wilcox (112). Briefly, N-propyl-N-nitrosourea (obtained from Dr. Merrill Wilcox, Agronomy Department, University of Florida) was reacted with 40% KOH in water and trapped in peroxide-free ethyl ether. The etheral solution was stored over sodium sulfate in a polyethylene bottle in a freezer. To acetylate a sample, sufficient amounts of the solution were added so that a straw-yellow color persisted at the end of the reaction period.

Alternative esterification methods with diazomethane and diazobutane were used to aid in the identification of aromatic acids. The diazomethane reagent was prepared as outlined by Williams (113). Briefly, N-methyl-N'-nitro-N'-nitrosoguanidine (Aldrich Chemical Co., Milwaukee) was added to 20% KOH and trapped in ethyl ether. The diazobutane was prepared in a manner similar to the diazopropane except substituting N,

N-butyl-N-nitrosourea (obtained from Dr. Merrill Wilcox, Agronomy Department, University of Florida) for the N-propyl-N-nitrosourea. With both diazomethane and diazobutane, the initial esterification period, 30 minutes, was the same as with diazopropane.

In tests where esterification was slow for the carboxyl group or where acetylation of hydroxyl groups on the ring was slow or non-existent, 0.7% methanolic boron trifluoride was added to these diazo-compounds and the reaction was allowed to proceed at room temperature for 3 hours.

Standards of chemicals and fractions of extracts were dissolved in ethyl acetate for gas chromatography. Weights and volume on seed and solvent fractions were kept so that quantities could be expressed as seed equivalents. Standards had a final concentration of 1 mg per ml.

Conditions for gas chromatography: Separation of compounds of the acidic fraction of the ethanol extract was on a model 400 F and M gas chromatograph equipped with a flame-ionization detector. The column consisted of 1/4 inch stainless steel tubing 6 feet long packed with 8% S.E. 30 on 60-80 mesh Chromosorb W. Helium was used as a carrier gas with flow rate of 70 ml per minute. Temperatures for the system were as follows: oven, 180° C; injection port, 260° C; and detector, 250° C, except as noted in the results.

Identification of extracted compounds was made by comparison of their retention times with those of the known compounds. Matched retention times of several derivatives of knowns to those of identically treated unknowns lent greater support to tentative identification.

Alfalfa bioassay: Peruvian alfalfa seed were separated into red and yellow seeds. The red seeds were discarded because of their low germination capability (111) and the yellow seeds were used for the bioassay.

The bioassay was conducted in petri dishes with either filter paper disks or chromatography paper strips as a moisture holding absorbent, depending upon the test. Generally 40-50 seeds per dish were used for each assayed fraction. Before placing the seeds on the moistened paper, they were soaked in distilled water for a few seconds to improve the rate of imbibition of the seeds. Once the seeds had been placed in the dishes, the dishes were placed in the dark at $20^{o} \pm 2^{o}$ C. After 24 hours, observations were made on the number of germinated and non-germinated seeds per dish. A seed was considered to have germinated upon protrusion of the radicle.

Inhibitor characterization: Bioassays were conducted on 80% ethanolic extracts and fractions paper chromatographed in isopropanol: ammonia: water (80:1:19, v/v/v) solvent on Whatman 3 MM chromatography paper. Chromatograms were divided into sections of 10 R_f units and assayed, using the alfalfa seed bioassay (18).

Extracts of peach seeds were also subjected to acid hydrolysis (pH 2) with acetic acid, alkaline hydrolysis (pH 10) with ammonium hydroxide, dialysis against distilled water for 24 hours; and heating for 10 minutes at 50, 75, and 100^{o} C. Changes in inhibitory activity were monitored, using the alfalfa bioassay.

Solubility of components of the inhibitor complex in various organic solvents was investigated. Sections of the paper chromotograms containing the inhibitory zone were cut into strips representing the equivalent of a 0.5 g seed sample. These strips were steeped in various solvents for 2 hours. The solvents were decanted into small petri dishes containing a Whatman No. 4 filter paper disk and the residue deposited on the paper by evaporation. Distilled water (1.5 ml) was added to the

petri dishes, and to appropriate controls, and then bioassayed. Redistilled solvents of water, hexane, acetonitrile, ethyl ether, chloroform, methanol, ethyl acetate and carbon disulfide were used for the solubility studies.

Measurement of benzaldehyde and mandelonitrile: The quantity of benzaldehyde and mandelonitrile present in seeds under various treatments was determined. All seeds were fully turgid since they were placed in moist vermiculite for 42 hours at 20° C prior to treatment. Treatment 1 was seeds steeped in 3×10^{-2} M thiourea for 6 hours, blotted and kept in a moist medium until sampled. Treatment 2 was embryos removed from the seed coat and associated tissue after 48 hours from the start of the experiment. Treatment 3 was the control of intact seeds. Seeds in each treatment were kept at 20° C and a 4.8 g sample wet weight, equivalent to approximately 3 g dry weight, were taken at the following times from the start of seed imbibition: 48, 60, 72, 80, 88, 96, 104, 112, 120, 132, 144, 156, and 168 hours. The samples were frozen immediately to -70° C and then placed in a freezer at -30° C until ground, approximately 8 hours. The frozen seeds were ground in a Wiley mill with a 20-mesh sieve. The mill had been thoroughly cooled by passing large quantities of dry ice through it before the samples were ground. Also, sufficient amounts of powdered dry ice were passed through the mill along with the frozen seeds to keep the grinding head at approximately the temperature of the dry ice. The ground seeds plus powdered dry ice were collected together and added to ethyl ether at -70° C. After the dry ice had sublimed from the ethyl ether (generally 30-40 minutes at room temperature) the solutions were allowed to warm to approximately -5° C before they were placed in a -30° C environment

for 3 hours. This warming and steeping in a freezer was needed to obtain benzaldehyde and mandelonitrile in the ether phase. This etheral solution was subjected to gas chromatography under conditions noted with the results. Weight and volume were taken quantitatively so the data could be expressed in the amount of chemical per seed equivalent.

Under the conditions of gas chromatography, benzaldehyde and mandelonitrile chromatographed as benzaldehyde since heat caused mandelonitrile to decompose to HCN and benzaldehyde. Therefore, the following series of reactions were used to separate the 2 components. Firstly, the quantity of both compounds was obtained from gas chromatographic analysis of an aliquot of an extract. Secondly, the quantity of mandelonitrile remaining in an etheral solution was determined after quantitatively removing benzaldehyde by reacting with sodium bisulfite. This was accomplished by solvent partitioning between the etheral solution and aqueous 40% sodium bisulfite. Thirdly, quantitative analysis was again done on the ether phase after 40% potassium cyanide was added to the aqueous sample layered under ethyl ether and the mixture shaken vigorously. This converted the sodium bisulfite addition product of benzaldehyde to mandelonitrile which allowed it to pass back into the ether phase. After allowing the mixture to stand for 5 minutes in the cold, the ether phase was subjected to gas chromatographic analysis the third time. Quantitative determinations were made using the area under the peak as a measure of both compounds and the peak area of the sample after addition of sodium bisulfite. The latter represents that due to mandelonitrile. The difference between the two peak areas was assumed to be that due to benzaldehyde.

The conversion of benzaldehyde to a sulfite derivative soluble in water and then conversion to mandelonitrile is a well-known reaction (26). The sodium bisulfite reacts with the carbonyl group of benzaldehyde to form the sulfite addition product. Addition of potassium cyanide acts as a base and neutralized the sodium bisulfite in equilibrium with the bisulfite compound to form potassium bisulfite; the simultaneously liberated benzaldehyde and hydrogen cyanide then combine to give mandelonitrile (26).

Chilling study: Determinations were made of the inhibitor complex benzaldehyde and mandelonitrile after periods of chilling. Fully turgid seeds, attaining this condition after 48 hours in moistened vermiculite at 20° C, were placed at 4° C for 0, 168, 336, 504, and 672 hours. At the time of sampling, one sample was removed and extracted immediately and another sample was placed for an additional 40 hours at 20° C. A control lot of seeds was maintained at 20° C for sampling at equivalent times. At each time of sampling, seeds equivalent to 5 g dry weight were taken in duplicate. The quantity of benzaldehyde and mandelonitrile in the seeds was determined as outlined previously and the level of non-volatile inhibitors, presumably the beta-inhibitory complex (8), was assayed as follows. A sample of treated seeds was subjected to extraction with 80% ethanol after grinding, as previously noted. The solution was taken to near dryness by vacuum distillation, keeping the distilling chamber at less than 5° C. The residue was redissolved in 80% ethanol, applied to chromatographic paper (Whatman 3 MM) and developed in isopropanol: ammonia: water (80:1:19 v/v/v) solvent, using decending techniques. The inhibitory zone, as determined by R_f, were sectioned from the chromatograms, and solutes eluted from the paper with

glass distilled water. The eluates were then diluted in such a way that equivalent seed weights in the solutions were 1.0 g, 500 mg, 300 mg, 100 mg and 0 mg. The solutions were placed in small petri dishes on Whatman No. 4 filter paper disk, frozen and water removed by sublimation under vacuum. After again moistening the filter pads with 1.5 ml of H_2O, they were bioassayed with the alfalfa bioassay using 40 seeds per disk. Inhibitory levels were determined by calculations from a dilution curve based on relative seed weight.

EXPERIMENTAL RESULTS

Thiourea and seed coat excision: The influence of thiourea on germination of dormant 'Okinawa' peach seed and on anomalous seedling development is shown in Table 1. It was quite evident from this data that thiourea greatly increased the per cent germination, but enhanced anomalous development in the seedlings. In both cases, the higher the concentration, the greater the effect. The data indicates further that increases in germination and abnormal growth were statistically significant with concentrations of thiourea stronger than 10^{-2} M.

In determining the possible interaction between thiourea and seed coat on germination and subsequent seedling growth, the most striking finding was the absence of abnormal seedlings in any of the treatments; yet very good germination was obtained, as shown in Table 2. The length of time after imbibition and thiourea treatment for embryo excision seemed to have little effect on germination.

Influence of Benzaldehyde, cyanide, and mandelonitrile on seed germination: The data in Table 3 indicates that cyanide does not drastically reduce germination, except in very high concentrations (1.0 M). Data taken 20 days after start of imbibition showed that the 1.0 M concentration was still significantly different from the lower concentrations used. No abnormalities were noted in the seedlings from any of the treatments, and, interestingly, the 1.0 M cyanide did not kill the seeds.

Table 1.--Influence of thiourea concentrations on germination and per cent of production of abnormal seedlings from 'Okinawa' peach seeds.

Thiourea concentration, M[x]	Mean % germination[y]	Mean % abnormal[y,z]
0 (Control)	5.8 a	0.0 a
10^{-3}	5.8 a	0.0 a
10^{-2}	26.8 ab	21.9 b
10^{-1}	45.8 bc	40.1 b
3×10^{-1}	62.5 c	61.4 c
1.0	58.3 c	79.0 c

[x]Each treatment was replicated 3 times with 40 seed per replication.

[y]Means not having a following letter in common are significantly different at the 1% level.

[z]Percentage based on the total number of seed germinated.

Table 2.--Effect of thiourea concentration and embryo excision on germination of 'Okinawa' peach seeds 12 days after start of imbibition and on abnormal seedling production 32 days after start of imbibition.

Treatments[x]			Mean % germination[y]	% atypical seedling
Chemical	Seed coat			
Control	Intact		0.0 a	0.0
	Excised;	42 hrs[z]	100.0 e	0.0
		72 hrs	92.6 cd	0.0
		96 hrs	100.0 e	0.0
		120 hrs	100.0 e	3.7
10^{-1}M Thiourea	Intact		81.5 b	7.4
	Excised;	42 hrs	96.3 de	0.0
		72 hrs	92.6 cde	0.0
		96 hrs	100.0 e	0.0
		120 hrs	100.0 e	3.7
3×10^{-1}M Thiourea	Intact		88.9 c	3.7
	Excised;	42 hrs	96.3 de	0.0
		72 hrs	100.0 e	0.0
		96 hrs	100.0 e	0.0
		120 hrs	100.0 e	3.7

[w]Seeds were imbibed 42 hours, then treated with chemicals for 6 hours before planting.

[x]Each treatment consisted of 3 replications of 9 seed each.

[y]Means not having a following letter in common are significantly different at the 1% level.

[z]Hours after start of imbibition.

Table 3.--Per cent germination of 'Okinawa' peach seeds 7 and 20 days after start of imbibition as influenced by cyanide[x].

Cyanide concentration, M[y]	Mean % germination[z]	
	7 days	20 days
1.0	0.0 a	0.0 a
10^{-1}	88.9 b	94.4 b
3×10^{-2}	100.0 c	100.0 b
10^{-2}	100.0 c	94.4 b
Control	100.0 c	100.0 b

[x]Seeds were imbibed for 42 hours, then treated with the designated cyanide concentrations for 6 hours.

[y]Each concentration consisted of 3 replications with 6 seed per replication.

[z]Means not having a following letter in common are significantly different at the 5% level.

The treating of samples of excised embryos with various concentrations of mandelonitrile and benzaldehyde resulted in the inhibition of germination with some of the stronger concentrations (Tables 4 and 5). Mandelonitrile at 1.4 to 140.0 mg/g completely inhibited germination, while all other concentrations except 0.42 mg/g inhibited only slightly. Data taken 5 days after removing the seeds from the chemical showed that 1.4 mg/g exhibited very little inhibitory influence. Concentrations of 4.2 to 140.0 mg/g were still strongly inhibitory (Table 4). Concentrations of benzaldehyde of 11.0 and 110.0 mg/g completely inhibited germination, while a concentration of 3.3 mg/g resulted in only 16.7% germination. Concentrations lower than 3.3 mg/g had no measurable influence (Table 5). Five days after removal of seeds from the benzaldehyde media, germination occurred to an appreciable extent in the 3.3 mg/g treatment but 11.0 and 110.0 mg/g were still inhibitory. As found with cyanide, the anomalous growth patterns were not present on seedlings produced from seeds treated with either mandelonitrile or benzaldehyde. The influence of the benzaldehyde and mandelonitrile on seed germination is portrayed graphically in Figure 1.

Aromatic acids investigation: Tentative identification of the components isolated from the propyl esters of the acidic fraction of an ethanol extract of peach seeds was made by comparing the retention times on gas chromatograms with those of known compounds. A gas chromatogram of the fractions is shown in Figure 2.

The phenolic compounds tentatively identified were benzoic, mandelic, o-hydroxycinnamic, 2,6-dihydroxybenzoic, o-hydroxybenzoic, p-hydroxybenzoic and 2,4-dimethoxybenzoic acids (Table 6). The gas chromatograms of the standards for the known compounds listed above can be found in the Appendix.

Table 4.--Per cent germination of 'Okinawa' peach seeds 7 and 12 days after start of imbibition as influenced by mandelonitrile[x,y].

Mandelonitrile mg/g of perlite	Mean % germination[z]	
	7 days	12 days
140.0	0.0 a	0.0 a
14.0	0.0 a	0.0 a
4.2	0.0 a	0.0 a
1.4	0.0 a	83.3 b
0.42	100.0 c	100.0 c
0.14	94.4 bc	100.0 c
0.042	94.4 bc	100.0 c
0.014	94.4 bc	100.0 c
Control	88.9 b	88.9 bc

[x]Seeds were allowed to imbibe for 2 days, then placed in perlite containing mandelonitrile for 5 days. The indicated quantity of mandelonitrile was applied to the perlite.

[y]Each treatment was replicated 3 times with 6 seed per replication.

[z]Means not having a following letter in common are significantly different at the 1% level.

Table 5.--Per cent germination of 'Okinawa' peach seeds 7 and 12 days after start of imbibition as influenced by benzaldehyde[x,y].

Benzaldehyde mg/g of perlite	Mean % germination[z]	
	7 days	12 days
110.0	0.0 a	0.0 a
11.0	0.0 a	0.0 a
3.3	16.7 b	94.4 b
1.1	100.0 c	100.0 c
0.33	100.0 c	100.0 c
0.11	100.0 c	100.0 c
0.033	100.0 c	100.0 c
0.011	100.0 c	100.0 c
Control	100.0 c	100.0 c

[x]Seeds were allowed to imbibe for 2 days, then placed in perlite containing benzaldehyde for 5 days. The indicated quantity of benzaldehyde was applied to the perlite.

[y]Each treatment was replicated 3 times with 6 seed per replication.

[z]Means not having a following letter in common are significantly different at the 1% level.

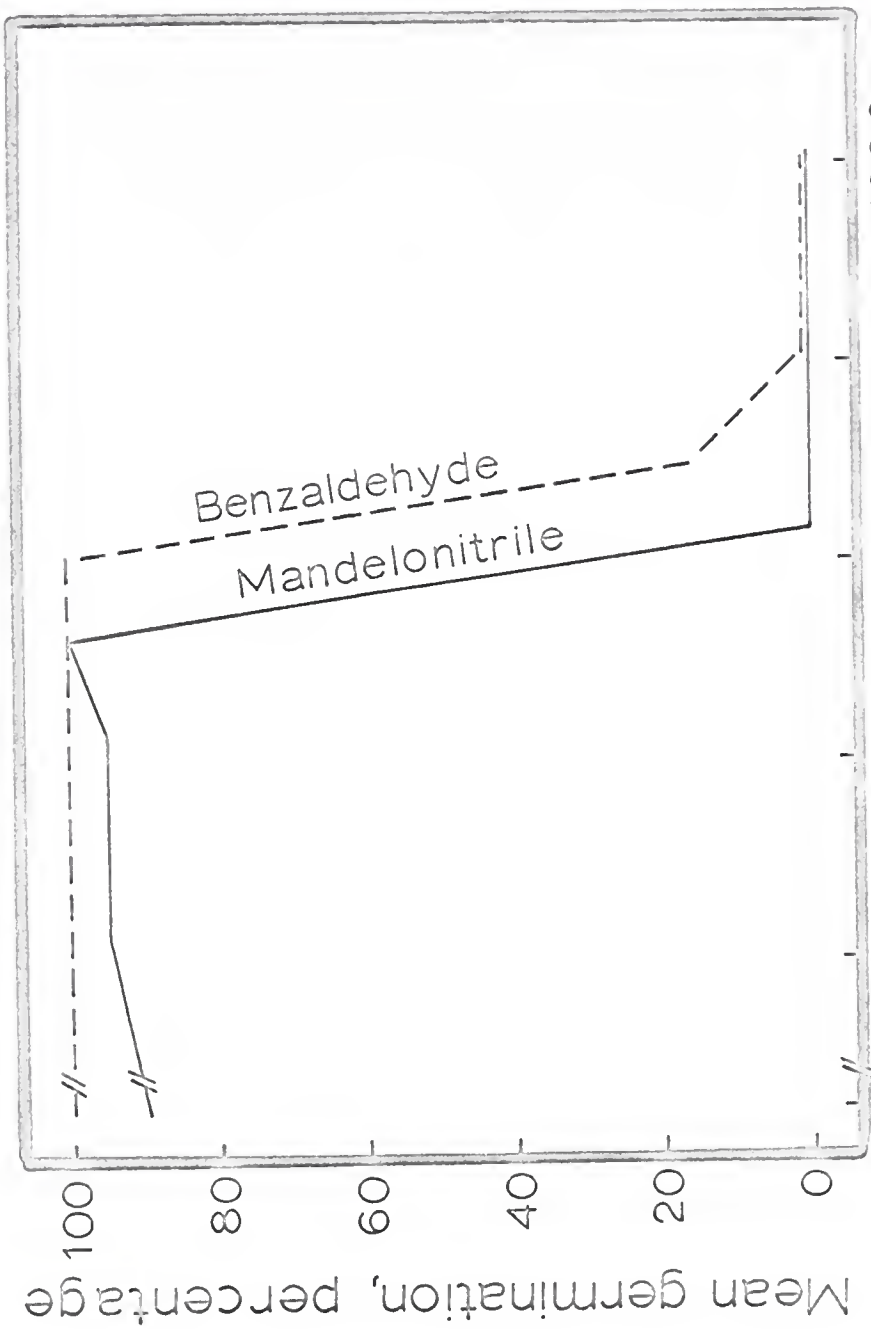


Fig. 1. Influence of benzaldehyde and mandelonitrile on peach seed germination. (Quantities given, mg of material per g of perlite, in legend are those available to the aqueous phase within the container.) (See Tables 4 and 5 for parameters.)

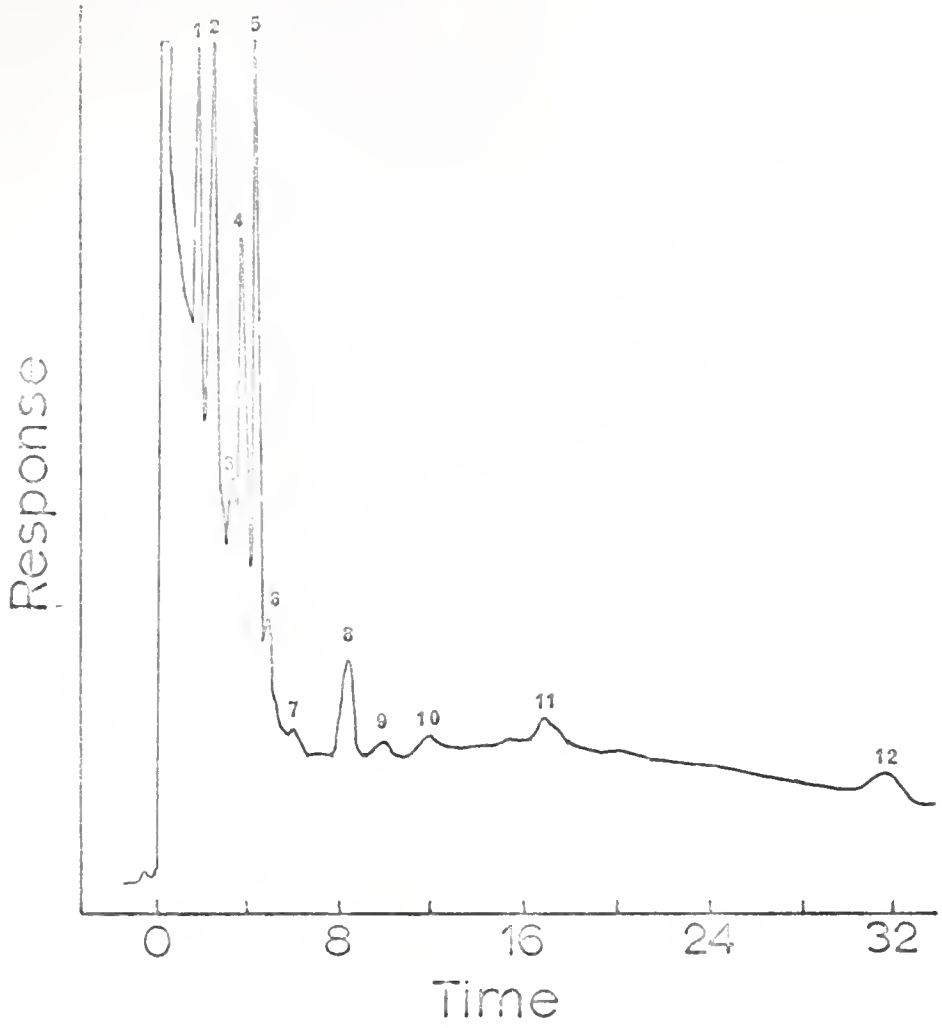


Fig. 2. Gas chromatogram of the propyl esters of the acidic fraction from an ethanol extract of peach seeds. Time is in minutes. (See Table 6 for gas chromatograph parameters.)

Table 6.--Relative retention time and possible identity of components separated by gas chromatography of the propyl esters of the acidic fraction from an ethanolic extract of peach seeds[x].

Peak No.	Relative retention time[y]	Possible identity of acids[z]
1	1.00	Benzoic
2	1.37	Succinic
3	1.91	Malic or mandelic?
4	2.13	o-hydroxycinnamic
5	2.50	Fumaric ?
6	2.89	2,6-dihydroxybenzoic
7	3.59	o-hydroxybenzoic
8	4.99	p-hydroxybenzoic
9	5.89	2,4-dimethoxybenzoic
10	7.11	?
11	10.21	Citric
12	19.29	?

[x] - Instrument: F & M model 400, flame detector.
Column: 8% S.E. 30 on 60-80 mesh chromasorb W, acid washed, silane treated; 1/4" O.D. stainless steel 6' in length.
Carrier gas: He. Outlet flow rate: 70 ml/min.
Oven temp: 180° C. Injection port temp: 260° C.
Detector temp: 250° C.
Range and attenuation: 10 x 8. Chart speed: 1/4" per min.

[y] - Relative retention time is based on benzoic acid.

[z] - Possible identity based on matched retention times.

Inhibitor characterization: Using the Peruvian alfalfa bioassay, it was found that extraction of 8 g wet weight of dormant peach seeds with 80% ethanol yielded a strongly inhibitory complex (0% germination). Specific gravity measurements indicated that the inhibitory influence was due to factors other than osmotic ones. Paper chromatography of the ethanol extract, using an isopropanol: ammonia: water (80:1:19 v/v/v) solvent system, yielded a strong inhibitory complex between R_f's 0.6 and 0.8 when bioassayed with the alfalfa seed test (Table 7).

From tests on the influence of acids, bases and heat on the stability of the inhibitory complex, the data on per cent germination from the alfalfa bioassays (Table 8) would seem to indicate that the inhibitory complex was reasonably stable since none of the treatments destroyed the inhibitory capacity of the extract.

Comparison of various organic solvents (Table 9) for the solubilization of the inhibitory complex showed that the more polar solvents (water and alcohol) serve as suitable solvents for the inhibitor. The data indicated also that the inhibitor may be only partially soluble in acetonitrile.

Isolation and characterization of benzaldehyde and mandelonitrile from peach seeds: Benzaldehyde and mandelonitrile were isolated and characterized from peach seeds by several techniques. Crushed peach seeds evolve an aroma similar to that of benzaldehyde and mandelonitrile. Co-chromatography of the pure chemicals and the extract components from peach seeds by chromatography yielded identical R_f's and retention times, respectively. Ultra-violet fluorescence (3200 A^o and 2537 A^o) of benzaldehyde and a fraction from an ethanol extract from peach seeds on paper chromatograms were identical. Also, benzaldehyde and the extracted

Table 7.--Paper chromatographic separation of the inhibitory complex from dormant peach seeds.

R_f Value[x]	% Germination[y]
0.0 - 0.1	92.5
0.1 - 0.2	85.0
0.2 - 0.3	87.5
0.3 - 0.4	85.0
0.4 - 0.5	55.0
0.5 - 0.6	0.0
0.6 - 0.7	0.0
0.7 - 0.8	0.0
0.8 - 0.9	85.0
0.9 - 1.0	80.0

[x]Solvent system: Isopropanol: ammonia: water (80:1:19 v/v/v).

[y]Bioassayed with the alfalfa seed test. Control = 90%.

Table 8.--Influence of acids, bases and heat on the inhibitory complex from peach seeds after paper chromatography.

Test	% Germination[x]
Acid hydrolysis (pH 2)	0.0
Alkaline hydrolysis (pH 10)	0.0
Dialysis, inside tubing[y]	0.0
outside tubing	0.0
Heating for 10 minutes:	
50° C	0.0
75° C	0.0
100° C	0.0
Extract control	0.0
Water control	90.0

[x]Bioassayed with the alfalfa seed test. Seed equivalent of the extract was 0.5 g dry weight.

[y]Dialysis was conducted with seamless cellulose tubing against distilled water for 24 hours.

Table 9.--Solubility of the inhibitor-complex in various organic solvents as determined by the alfalfa bioassay.

Solvent	% Germination	
	Eluate[x]	Chromatogram Section[y] (R_f 0.6-0.8)
Water	0.0	80.0
Hexane	92.5	0.0
Acetonitrile	30.0	0.0
Ethyl ether	85.0	0.0
Chloroform	90.0	0.0
Methanol	0.0	82.5
Ethyl acetate	87.5	0.0
Carbon disulfide	92.5	0.0

[x]Elution fraction from chromatogram section of R_f 0.6-0.8.

[y]Chromatogram section containing the inhibitory complex after eluting with the respective solvent.

component reacted similarly to aldehyde indicators. Component of an ethanol extract and benzaldehyde formed a sodium bisulfite addition product which then generated mandelonitrile on treatment with KCN.

Ultra-violet fluorescence (3200 A^{o} and 2537 A^{o}) of mandelonitrile and a fraction from an ethanol extract from peach seeds on paper chromatograms were identical. The extract components and mandelonitrile form benzaldehyde and cyanide when subjected to high temperatures (200-250^{o} C). Mandelonitrile and components of the extract reacted alike when tested with hydrocyanin indicators.

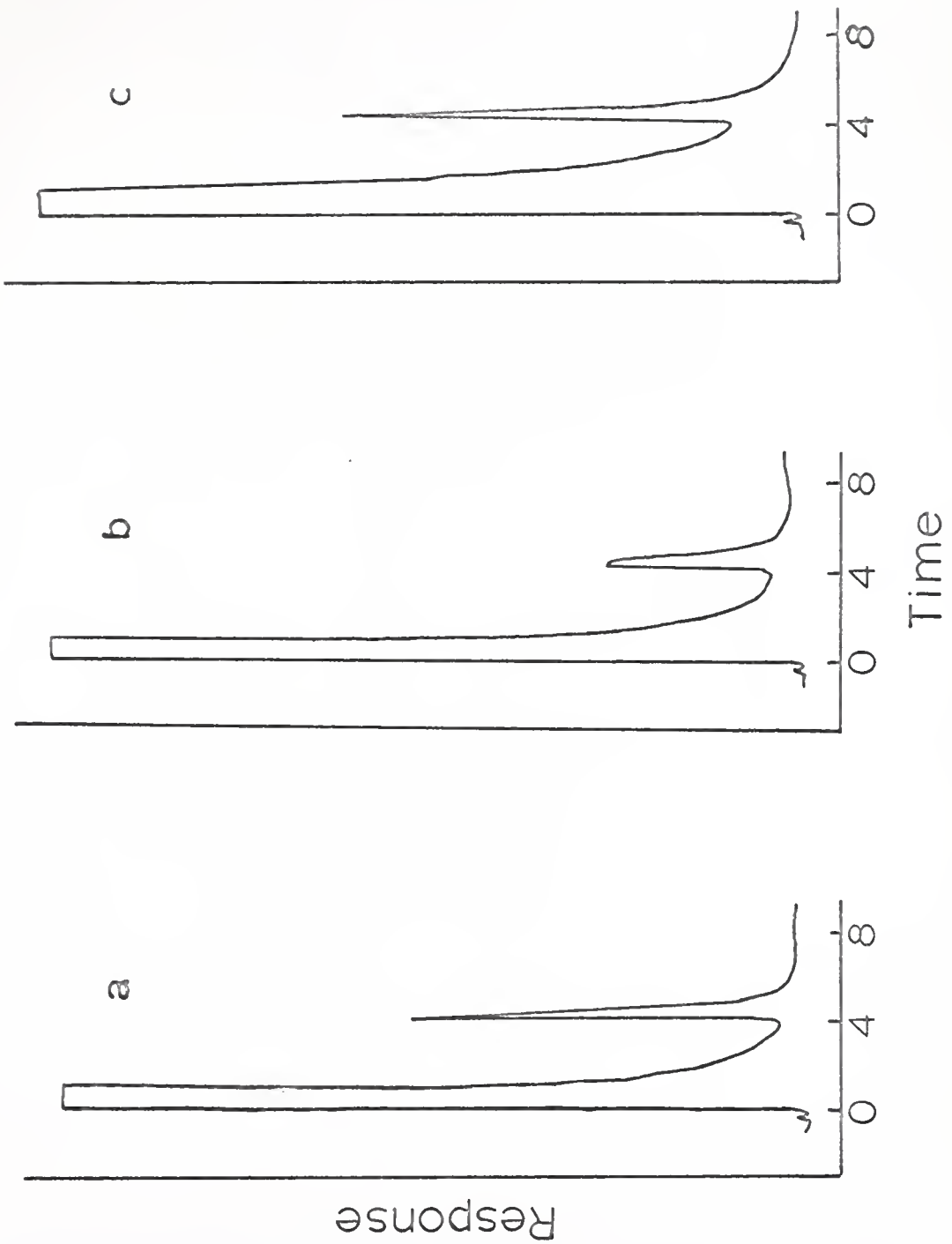
Quantitative determinations of benzaldehyde and mandelonitrile: Benzaldehyde and mandelonitrile were determined using procedures established in identifying the 2 compounds. Briefly, this was gas chromatographic analysis of the ethereal extract before making a sodium bisulfite-addition product, after the reaction to assay the level of decrease and again after converting the benzaldehyde to mandelonitrile by KCN. A chromatogram of the composite of both compounds is shown in Figure 3a. After treating with sodium bisulfite, the peak is reduced (Figure 3b) and increased after the subsequent addition of potassium cyanide (Figure 3c).

Comparison of the influence of thiourea treatment, and embryo excision, as compared to a non-germinating control of intact seeds, on the rate of release of benzaldehyde and mandelonitrile both collectively and individually is shown in Figures 4, 5 and 6. The graphs indicated that intact seeds and thiourea-treated seeds had peak times of production of benzaldehyde and mandelonitrile at about the same time, 72 hours, while the excised seeds had a delay in the maximum period of production by 16 hours. The thiourea-treated seeds had a second peak of production at

Fig. 3. Gas chromatograms of a known composite sample of an etheral solution of benzaldehyde-mandelonitrile: (a) initial solution; (b) after addition of a solution of sodium bisulfite; and (c) after addition of potassium cyanide.

Gas chromatograph parameters.

Instrument:	F & M model 400, flame detector.
Column:	8% S.E. 30 on 60-80 mesh Chromosorb W, acid-washed, silane treated; 1/4" O.D. stainless steel 6' in length.
Carrier gas:	Helium. Outlet flow rate: 70 ml/min.
Temperatures:	Oven, 100° C; Injection port, 150° C; Detector, 160° C.
Range and Attenuation:	10 X 8
Chart Speed:	1/4" per minute.


a
b
c
0
4
8
Response
Time

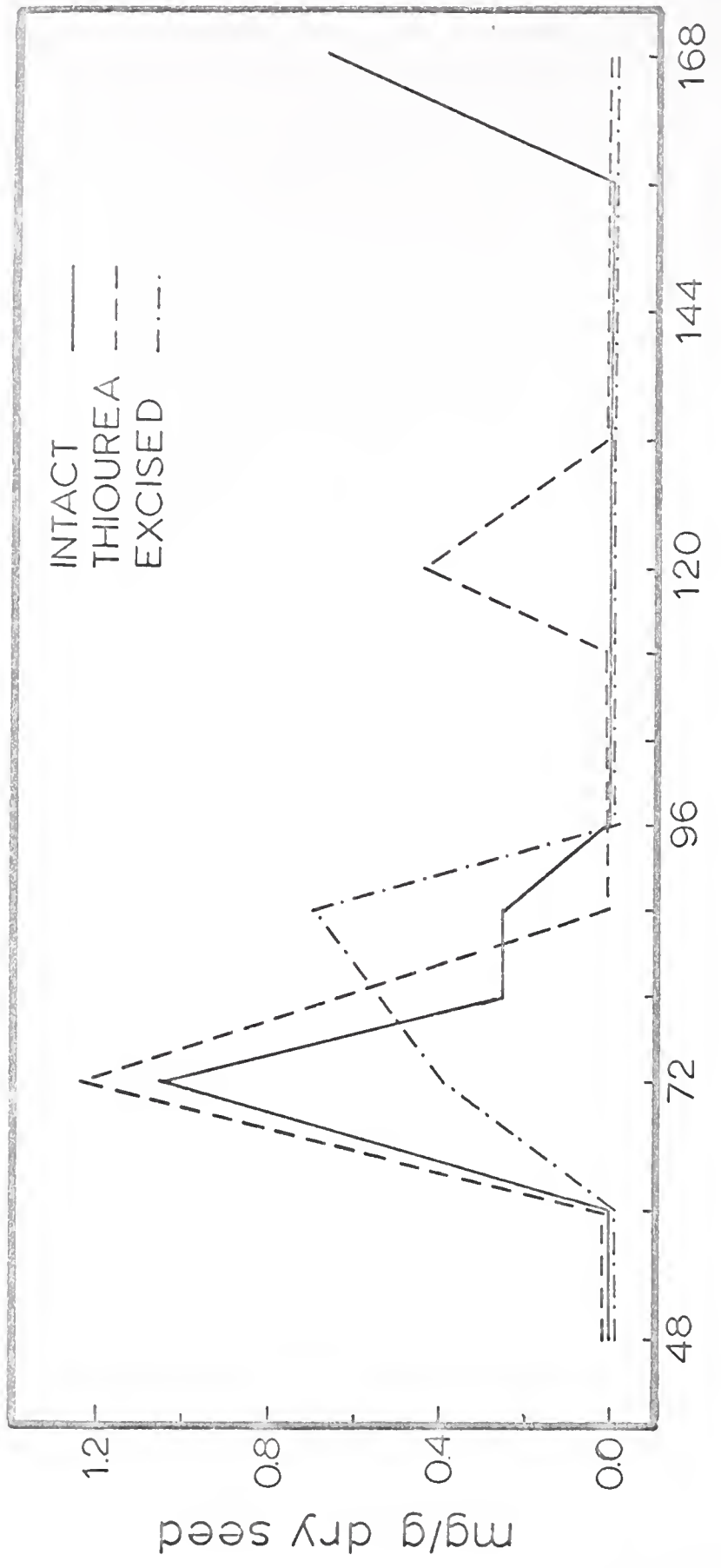


Fig. 4. Changes in benzaldehyde-mandelonitrile content of peach seeds as measured at various intervals after start of imbibition under the designated treatments.

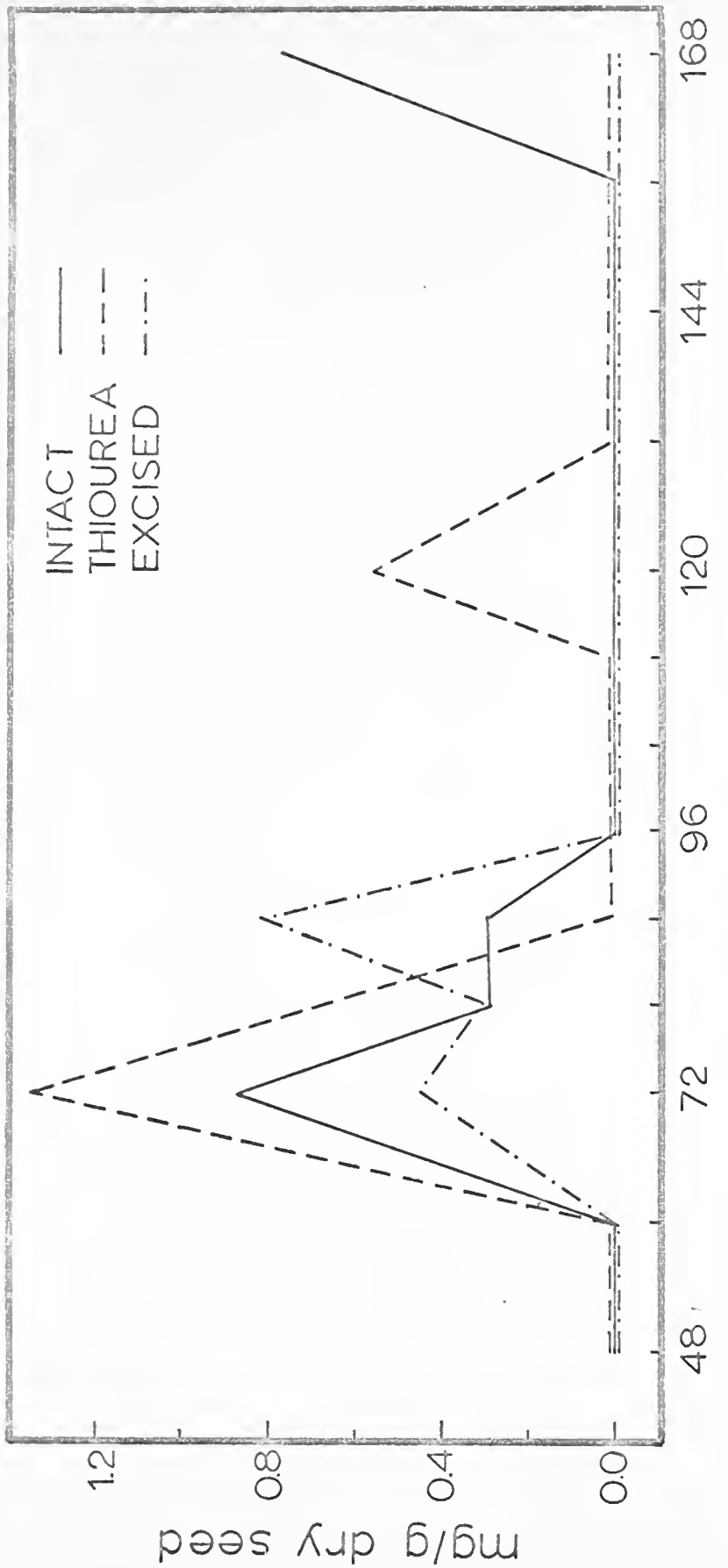


Fig. 5. Change in the content of mandelonitrile in peach seeds at various intervals after start of imbibition under the designated treatments.

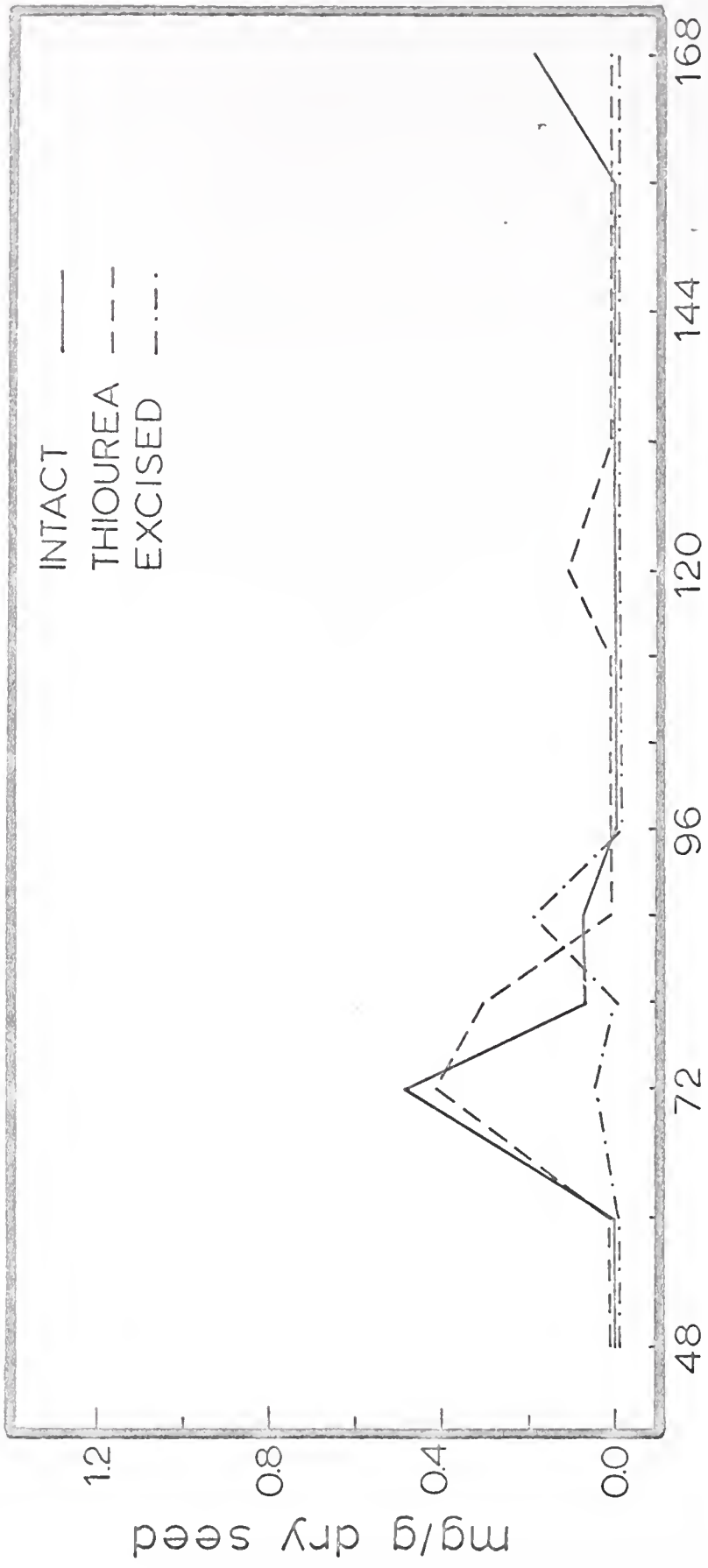


Fig. 6. Change in the content of benzaldehyde in peach seeds at various intervals after start of imbibition under the designated treatments.

120 hours after the start of imbibition. Relatively larger amounts of mandelonitrile than benzaldehyde were in the extracts from the seeds.

The effect of the 3 treatments on peach seed germination is shown in Figure 7. The excised seeds attained 100% germination at approximately 132 hours or about 44 hours after the peak in production of benzaldehyde and mandelonitrile. However, those seeds treated with thiourea required a much longer period of time after the peak production time in order to attain nearly 100% germination. This was true even when the time from the second peak at 120 hours was considered.

Quantitative determination of benzaldehyde, mandelonitrile and the inhibitory complex of seeds subjected to various degrees of chilling: Gas chromatographic determination of the quantities of benzaldehyde and mandelonitrile in peach seeds at weekly intervals during the chilling period indicated only trace amounts were present. Calculations indicated that the tissue level of both chemicals was below 1.0 ug/gm dry weight of tissue. Only trace amounts of benzaldehyde and mandelonitrile were detected by gas chromatography on seeds placed at 20° C for 40 hours after removal from various intervals of chilling.

The level of the 80% ethanol soluble inhibitory complex of peach seeds, as determined by the alfalfa bioassay, does not decrease during the chilling period (Figure 8). Slight week-to-week fluctuations were present but the overall analysis showed little change in the level. However, the per cent germination of seed periodically removed from the chilling temperatures and placed at 20° C indicated that 504 hours of chilling was sufficient to terminate dormancy in over 80% (Figure 9) of the population of the seeds.

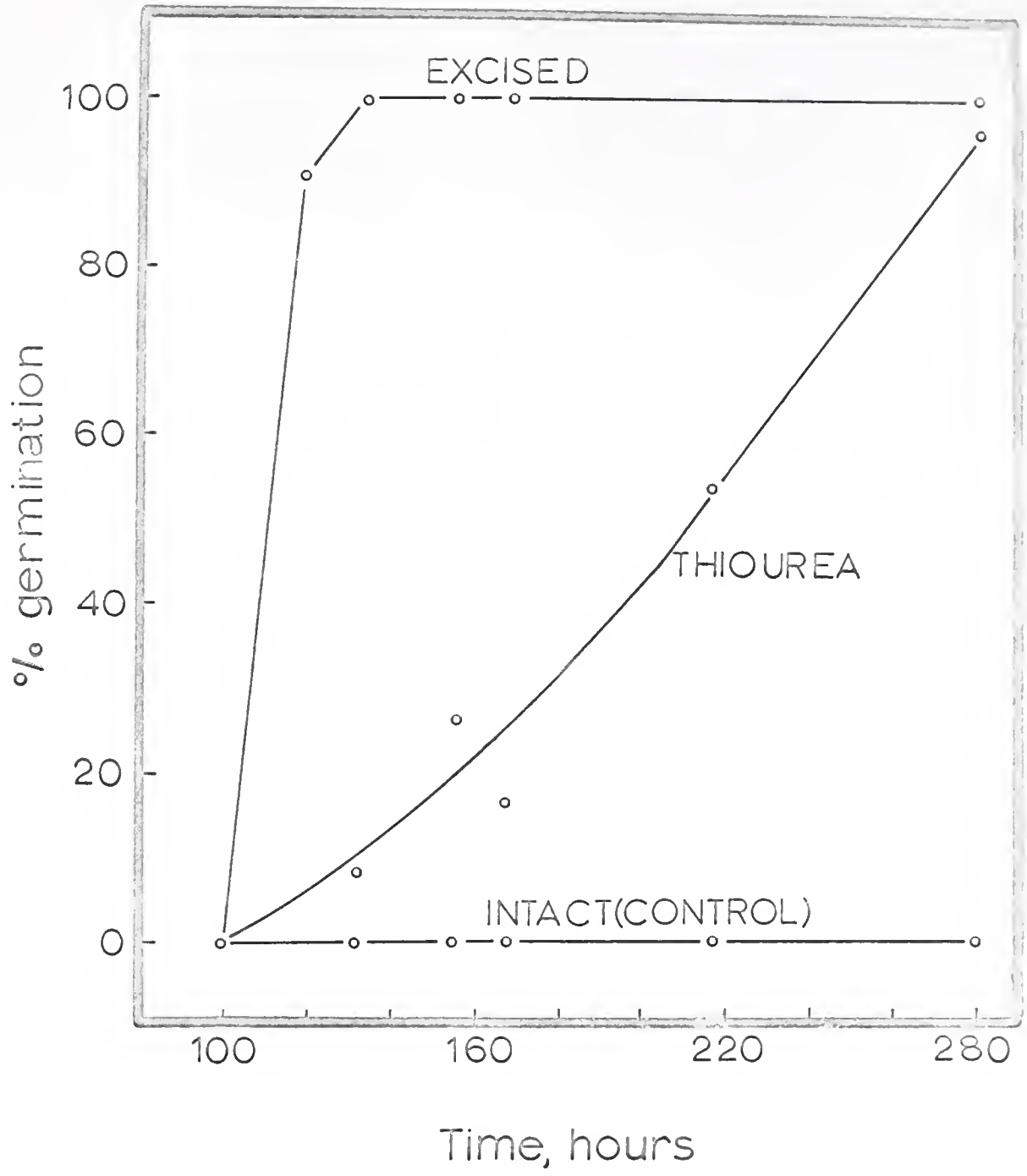
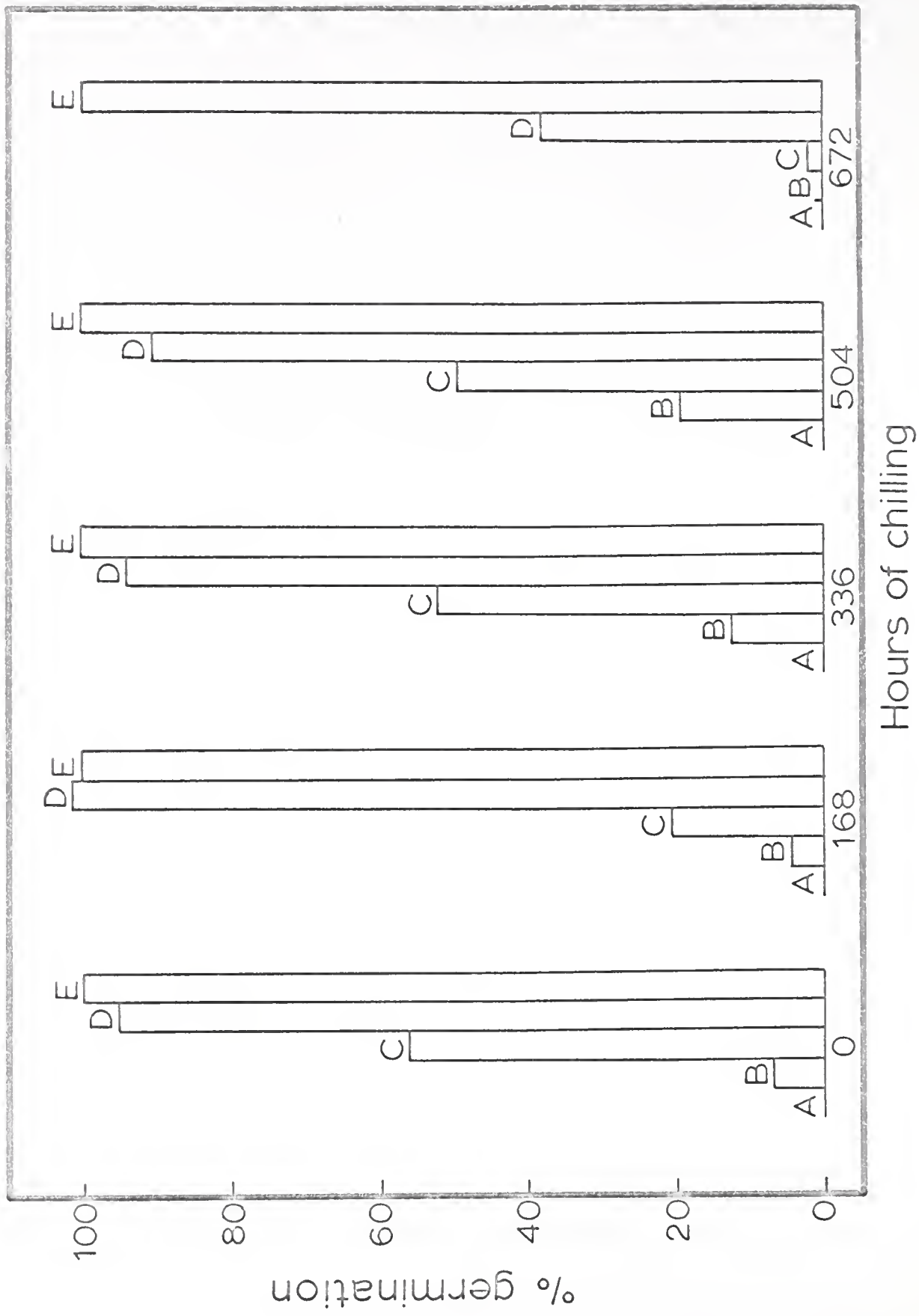


Fig. 7. Germination of peach seed as influenced by embryo excision, and thiourea treatments as determined periodically after the start of imbibition. (Growth of excised embryos was taken to be equivalent to germination when the radicle had elongated to 2 mm.)

Fig. 8. Relative inhibitory activity, as measured by the alfalfa bioassay, of the inhibitory complex in an ethanolic extract of peach seeds chromatographing between R_f's 0.6 to 0.8. (Hours of chilling were just prior to extraction; and seed equivalents were A= 1.0 g. B= 0.5 g, C= 0.3 g, D= 0.1 g and E= control.)


% germination
100
80
60
40
20
0
A
B
C
D
E
0
168
336
504
672
Hours of chilling

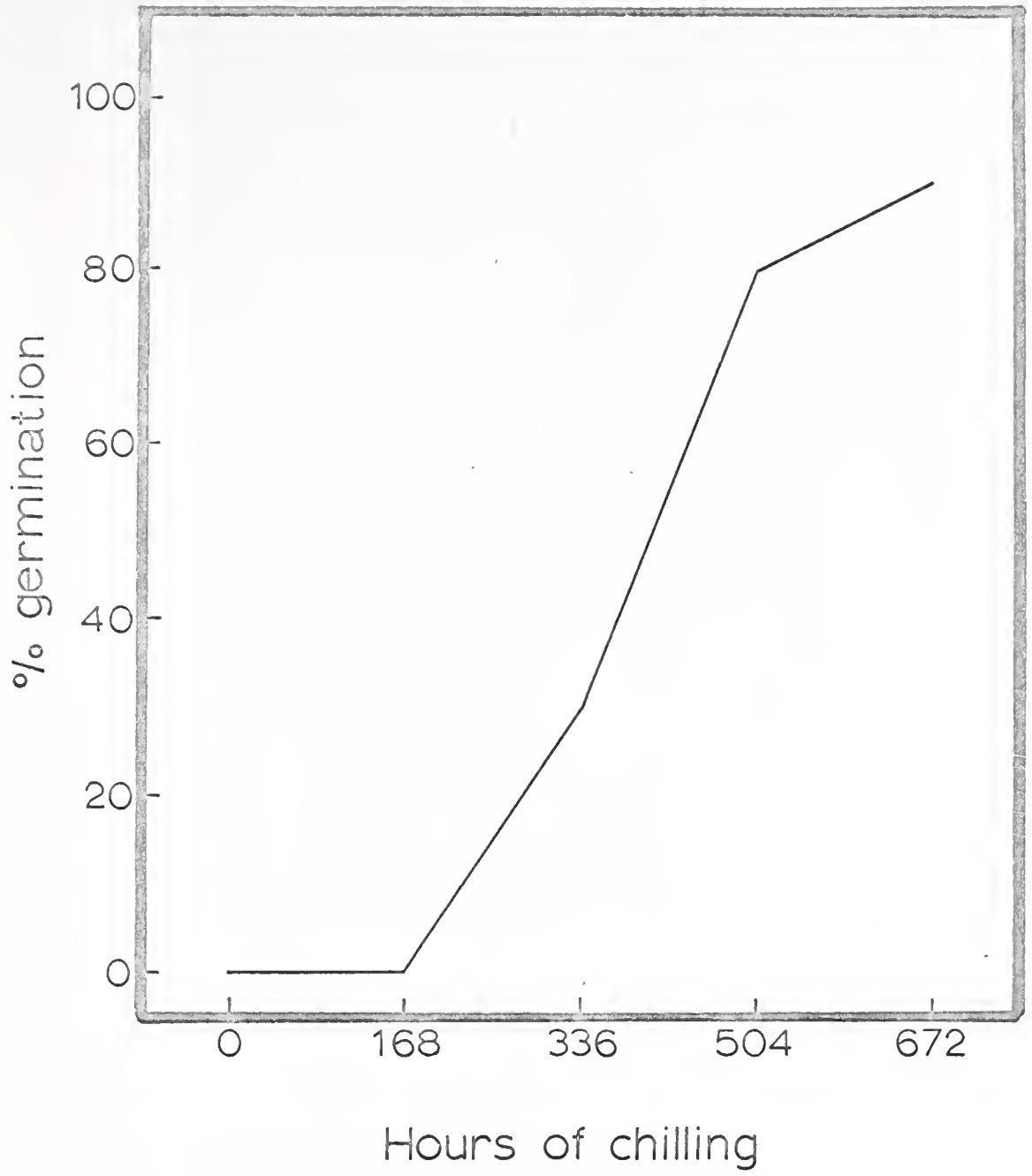


Fig. 9. Germination of peach seeds as influenced by 5° C of varying durations.

Influence of chemicals on possible stimulation of peach seed germination: The influence of benzaldehyde, mandelonitrile, benzoic acid and p-hydroxybenzoic acid on the breaking of seed dormancy was determined and the test differed from that for inhibition of germination in that they were applied to intact seeds. Using benzaldehyde and mandelonitrile at various concentrations, there was no evidence for a stimulatory effect on seed germination (Table 10). However, the 2 chemicals were active in inhibiting the weak capacity for germination, which supported the data of an inhibitory influence as shown earlier.

Benzoic and p-hydroxybenzoic acids were used on intact seed at concentrations ranging from 10^{-3} to 10^{-1} M. The data in Table 11 indicates that p-hydroxybenzoic acid at 10^{-1} and 3×10^{-2} M may have slightly stimulated seed germination as compared to the control; yet this is of doubtful significance since the control had a weak capacity to germinate (compare Tables 10 and 11). Benzoic acid had little influence on germination under the conditions of these tests.

L-mandelic and p-hydroxybenzoic acid determinations: Gas chromatograms of the control, peach seed extract, L-mandelic acid and p-hydroxybenzoic acid after treating with diazomethane for 30 minutes are shown in Figures 10, 11, and 12. These should be primarily the esters of the aromatic acids. These were separated on the gas chromatograph at an oven temperature of 180° C. The methoxy and butoxy esters were separated by gas chromatography after acetylation by diazomethane and diazobutane, respectively. Since these derivatives were more volatile, an oven temperature of 150° C was used.

Based upon the comparison of retention times of the components in the extract with those of the standards (Table 12), it was concluded

Table 10.--Mean per cent germination of dormant 'Okinawa' peach seeds 30 days after start of imbibition as influenced by benzaldehyde and mandelonitrile concentrations[x].

Concentration, M[y]	% Germination	
	Benzaldehyde[z]	Mandelonitrile[z]
1.0	0.0 a	0.0 a
10^{-1}	0.0 a	0.0 a
3×10^{-2}	16.7 b	0.0 a
10^{-2}	22.2 b	38.9 b
0	33.4 b	33.4 b

[x]Seeds were imbibed for 2 days, then exposed to chemical concentrations for 5 days.

[y]Each treatment was replicated 3 times with 6 seed per replication.

[z]Means not having a following letter in common were significantly different at the 5% level.

Table 11.--Mean per cent germination of dormant 'Okinawa' peach seeds 30 days after start of imbibition as influenced by benzoic and p-hydroxybenzoic acid concentrations[x].

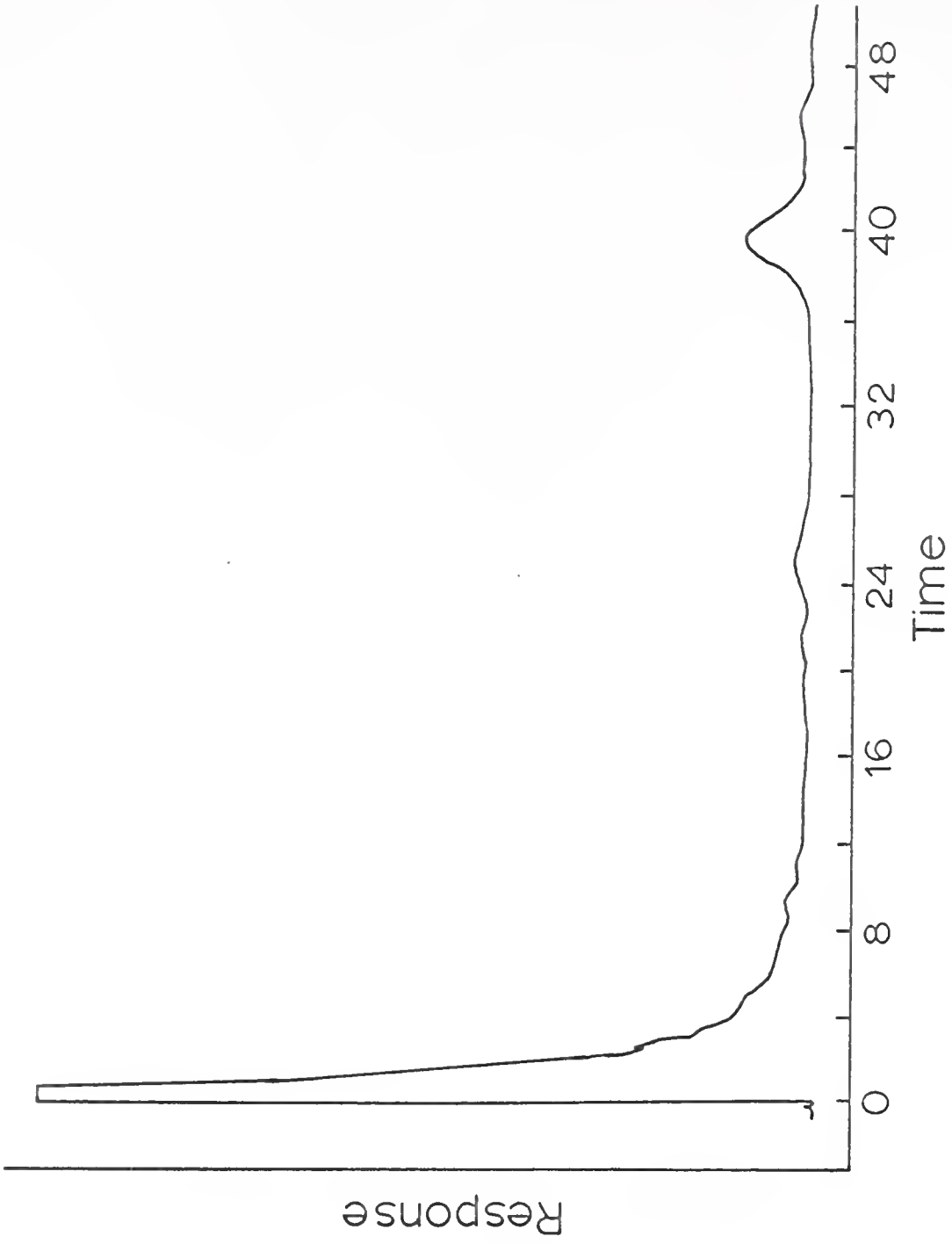
	% Germination	
Concentration, M[y]	Benzoic[z]	p-Hydroxybenzoic[z]
10^{-1}	5.5 a	38.9 b
3×10^{-2}	0.0 a	38.9 b
10^{-2}	16.7 a	11.2 a
10^{-3}	16.7 a	11.2 a
0	16.7 a	16.7 a

[x]Seeds were in moist medium for 2 days, then exposed to chemical concentrations for 5 days.

[y]Each treatment was replicated 3 times with 6 seed per replication.

[z]Means not having a following letter in common are significantly different at the 5% level.

Fig. 10. Gas chromatogram of diazomethane-solvent control. Retention time is in minutes. (See Table 6 for parameters of gas chromatograph.)


Response
0
8
16
24
32
40
48
Time

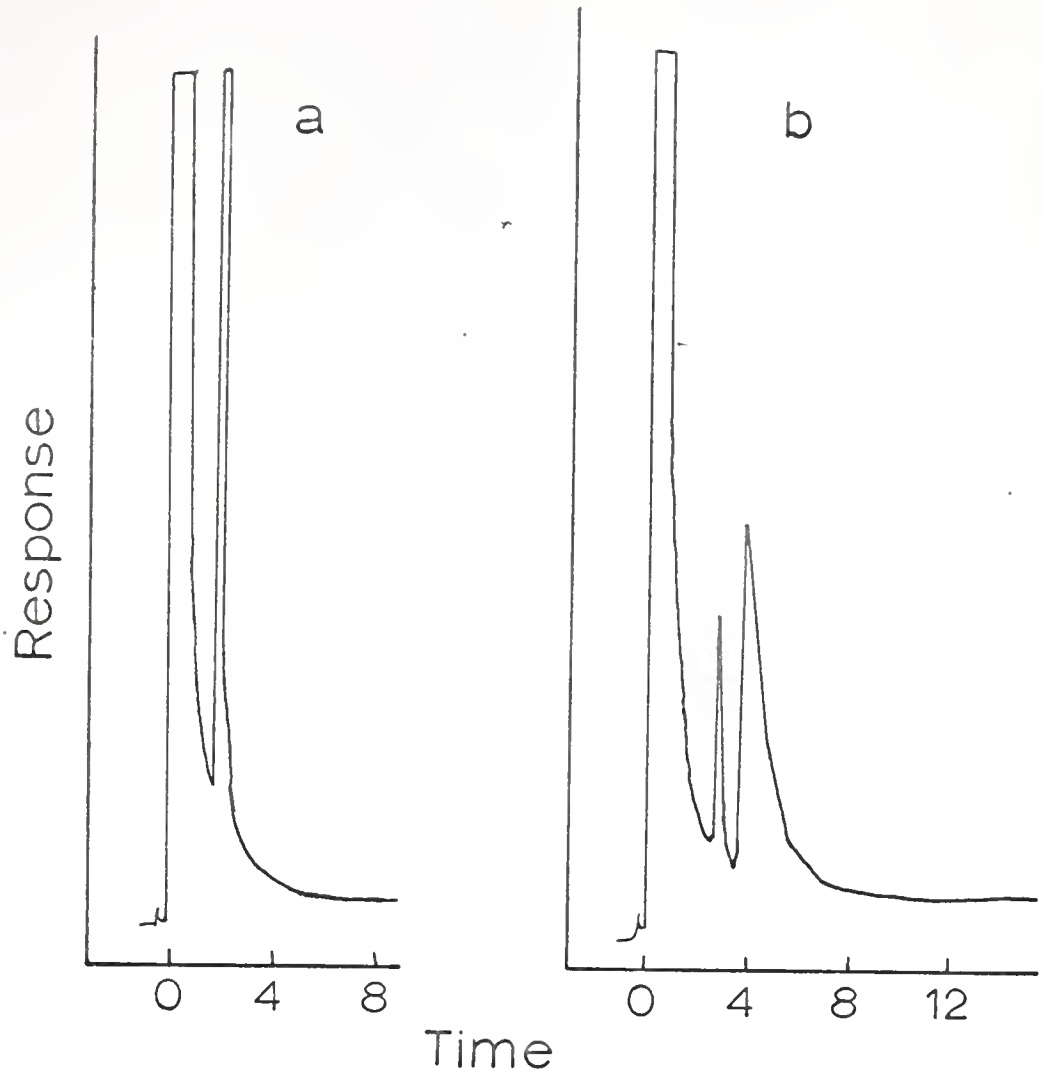


Fig. 11. Gas chromatograms of L-mandelic acid(a) and p-hydroxybenzoic acid(b) treated for 30 minutes with diazomethane. Retention time is in minutes. (See Table 6 for parameters of gas chromatograph.)

Fig. 12. Gas chromatogram of an ethanol extract from peach seeds treated for 30 minutes with diazomethane. Retention time is in minutes. (See Table 6 for parameters of gas chromatograph.) (M= L-mandelic acid and pHBA= p-hydroxybenzoic acid.)

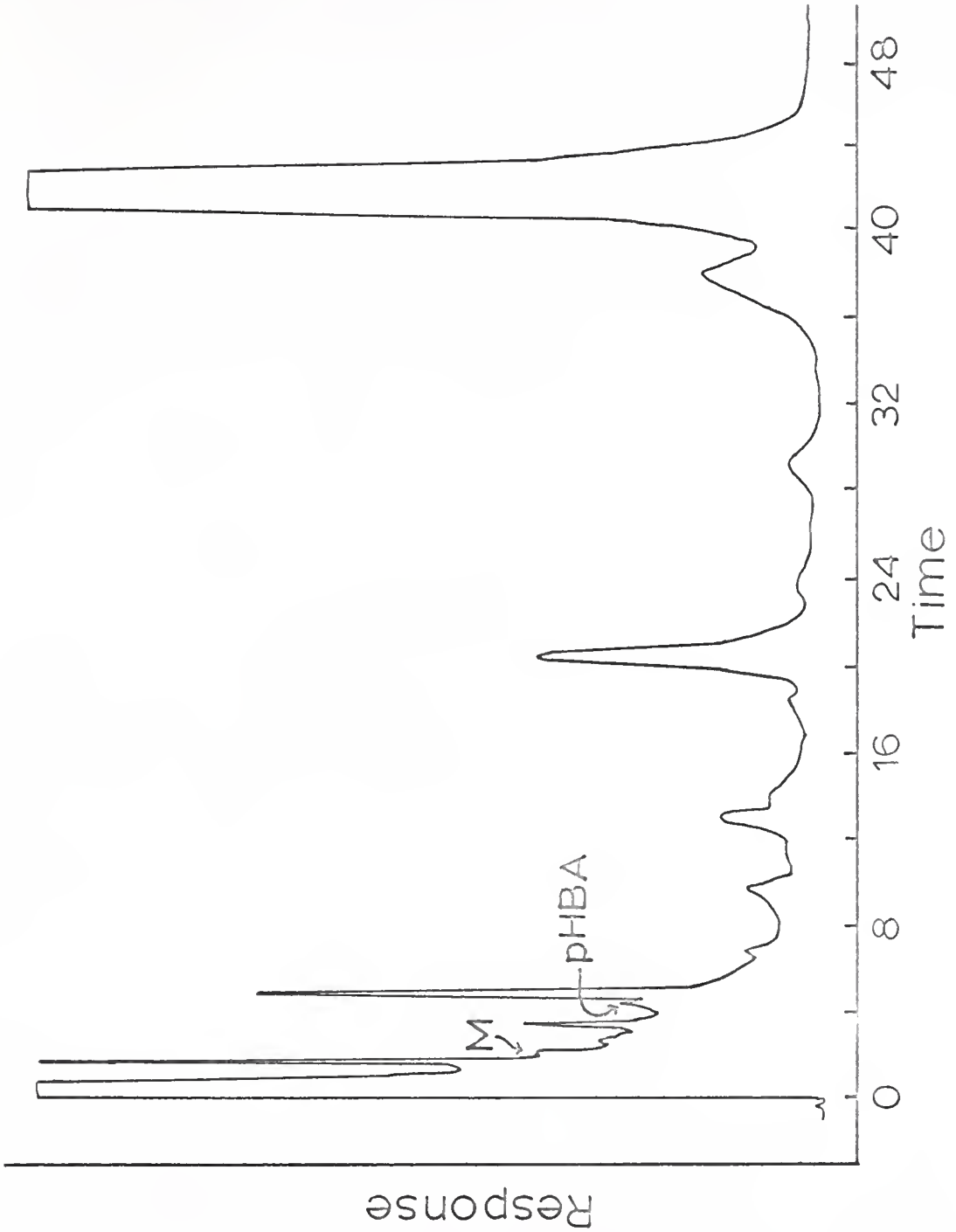

Response
0
8
16
24
32
40
48
Time
pHBA

Table 12.--Comparison of retention times of p-hydroxybenzoic acid and L-mandelic acid as influenced by various acetylation procedures.

Acetylation procedure[x]	Retention times (minutes)	
	Extract	Standard
1-diazomethane, 30 minutes:		
p-Hydroxybenzoic acid	4.10	4.10
L-Mandelic acid	2.05	2.05
2-diazomethane, 3 hours:		
p-Hydroxybenzoic acid	11.80	11.80
L-Mandelic acid	4.57	4.57
3-diazobutane, 3 hours:		
p-Hydroxybenzoic acid	12.60	12.90
L-Mandelic acid		
Methyl derivative	4.48	4.48
Butyl derivative	6.70	6.35

[x]Refer to Materials and Methods for the details of each procedure.

that L-mandelic acid and p-hydroxybenzoic acid were present in peach seeds after 48 hours' imbibition. However, only trace amounts of L-mandelic acid could be detected in the extract (Table 13). On the other hand, sufficient quantities of p-hydroxybenzoic acid were present for an estimation of amounts in the tissue. Based on peak area comparison of components of the extract with the standard, it was estimated that approximately 0.12 ug of p-hydroxybenzoic acid was present in 1 g wet weight of seed tissue under the conditions of the tests.

Table 13.--Comparison of peak areas of p-hydroxybenzoic acid and L-mandelic acid as influenced by various acetylation procedures.

Acetylation procedure[x]	Peak area, mm^2	
	Extract	Standard[y]
1-diazomethane, 30 minutes:		
p-Hydroxybenzoic acid	45.0	540.0
L-Mandelic acid	Approx. 5.0	597.0
2-diazomethane, 3 hours:		
p-Hydroxybenzoic acid	49.0	342.0
L-Mandelic acid	Approx. 5.0	675.0
3-diazobutane, 3 hours:		
p-Hydroxybenzoic acid	30.0	545.0
L-Mandelic acid		
Methyl derivative	Approx. 5.0	996.0
Butyl derivative	Approx. 5.0	812.0

[x]Refer to Materials and Methods for the details of each procedure.

[y]Each standard represents 2 ug of the respective compound.

DISCUSSION

A quantitative method was devised to determine the amount of benzaldehyde and mandelonitrile in peach seeds. The method was designed to quantitatively obtain the 2 volatile components in an ethereal solution so it could be analyzed by gas chromatography. Grinding of the frozen tissue and extraction at a low temperature prevented enzymatic release and destruction of benzaldehyde and mandelonitrile. Keeping the ethereal solution cool and immediate analysis prevented loss by volatilization. Also, the analysis by gas chromatograph was done before and after reaction with aqueous sodium bisulfite and again after reacting the benzaldehyde-sulfite addition product with potassium cyanide to form mandelonitrile. The chemical reactions used are well known (26), and the determination of pure benzaldehyde and mandelonitrile by the technique described was shown to be quantitative.

Using this procedure, the quantity and rate of release of mandelonitrile and benzaldehyde were studied in relation to germination. The data indicated that a lag time existed in excised seeds for the maximum release of mandelonitrile and benzaldehyde. The intact seeds and those treated with thiourea differed only in magnitude and the thiourea-treated seeds exhibited a secondary peak at 120 hours.

The maximum period of germination of the excised seeds was about 48 hours after the maximum period of release of benzaldehyde and mandelonitrile whereas the greatest period of germination of seeds treated with thiourea occurred about 160 hours after the second peak of release of

benzaldehyde and mandelonitrile. Thus, there was no indication that either benzaldehyde or mandelonitrile was correlated with an inhibition of germination under conditions of these tests. Yet, when concentrations of benzaldehyde and mandelonitrile are present in tissues at concentrations of 11.0 and 4.2 mg/g respectively, they would be affecting the system. Thus, the 2 compounds may have a temporary influence on germination, and it could be possible under certain circumstances a factor contributing to dormancy.

Determinations were also made of the content of mandelonitrile and benzaldehyde present in peach seeds at weekly intervals during chilling. Only trace amounts were observed at the various times of sampling. Thus, it seems that the majority of mandelonitrile and benzaldehyde was released between about 72 and 96 hours after the start of imbibition.

This was the first reported instance of the detection and measurement of mandelonitrile in peach seeds. It may be significant that the quantity of mandelonitrile present was much greater than that of benzaldehyde. This would indicate that the hydrolysis of amygdalin in intact seeds was not the same as in seed homogenates. In the latter case the products formed are benzaldehyde and cyanide with the mandelonitrile considered an unstable intermediate (104).

Interest was also directed at the presence of phenolic acids in peach seeds. Using gas chromatographic techniques, benzoic, o-hydroxycinnamic, 2,6-dihydroxybenzoic, o-hydroxybenzoic, p-hydroxybenzoic, 2,4-dimethoxybenzoic and mandelic acids were isolated and tentatively identified. Of primary interest was p-hydroxybenzoic acid since it had been reported in the literature as having growth regulator actions (105). With this in mind, it was necessary to determine if p-hydroxybenzoic acid

existed in peach seeds. Using extraction procedure for phenolic acids (113), gas chromatographic analysis of various derivatives were made of the extracted components. Positive identification was made for p-hydroxybenzoic and a strong indication was noted that L-mandelic acid was present in the seeds. The latter has been reported to occur in peach seeds (R. H. Biggs, Unpublished data), and found to inhibit germination of alfalfa seeds at 10^{-6} M concentrations. Jones and Enzie (46) identified a growth-inhibiting substance from peach flower buds as being mandelonitrile.

Since degradation products of amygdalin were found to occur in seeds, attempts were made to assess the influence of cyanide, benzaldehyde and mandelonitrile on germination. Cyanide treatments indicated that only at the highest concentration tested, 1.0 M, was an inhibitory influence shown. Recently, it has been reported that some plants, particularly Vicia sp., have the capability of metabolizing cyanide and converting it into non-toxic compounds (34, 69). It was observed that hydrogen cyanide (^{14}C) was incorporated into asparagine in a number of plant species. This was thought to be accomplished by cyanide coupling with serine directly to form the 4-carbon chain of beta-cyanoalanine. The beta-cyanoalanine could then form asparagine, or by addition of a gamma-glutamyl group, form gamma-glutamyl-beta-cyanoalanine. It was concluded that cyanide had little influence on germination, except at concentrations considered quite high. Interestingly, this indicates that peach seed do contain a cyanide-resistant mechanism for respiration. Furthermore, the subsequent seedlings were much greener and exhibited other characteristics that accompany nitrogen fertilization. Thus, it was concluded that the tissues were incorporating cyanide.

In contrast to the results of cyanide treatments, concentrations of 4.2 mg/g mandelonitrile and 11.0 mg/g benzaldehyde completely inhibited germination of excised embryos. It was noted that mandelonitrile inhibited at a weaker concentration and that the intermediate concentration of both compounds had an action that was reversible. Thus, if high enough concentrations of benzaldehyde or mandelonitrile did occur in seeds they could be inhibitory and the action could be transitory if the compounds were subsequently degraded (104).

The possibility that subsequent derivatives of benzaldehyde could be involved in seed germination was investigated. Thus, the influence of benzoic and p-hydroxybenzoic acids on dormant peach seeds was tested. Benzoic acid had little influence, but concentrations of p-hydroxybenzoic acid at 3×10^{-2} and 10^{-1} M significantly increased the degree of germination as compared to the control.

The fact that p-hydroxybenzoic acid has been found to have growth regulatory properties (105) and its presence in peach seeds suggested that it may play a role in dormancy. The growth regulatory activity of p-hydroxybenzoic acid has been established for woody cuttings of Ribes rubrum (105). Pilet (78) has reported that p-hydroxybenzoic acid at low concentrations causes a stimulation of the growth of stem sections, while at high concentrations it inhibits growth. The inhibition was apparently due to the stimulation of IAA-oxidase and subsequent decrease in auxin level (116). Also, the activation observed for lower concentrations of p-hydroxybenzoic acid indicated that it acted on several other biochemical processes which were connected with growth (78).

In Ribes rubrum, p-hydroxybenzoic acid was present in the range of 0.2 - 1.0 ug/g of fresh tissue (105). The quantity found in peach seeds was approximately 0.12 ug/g of fresh tissue. Thus it appears that the

tissue-levels of p-hydroxybenzoic acid in both dormant Ribes woody stems and dormant peach seeds were similar. In the case of Lens stems, internode sections were stimulated to elongate at 10^{-6} M concentration (78). At higher concentrations, the growth of stem sections was inhibited. The quantity isolated from peach seeds was in the range that was inhibitory in the Lens bioassay. Thus, it could be inhibitory to the seeds. However the data was such with peach seeds that this point can be considered a matter of conjectual. Yet, it was shown that p-hydroxybenzoic acid would stimulate peach seed germination at 3×10^{-2} to 10^{-1} M concentration. This stimulation was from adding p-hydroxybenzoic acid to the external media for 120 hours. Thus, the internal concentration could have been much lower. The bioassay would not demonstrate an inhibitory effect.

The ethanolic extracted inhibitory-complex obtained from peach seeds was studied with the use of paper chromatography and other physical treatments in order to obtain some clues as to its identity. The R_f values obtained on paper chromatography correspond with the inhibitory area obtained by Bennet-Clark and Kefford (8) using the same solvent with an alcoholic extract from Ribes sp. This inhibitory area was termed the beta-inhibitor complex. Recently, the beta-inhibitor concentrated from an acidic fraction from extracts of dormant maple buds was thought to be a complex of phenolic substances (86). However, the phenolic compounds described were found not to be identical with any of the phenolic compound previously proposed as being members of the beta-inhibitor complex. Recently, many of the phenolic compounds associated with the beta-inhibitor complex have been identified. Koves and Varga (53) reported the identification of many phenolic compounds, among which were several of the hydroxybenzoic acids.

The data on the level of the inhibitor-complex during the chilling period showed that it did not change drastically. In fact, at the end of chilling period, the level seemed to be greater than anytime during chilling. This finding was in line with that found by Villiers and Wareing (106, 107) for dormant organs of Fraxinus excelsior. Briefly, chilling has no effect on the level of inhibitors in the tissue but termination of dormancy was accompanied by a buildup in growth promotors in the seed. This may be the case for peach seeds since the capacity to germinate increased with increases in the duration of the chilling period.

The products of amygdalin degradation, mandelonitrile, benzaldehyde and cyanide do not appear to directly influence the breaking of peach seed dormancy. However, it would seem that a hydroxylated derivative, p-hydroxybenzoic acid, of the benzaldehyde oxidation product, benzoic acid, exhibits some stimulatory influence upon dormant peach seeds. Furthermore, L-mandelic acid, as well as other phenolic compounds, may be involved in peach seed dormancy.

The induction of germination of peach seed by thiourea substantiated previous reports that this chemical will terminate seed dormancy (76, 97). Furthermore, it supported earlier observations (30, 80) that the growth of the subsequent seedlings was not abnormal if the proper environmental condition were maintained during germination.

SUMMARY AND CONCLUSIONS

Investigations were initiated to determine the relation of certain phenolic compounds to peach seed germination. The phenolic compounds of primary interest were those which are degradation products of the glucoside, amygdalin, namely, mandelonitrile and benzaldehyde, and their immediate by-products. The following conclusions were made based on the research conducted:

1. Mandelonitrile and benzaldehyde at 1.4 to 11.0 mg/g of perlite inhibited germination of excised embryos, but did not stimulate dormant seeds to germinate. Quantitative determinations of these 2 compounds from peach seeds by gas chromatography indicated that the majority of the mandelonitrile and benzaldehyde was released between 72 and 96 hours after the start of imbibition and thereafter only trace amounts could be observed. Only at this time was the tissue-level high enough to be considered inhibitory to germination, yet it showed no correlation with germination. Furthermore, determinations made at weekly intervals during chilling indicated that only trace amounts were present at any of the sampling times during chilling. Therefore, it was concluded that mandelonitrile and benzaldehyde have no direct, inhibitory or promotive influence on the germination of peach seeds.

Cyanide had little effect on reducing the per cent germination at concentrations less than 1.0 M. From observations on the increased size of seedlings in several cyanide treatments, it was postulated that the tissues were incorporating cyanide, however no measurements of the increase in glucoside content was made.

2. The fact that phenolic acids could be produced in dormant peach seeds as a result of the metabolism of mandelonitrile and benzaldehyde, led to an investigation of phenolic acids in dormant peach seeds, and several phenolic acids, including the hydroxy and methoxy derivatives of benzoic acid, were found. Of prime interest was the finding that p-hydroxybenzoic acid at concentrations of 3×10^{-2} to 10^{-1} M would slightly stimulate the germination of dormant peach seeds.. However, quantitative determinations showed that approximately 0.12 ug of p-hydroxybenzoic acid was present per 1.0 g of tissue on a wet weight basis which was below that found to be necessary in the external media for germination. Determinations of the tissue-levels of p-hydroxybenzoic acid in germinated seed was not conducted. L-mandelic acid, a product of mandelonitrile hydrolysis, was also shown to be present in amounts in the range of 0.005 to 0.05 ug/g of fresh tissue. The influence of L-mandelic acid on peach seed germination was not studied.

3. The inhibitor-complex level of peach seeds which appeared on paper chromatograms at an R_f of 0.6 - 0.8 was found to be essentially the same after chilling as prior to chilling. Thus this complex does not appear to be involved in the maintenance of dormancy of peach seeds. The inhibitory-complex had similar characteristics to the beta-inhibitor complex reported to be found in other plant tissues.

4. Experiments with thiourea supported previous research and showed that the time of embryo removal from the seed coat and associative tissue after seed imbibition had little influence on the amount of abnormal seedling production.

APPENDIX: GAS CHROMATOGRAMS OF STANDARDS

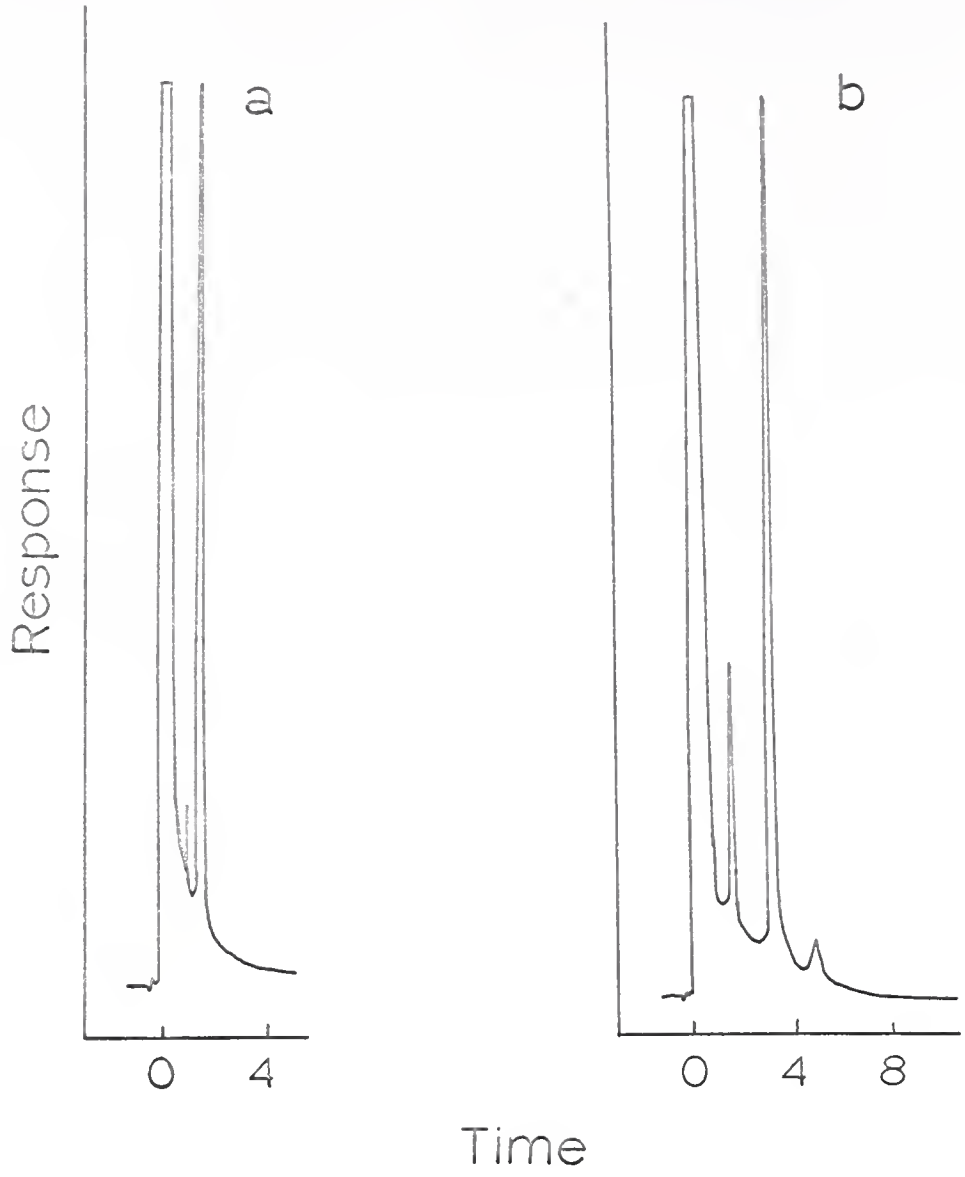


Fig. 13. Gas chromatograms of the propyl esters of benzoic acid(a) and mandelic acid(b). Retention time in minutes. (See Table 6 for parameters of gas chromatograph.)

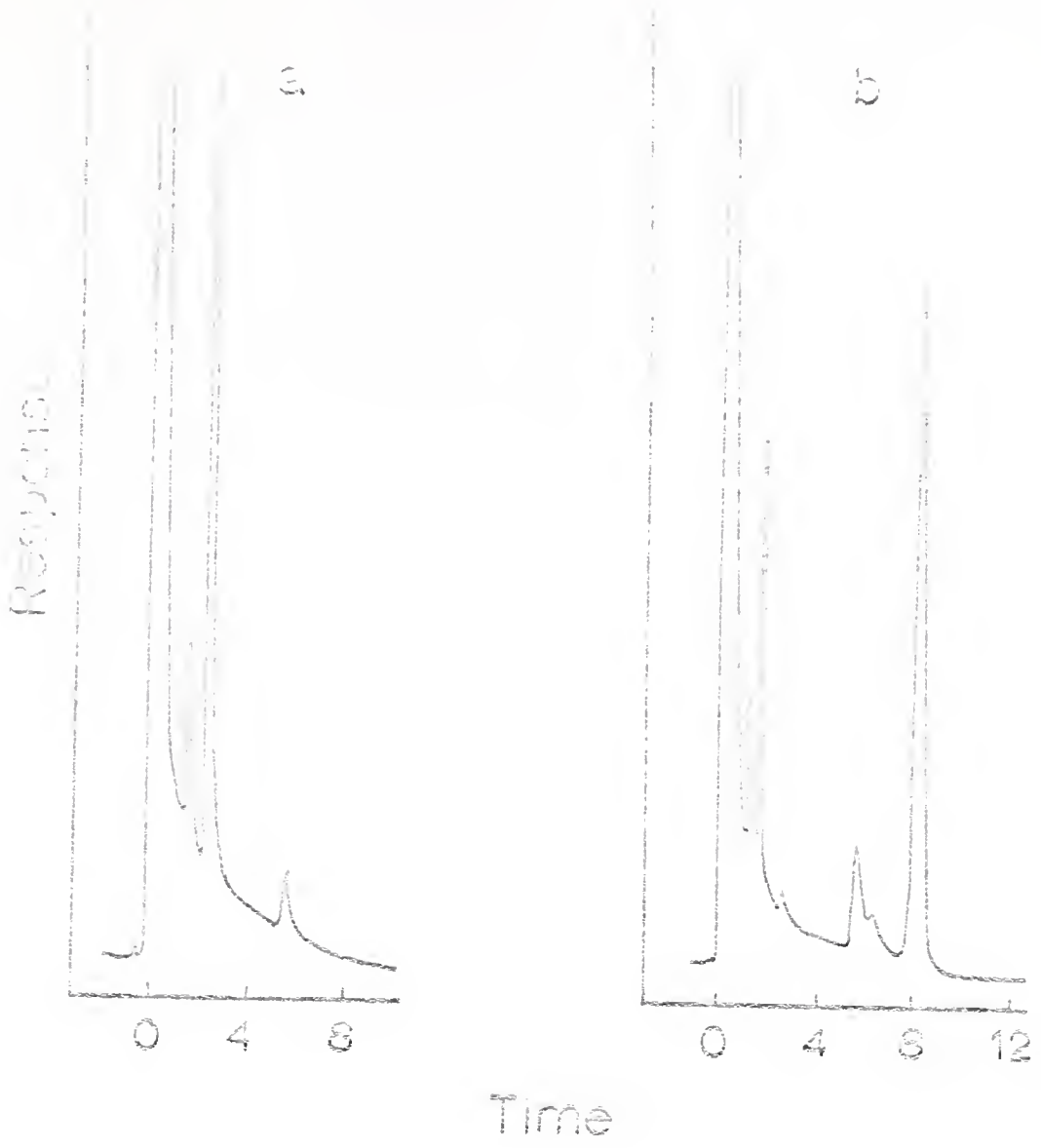


Fig. 14. Gas chromatograms of the propyl esters of o-hydroxybenzoic acid(a) and p-hydroxybenzoic acid(b). Retention time in minutes. (See Table 6 for parameters of gas chromatograph.)

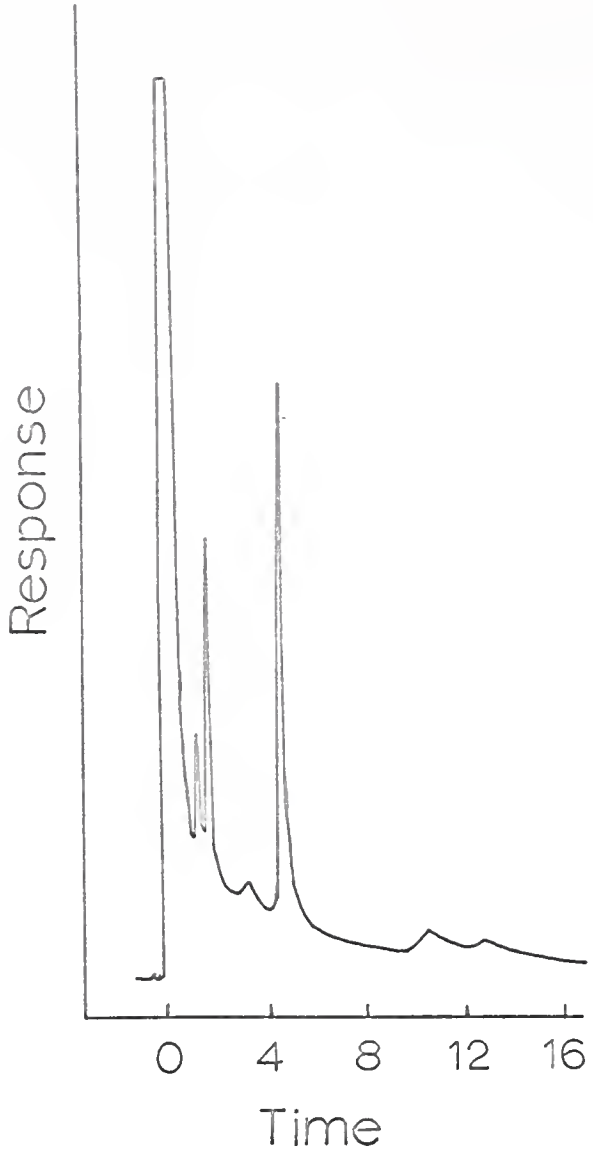


Fig. 15. Gas chromatograms of the propyl ester of 2,6-dihydroxybenzoic acid. Retention time in minutes. (See Table 6 for parameters of gas chromatograph.)

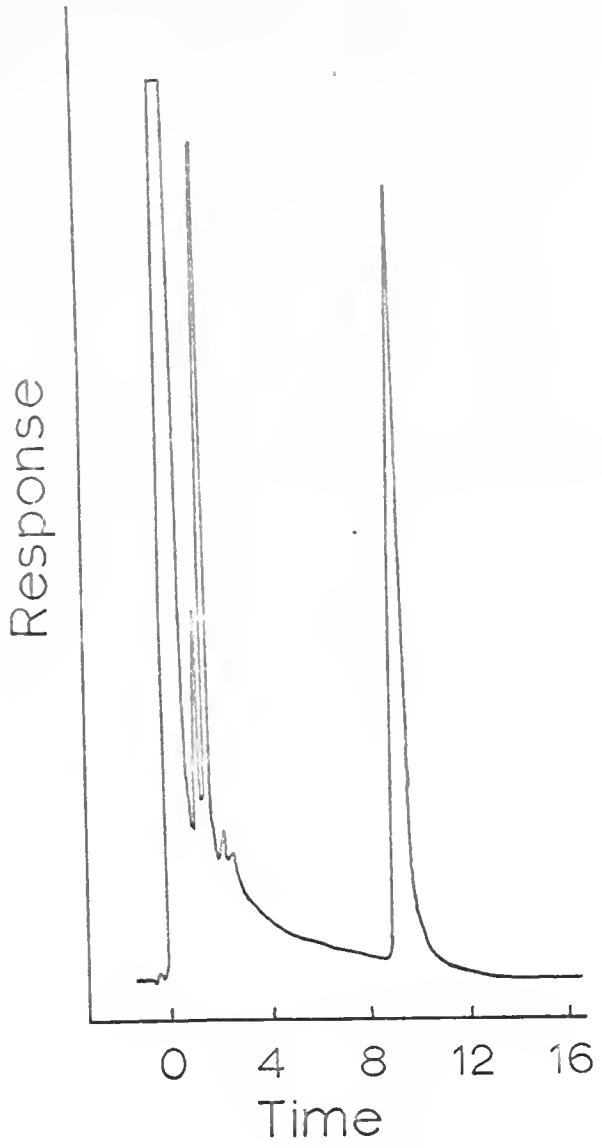


Fig. 16. Gas chromatograms of the propyl ester of 2,4-dimethoxybenzoic acid. Retention time in minutes. (See Table 6 for parameters of gas chromatograph.)

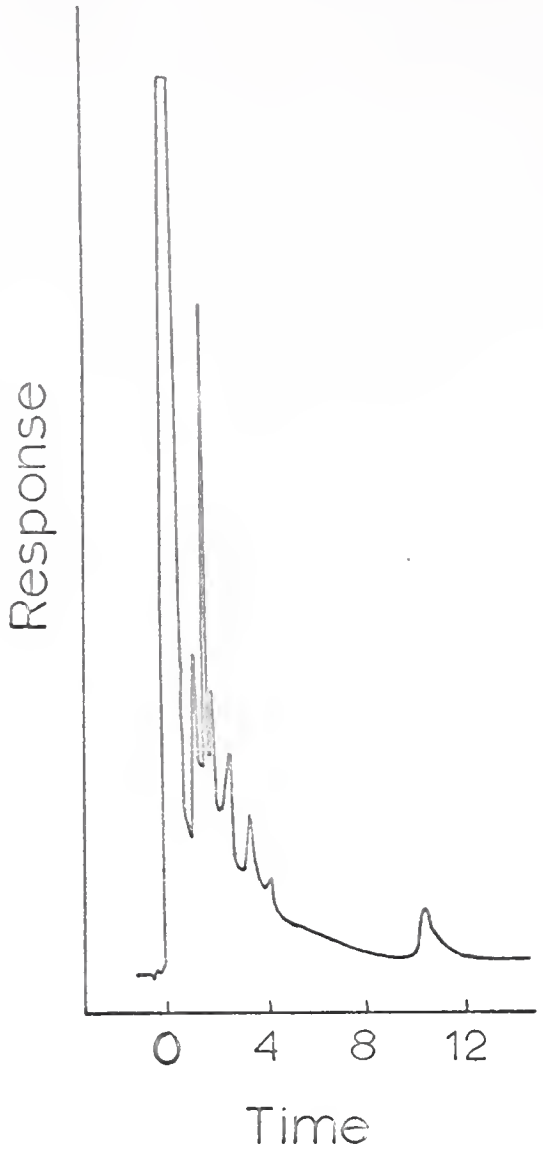


Fig. 17. Gas chromatograms of the propyl ester of o-hydroxycinnamic acid. Retention time in minutes. (See Table 6 for parameters of gas chromatograph.)

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BIOGRAPHICAL SKETCH

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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