THE INFLUENCE OF SIMAZINE ON THE QUALITY AND COMPOSITION OF POME FRUIT

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ABSTRACT

THE INFLUENCE OF SIMAZINE ON THE QUALITY AND COMPOSITION OF POME FRUIT

by Donald Bernard Carlson

The successive annual application of simazine is a common practice in many Michigan fruit orchards. It was therefore postulated that simazine may have an obscure influence on fruit quality. The influence could be due to the direct effect of the chemical, or the well documented indirect effect of increasing the foliage nitrogen; which may be a critical factor in the quality of pome fruit.

Simazine applications increased the foliage nitrogen in only one pear and one apple orchard, out of a total of nine experiments conducted during 1965 and 1966. The increase in nitrogen level of apple foliage was not reflected in the protein content of the buds, fruit firmness, total pectic material, soluble solids or titratable acidity. In addition, protein content and the respiration rate of whole apple fruit were not altered.

In one experiment one pound of supplemental nitrogen per tree, as compared with non and one-half pound, increased the apple foliage nitrogen.

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Simazine slightly reduced the level of soluble solids in one apple and one pear orchard in 1965. No other effects of simazine were observed for fruit from these orchards, and a similar reponse was not observed for fruit from the apple orchard in 1966, negating the practical significance of this observation. The application of simazine to the remaining orchards had no influence on either the nitrogen metabolism, or any of the aforementioned quality or composition parameters. Under the conditions of these experiments simazine had no obscure effects on the quality of apple or pear fruit.

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By

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INTRODUCTION

The controversial book "Silent Spring", by Rachel Carson (16), called attention to the widespread use of pesticides in our environment. The publicity and reviews following the appearance of this publication acted as a major stimulus to numerous investigations. In 1950, twelve years prior to the printing of this text, the 81st Congress of the United States passed a resolution to create a committee to conduct an investigation of "the nature, extent, and effect of the use of chemicals, compounds and synthetics on the production, processing, preparation and packaging of food products." (86). The purpose was to determine the effect of the use of these materials. This was in the interests of the health and welfare of the nation, and the stability and well-being of the agricultural economy.

Unfortunately, little attention has been given to the possibility that the pesticides we use may not only have detrimental effects upon the organisms exposed to them, but may have harmful effects on the crops they are often used to protect. A deleterious effect on the quality or composition of a foodstuff could outweigh the advantages gained through control of a plant pest.

Simazine (2-chloro-4,6-bis(ethylamino)-S-triazine), introduced in 1955 (90), has become a widely used selective herbicide for the control

of broadleaf and grassy weeds in numerous horticultural crops. This chemical has been found applicable to weed problems in deciduous orchards.

The objective of this research was to determine the effects of simazine on the quality and composition of deciduous tree fruit, and the composition of selected fruit tree portions, with primary emphasis being placed on apples of the McIntosh cultivar.

REVIEW OF LITERATURE

Physical Properties of Simazine

Simazine, first reported to have herbicidal activity in 1956 (34), is a white, crystalline material, with a molecular weight of 201.7 and a melting point of 225-227°C (90). It is nonflammable and noncorrosive under normal use conditions. A low solubility in water, (varying from 2.0 ppm at 0°C, and 5.0 ppm at 20°C to 84 ppm at 85°C), and the adsorption to certain soil constituents, limits the leaching of simazine. Tests have shown that after application the greatest portion of the compound is found in the top two inches of the soil (15, 6). This gives a soil placement selectivity which is important in the case of some deep-rooting perennial crops.

Mode of Action -- The low solubility of simazine in water is accompanied by a low lipoid solubility, and as a result its toxicity, when applied to plant foliage is negligible (40). Davis et al. (21) found that unless the cuticle was disrupted in some manner, the foliar absorption of ^{14}C-labeled simazine, was limited, indicating that the intact cuticle is a rather effective barrier to the absorption of this compound. Simazine is readily taken up by the roots, and moves to the upper portions of the plant (21, 72). Sheets (72), using ^{14}C-labeled simazine, determined that uptake by the roots of seedling oats (Avena sativa, L. Cv. Kanota), and distribution

throughout the plant occurred within three hours. Absorption and translocation of the herbicide were greater at 37°C than at 26°C. In addition, with a constant temperature, absorption and translocation were greater in an environment of low relative humidity. This indicated that the amount of material in the plant is dependent on the transpiration rate.

Absorption involves entrance into the stele of the root, migration to the apoplast, and movement in the xylem (20). Once it reaches the leaf there is a marked tendency for marginal accumulation in wide leaves, accumulation at the tip of grass leaves, and a general distribution in the leaves of the tolerant corn species (20, 21). Following translocation it enters the living cells of the chlorenchyma, and, in the presence of light, is believed to block the photolysis of water and the evolution of oxygen. (Hill reaction). (27, 40, 59, 60). Photosynthesis is very likely the physiological system most sensitive to the activity of triazines. A 50 percent inhibition of the Hill reaction in isolated chloroplasts from corn (Zea mays L.) and spinach (Spinacia oleracea L.) was reported by Exer (27), using simazine at a concentration of 7×10^{-7} M. Moreland et al. (59), working with isolated barley (Hordeum vulgare L.) chloroplasts, and Moreland and Hill (60) using chloroplasts isolated from turnip greens (Brassica spp), found a 50 percent reduction of photochemical activity at simazine concentrations of 4.6×10^{-6} M and 5.9×10^{-6} M, respectively.

Further proof of simazine's action on the photosynthetic process is its prevention of the accumulation of starch (32). However, plants may be kept alive and growing, in the presence of otherwise lethal concentrations of simazine, by supplying glucose through severed leaf tips (59).

Various investigations have demonstrated that simazine inhibits O_2 release in Elodea (68), CO_2 utilization by intact plants (87), and $^{14}CO_2$ fixation by illuminated bean leaves (5). Clearly, many of the toxic effects of triazine herbicides are related to photosynthesis. One exception to this mode of action is that suggested by Ries et al. (68). They suggest that simazine toxicity may be the result of excess production of nitrite or ammonia, or both.

Ashton et al.(4), found that the fine structure of chloroplasts in Phaseolus vulgaris L., was greatly altered by treatment with atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), a triazine with a similar mode of action to simazine. These changes occurred when the plant was kept in the light, but the chemical had no effect in the dark. These workers proposed that the alterations were brought about by the formation of a toxic substance, or substances, involving the interaction of atrazine and light in the presence of chlorophyll.

In addition to the effect on photosynthesis, there are reports in the literature on the influence of simazine and triazines in general,

on other physiological processes in plants. Increases (1, 63) and decreases (24, 31) in respiration have been detected. Differences under light and dark conditions in the distribution of ^{14}C in compounds, following ^{14}C-sucrose uptake in atrazine treated plants, indicated not only interference of photosnythesis, but also of other metabolic processes. (3)

Metabolism of Simazine -- Roth (68) was the first to observe that simazine was degraded by expressed corn sap, whereas juice from a susceptible plant such as wheat did not degrade the herbicide. Further studies disclosed that simazine was converted, both in vitro and in vivo, to the non-phytotoxic substance 2-hydroxy-4,6-bis(ethylamino)-S-triazine. (17, 43 58). Metabolites other than hydroxysimazine have been found in barley (Hordeum vulgare L.), oats (Avena sativa L.) and sorghum (Sorghum vulgare) by Hamilton (42) opening the possibility of other degradative mechanisms. A dealkylated product of atrazine was reported by Shimabukuro (73) in both the roots and shoots of young pea plants. Funderburk and Davis (31) found $^{14}CO_2$ released from plants treated with chain-labeled simazine, suggesting the possibility of dealkylation of this herbicide.

Though the detoxification to hydroxy-simazine accounts for resistance in some crops, such as corn, little is known of the tolerance of fruit trees to herbicides such as simazine. In a study

employing reciprocal grafting of peach and apricot seedlings (81), it appeared that the greater tolerance of young peach trees over apricot trees to the triazines simazine and prometryne (2,4-bis(isopropylamino)-6-methylmercapto-5-triazine), was the result of a physiological detoxification occuring in the scion. (81). Although simazine is readily absorbed and translocated by the plant, sensitive residue determinations (0.02 - 0.06 ppm), of apple and pear fruit from field trial plots and treated commercial crops, have not disclosed detectable residues of simazine in any edible parts when the chemical was used at the recommended rate and time of application (30).

Simazine and Nitrogen Metabolism -- In 1963, Ries et al. (67) reported that peach and apple trees growing in soil sprayed with simazine had higher leaf nitrogen and more terminal growth than trees where the weeds were controlled by hand-hoeing or black plastic mulch. Similar observations on fruit trees were made by Karnatz (48).

A study of the effect of simazine on Monterey Pine (Pinus radiata) and corn, as influenced by lime, bases and aluminum sulfate, disclosed that simazine always increased top-root ratios of corn, but generally decreased those of pine seedlings (22). Simazine significantly increased the uptake of nitrogen by corn in all soils, of magnesium and phosphorus in limed and of potassium in acidified soils.

Simazine applied at 0.6 ppm in solution culture increased the yield of corn tops and the uptake of nitrogen, phosphorus, magnesium and potassium (29). Applications of 1.5 ppm simazine to soil in pots increased dry matter yields and uptake of nitrogen in corn only when additional nitrogen was applied to the soil. It was also found that inoculation of soil with simazine did not increase mineralization of the soil nitrogen, nor did it have any effect on immobilization of nitrogen. It was therefore suggested that the herbicide increased plant growth by a direct effect on plant metabolism and not through any interaction with the soil.

Ries and Gast (64) disclosed that the addition of simazine to nutrient solutions, in which corn was being grown, increased the nitrogen content of corn. In a second test, conducted by the same workers, under more favorable environmental conditions for growth (higher temperatures, longer days and higher light intensities), the total quantity of nitrogen per plant was not increased, but the percent nitrogen was increased in the shoots of corn grown at a low nitrogen level.

The nitrogen content of corn forage was found to increase as nitrogen fertilizer, simazine, and atrazine rates were increased (28). The percent nitrogen rose more sharply with increasing herbicide rate on plots not receiving nitrogen fertilizer than on plots receiving nitrogen. Grain nitrogen content increased as the fertilizer level increased, but was unaffected by herbicide treatments.

Similar descriptions of higher nitrogen percentages in corn due to atrazine treatment have been presented by Gramlich and Davis (37). Enhanced growth and total leaf nitrogen in citrus trees, growing in soils receiving simazine treatments, were reported by Goren and Monselise (36).

Tweedy (80) found that simazine increased the nitrogen content of corn plants grown in nutrient culture solutions under conditions of low temperature and low nitrate level. If, however, the ammonium ion was substituted for the nitrate ion, or if the nitrate levels or temperature were optimum for growth, no increase in nitrogen was observed with simazine applications. Further studies disclosed that this increase in nitrogen content of corn plants, grown under sub-optimal conditions (low temperature, low nitrate) was due to an effect on nitrate reductase (82). A ten-fold increase was found in the nitrate reductase activity of corn leaf extracts from plants grown in low nitrate levels, and at low temperatures, in the presence of simazine. Though this phenomenon occurred only under sub-optimal growing conditions, the authors emphasize that low nitrate and low temperature conditions are not unusual during the growth of plants.

Current research has shown that the protein content of plants may be increased by treatement with simazine (63). Rye plants receiving 0.5 to 0.8 micromolar simazine contained up to 45 percent more water extractable protein per plant than controls. Pea plants, grown to maturity, contained more seed protein when treated with simazine.

Cold Hardiness and Nitrogen Compounds -- Cold hardiness is of great interest to fruit growers. Some researchers have suggested that various nitrogen compounds may be related to this phenomenon. As early as 1926, Hildreth (44), in a study on the determination of hardiness in apples, suggested there was some indication that high reserves of carbohydrates and organic nitrogen might be correlated with greater hardiness. In another study (79), protein nitrogen was found to be highest during the dormant season in apple trees, and decreased as the active growing season approached, reaching a minimum in June. More recently, Siminovitch and Briggs (74, 75) studied the changes which occurred in the water-insoluble protein nitrogen, the water-soluble protein nitrogen, water-soluble non-protein nitrogen, and the reducing and nonreducing sugars of the living bark of Black Locust (Robinia pseudo-acacia L.) in relation to the season variations in its frost hardiness. Of the nitrogen fractions, only the water-soluble proteins were found to increase in concentration in the fall, along with the development of frost hardiness. This constituent also decreased in concentration in the spring, with the disappearance of hardiness. The authors stated that the correlation observed between water-soluble protein and hardiness, suggested that this constituent bears some causal relationship to the mechanism of development of frost hardiness. Studies on the changes in metabolites of Red-Osier Dogwood (Cornus stolonifera Mich) by Li, et al. (53) disclosed that protein nitrogen increased in the early fall, decreased somewhat at the outset

of rapid cold acclimation, and then increased more slowly in the late fall. Since it has been established that the plasma membranes of plant cells become more permeable to water as they acclimate to cold, and that sugars increase during cold acclimation, the authors suggest the possible involvement of proteins in these phenomena, either as membrane components or specific enzymes.

The possibility that high nitrogen levels could cause excessive succulence, reducing the hardiness of the plant, should also be considered.

The increase in nitrogen, due to the use of simazine, would be of obvious importance in relation to the previous suggestions.

Orchard Weed Control -- In 1960, Larsen and Ries (51) reported commercially acceptable weed control under bearing and nonbearing fruit trees by employing simazine. A combination of either simazine plus dalapon (2,2-dichloropropionic acid), or simazine plus amitrole (3-amino-1,2,4-triazole), was reported to give excellent weed control and resulted in no injury to young apple trees (18). Benson and Degman (9), however, found that 5 lb/A of simazine alone did not provide adequate weed control around non-bearing apple and pear trees. The use of 10 lb/A of simazine resulted in tree injury, leading the authors to the conclusion that more testing of this herbicide was needed. More recently, Saidak and Rutherford (70), and Ries et al. (65) have reported that combinations of simazine plus amitrole-T (amitrole +

ammonium thiocyanate) gave satisfactory weed control on apples, non-bearing peaches and sour cherries. The former workers suggest that annual applications of simazine should not exceed 6 lb/A in order to avoid injury to young trees. The standard recommendation for apples in Michigan at the present time is 2-4 lb/A simazine (depending on whether the trees are non-bearing or bearing), plus 2 lb/A amitrole-T (62).

Apple Fruit Quality and Composition -- Smock and Neubert (77) use the term quality to describe those attributes of the apple that pertain to edibility. These factors affecting eating quality may be classified as follows, chemical factors, which includes acids, sugars and astringent materials (eg. true tannins, tannin derivatives, and coloring materials, such as flavonols); physiological-anatomical factors, including changes in protopectin, which cements cells together, to soluble pectin; and physical factors which would include size and color.

Malic acid is the primary acid in the fruit of apple. Citric may also be present, and lesser amounts of ascorbic, lactic, glyoxylic, m-tartaric, oxalacetic and an uronic acid have been reported (50, 77). Carboxyl groups of pectic substances are usually sufficiently esterified or engaged in salt formation to make their contribution to the total acidity insignificant. Acidity may be expressed as the total titratable acids (a representation of organic acids) or as hydrogen ion

concentration. Haller and Magness (41) found that the acidity of the apple varieties Ben Davis and Delicious, increased as the number of leaves per fruit increased.

Simple sugars constitute the major portion of the carbohydrates in apples, and of the soluble solid material in the flesh of the apple. Since the fruit is primarily dependent on the leaves as the source of sugar, high leaf/fruit ratios usually result in a greater percentage of sugars in the fruit, up to a certain point, beyond which more leaves per fruit does not result in sugar increases. According to Smock and Neubert (77), the amount of sugar present is an important consideration, however, the acid-sugar ratio is probably more critical. The authors point to the situation where on variety may be slightly higher in sugars than a second, but more sour to the taste; this greater sourness is probably due to the presence of a greater amount of acid.

The term pectic substances refers to complex carbohydrate derivatives which contain a large proportion of anhydrogalacturonic acid units in a chain-like combination (77). The carboxyl groups of these polygalacturonic acids may be partly esterified by methyl groups. Three principal types of pectic substances may be found in fruit: pectic acid, pectin, and protopectin (57). Pectic acid appears to be a long straight-chain molecule built up by the condension of a large

number of α-galacturonic acid molecules. In pectin, many of the carboxyl groups of the pectic acid have been esterified with methyl groups, and the chain length is greater. Protopectin applies to the water-insoluble parent pectic substances. The cells of the apple are cemented together with protopectin, and during the ripening of fruit, the insoluble pectin (protopectin) is hydrolyzed to soluble pectin, and the cells of the tissue are no longer held firmly together. Thus, it is this conversion from protopectin to pectin that is responsible for the softening of fruit. When apples become mealy or over-mature, there is a breakdown of the soluble pectic components into nonpectic substances. The amount of total pectic substances remains fairly constant until the fruit becomes overripe and mealy. Following this, there is a decrease in the quantity of these components.

In relation to previous sections of the literature review, it is important to consider that, "nitrogen exerts more influence on apple quality and storage life than any other element." (83) Investigations have shown an inverse correlation between leaf nitrogen and fruit firmness (11, 14, 26, 55, 76, 88). Though the decrease in firmness may not be critical if the fruits are marketed during the normal marketing season, it is generally agreed that higher nitrogen levels reduce the potential storage life (10, 55, 88).

Weeks et al. (88) found that the amount of red color development was associated with both nitrogen and potassium. Increases in leaf N were associated with reduction of fruit color, whereas increases in leaf K were associated with increased color. Hill et al. (46) also found that a widening of the N/K ratio was associated with a decrease in fruit quality. A leaf nitrogen level of approximately 2.1 percent has been reported to be associated with good quality fruit (25, 46).

Pesticides -- Crop Quality and Composition - An extensive review of the literature has disclosed a minimum number of references to the effect of pesticides on crop quality and composition. Studies by Garman et al. (32) showed that sprays (insecticides and fungicides) can affect the quality of apples. The fungicide glyodin (2-heptadecyl-glyoxylidine acetate) was found to significantly increase total sugars when included in the spray mixture. No significant effect of insecticides on sugars was detected. A complete spray schedule employing the fungicide phygon (2,3-dichloro-1,4-naphthoquinone) plus lead arsenate reduced acid content.

Palmiter and Smock (67) found that glyodin tended to advance the maturity of McIntosh apples. This may relate to the increased sugars noted by Garman (32) due to the same chemical, since sugars increase with maturity, up to a certain point.

The storage and ripening of Delicious apples and Anjou pears as influenced by DDT (dichlorodiphenyl-trichloroethane) and parathion

(0,0-diethyl-0-p-nitrophenyl phosphorothioate) has been studied (35). No significant difference was found in the storage behavior of sprayed and unsprayed fruit. Neither spray influenced the appearance, rate of ripening or the dessert quality of the fruit.

Studies on a number of herbicides in relation to taints in jams indicated that there is normally little risk of tainting from the use of a wide range of herbicides (88). This was expected since herbicides rarely come into direct contact with the fruit itself.

Arthey (2), using the herbicides chloroxuron (N-4-(4-chlorophenoxy) phenyl-N,N-dimethylurea),dalapon, diphenamid (N,N-dimethyl-2,2-diphenyl-acetamide) and lenacil (3-chlorohexyl-5,6-trimethyleneuracil) to determine if there was an effect on the flavor of canned and quick-frozen fruits and vegetables, found that none of the above listed materials left a taint in processed fruit. The author stresses the point that the tests had been conducted for one year only, and therefore must be interpreted with care. (According to Arthey, a minimum of three years is necessary to provide reliable information on the effect of an agricultural chemical on the flavor of a processed product).

Amiben (3-amino-2,5-dichlorobenzoic acid), dichlobenil (2,6-dichlorobenzonitrile), diphenamid and dalapon were found to have no adverse effects on the flavor, internal color, storage, or yield of sweet potatoes (89). NAP (N-1-napthylphthalamic acid), on the other hand, caused severe and objectionable roughening of the surface and a

marked enlargement of the surface veins of sweet potatoes. Cooke (19) found that monuron (3-(4-chlorophenyl)-1,1-dimethylurea) caused a large decrease in the sugar content of several legumes, and, at the same time, caused a considerable increase in the soluble nitrogen fraction of the plant.

Summary of Literature Review

Simazine is a triazine herbicide which is widely used in deciduous orchards. Several investigations indicate that simazine's mode of action is inhibition of photosynthesis. Recent studies open the possibility of nitrite or ammonia excesses being responsible for this chemical toxicity. Some resistant plants degrade simazine to the non-phytotoxic hydroxysimazine. However, the possibility of additional modes of degradation, such as dealkylation, have been suggested in recent investigations.

Simazine applications may also result in increased growth, nitrogen level, nitrate reductase activity and protein content of plants.

High nitrogen levels in pome fruit trees have been found to have adverse effects on fruit quality.

Reports of pesticide effects on quality and composition of edible products are limited. Some chemicals have a definite effect on the quality and/or composition of an edible product; however, the majority of pesticides have been used without any deleterious results.

MATERIALS AND METHODS

Field Studies

In 1965 and 1966 trials were conducted on pears (Pyrus communis, L. "Bartlett"), and apples (Malus sylvestris, Mill. "McIntosh").

Following the completion of fruit quality studies in 1965, plans were made to concentrate on apples and the number of experimental sites was increased in 1966. A summary of orchard locations and other pertinent data is found in the Appendix - Table 1. Future reference to an orchard will be by use of alphabetical listings from this table.

Herbicide treatments, using commercial formulations, were applied with a carbon dioxide-pressured small plot sprayer (66). The rate of chemical used is expressed as lb/A of active ingredient unless otherwise noted.

Supplemental nitrogen was applied as evenly as possible, with a perforated coffee can, around the tree out to the drip line. Herbicide and supplemental nitrogen treatments were applied in the early spring. The treatments listed by orchard may be found by reference to the Appendix-Table 2. It should be noted that simazine was applied at 4 lb/A alone or in combination with amitrole-T except in orchards B and C where an additional 8 lb/A rate was utilized.

The experiments in orchards B, D and E were single factor experiments. Those in orchards A, C, F, G, H, and J were factorial experiments. A randomized complete block design was utilized in all cases. All treatments were replicated three times, except in orchards F and H where four replications were utilized.

Fruit were harvested at approximately the same time the grower was harvesting. In 1965 orchard A was harvested on August 18. Orchards B and C were harvested during the week of September 13th. All 1966 harvests were made during the week of September 25-30. Random samples were obtained by picking at various heights, and depths within the tree crown. The fruit were held in cold storage at approximately 1-2°C.

Tests Conducted on Tree Portions

Nitrogen Analysis -- Leaf samples for nitrogen analysis were collected between July 1 and August 15 as recommended by Kenworthy and Larsen (49). After washing and rinsing, the plant tissue was dried in a forced air oven at 80-90°C. The dried tissues were then ground through a 20 mesh screen using a Wiley Mill. In 1965, samples were analyzed for nitrogen using macro-Kjeldahl procedures.[1] In 1966 samples were collected, dried and ground in the previously stated manner; nitrogen determinations, however, were made using the following micro-Kjeldahl procedure. A 30 mg sample was weighed in cigarette

[1] Plant Analysis Laboratory, Horticulture Dept., Michigan State University

paper, using a Mettler analytical balance. The sample, wrapped in the cigarette paper, was digested in a 50 ml Taylor digestion tube using the following reagents: 3 ml concentrated sulfuric acid, 0.5 g K_2SO_4, and 0.3 ml 1M $CuSO_4$ solution. Two glass beads were used in each tube to promote even boiling. A standard containing 1 ml of $(NH_4)_2SO_4$ solution (4.717 g $(NH_4)_2SO_4$/L) and a blank were included with each set. All samples were digested for approximately two hours, cooled, and then 1-2 ml of deionized water was added to each tube to dissolve any solids. The digest was then transferred to a one-piece distillation apparatus, rinsing the tube thoroughly four or five times. After adding 8-10 ml of 13 N NaOH, the sample was distilled, collecting the distillate in 8 ml of a 4 percent boric acid solution containing methyl red-methylene blue indicator. After collection, the sample was titrated to a gray end point using 0.01 M HCL. The percent nitrogen was calculated using the standard formula (7).

Nitrate-Reductase - Leaf and Root Tissue -- Both leaf and root tissue from Orchard G were assayed for enzyme activity. Crude enzyme preparations from leaf tissue were made in the following manner: one g of fresh tissue was ground, in a cold room with a mortar and pestle previously cooled in a freezer, in 5 ml of 0.1 M tris buffer plus 0.001 M cysteine pH 7.5. The homogenates were centrifuged, using an International High Speed Refrigerated Centrifuge at 10,000 R.P.M. for 20 minutes.

The crude enzyme preparations were assayed as follows: Five-tenths ml of 0.1 M potassium phosphate pH 7.5, 0.1 ml of 0.1 M KNO_3, 0.1 ml of distilled, deionized water, 0.1 ml of 0.001 M $NADH_2$, and 0.2 ml of the enzyme extract were added to a 12 x 100 mm test tube. The solutions were incubated in a water bath at 25°C. for 20 minutes. Triplicate samples of each extract were run. A fourth sample was stopped at zero time, serving as a blank for determining the quantity of nitrite produced during the incubation period. The reactions were stopped by adding 1 ml of 1 percent sulfanilamide in 3 N HCL. One ml of 0.02 percent N-1-napthylethylenediaminedihydrocholoride was added which forms a diazo derivative of nitrite having a reddish purple color. After allowing 15 to 20 minutes for color development, the optical density was measured with a Beckman DB-G grating spectrophotometer at a wavelength of 540 millimicrons. A standard curve was run, and 0.013 O.D. units equaled 1 millimicromole nitrite.

Root samples were prepared and assayed in the same manner except, prior to grinding they were rinsed for 15-20 minutes in a 5 percent sodium hypochlorite bleach solution. Following rinsing, the outer, easily removed layer, was stripped off the roots to reduce the chance of nitrate bacterial contamination.

Water Soluble Protein Determination - Apple Buds -- Lateral buds from trees in Orchard G were removed from twigs shortly after their collection in February, by making an incision at the base of the

leaf scar, and then carefully slicing the bud off the twig. A 250 mg sample of tissue was weighed, using an analytical balance, as rapidly as possible to avoid excess dehydration. The tissue was then ground with a mortar and pestle in 5 ml of tris buffer (tris-(hydroxymethyl)-aminomethane). After centrifugation at 10,000 x g for twenty minutes, a 2.5 ml allquot of the supernatent was precipitated with 2.5 ml of 20 percent trichloroacetic acid. The precipitate was rinsed twice with 10 ml of 10 percent trichloroacetic acid and centrifuged at 2,000 R.P.M. in a clinical centrifuge for at least ten minutes. After decanting, and discarding, the supernatent, the pellet was dried in an air stream, and then redissolved in 4 ml of 1N NaOH using a Vortex mixer. Protein assay was made using the method of Lowry, et al. (54). The optical density of the samples was determined at a wavelength of 700 millimicroms using a Beckman DB-G grating spectrophotometer.

Standards were prepared using 50, 100, 150 and 200 micrograms of bovine serum albumen. The actual protein content of the bovine serum albumen was determined by multiplying the micro-Kjeldahl nitrogen value by 6.25 The final results were expressed as mg protein/g fresh weight.

Tests Conducted on Fruit

Firmness -- Firmness of the fruit was determined using a plunger type pressure tester. Orhcards A, G, and D were tested prior to

storage and B and C after approximately four months storage. Ten fruit were randomly chosen from each treatment. A small slice was made with a paring knife on opposite sides of the fruit, exposing the flesh. The pressure in pounds required to force a plunger point 11 mm in diameter into the flesh of the fruit to a depth of approximately 7 mm was measured and recorded. The average of the two tests was computed, and an overall mean for that treatment was computed from these ten averages.

Soluble Solids -- Fruit juice collected during the firmness determinations was used for the soluble solids measurement, employing a hand refractometer. The results were expressed as percent soluble solids.

Total Pectic Material -- The total pectic material was determined, on fruit which had been stored for eight to nine months, using a method similar to that developed by McCready and McComb (56). Twenty-five grams of fresh fruit were macerated in a Waring blender for 5 minutes with 125 ml of 95 percent ethyl alcohol. The ethyl alcohol, containing the sugars, was then filtered and discarded. After washing twice with 75 percent ethyl alcohol, the moist pulp was transferred to a 250 ml beaker. The pectin was deesterified by mixing the pulp with 200 ml of a 0.5 percent EDTA (tetrasodium salt of ethylenediaminetetra-acetic acid) solution at pH 11.5 for 30 minutes. The mixture was acidified to pH 5.0 to 5.5 with acetic acid. One-tenth gram of pectinase

was added to the solution, which was transferred to a 250 ml Erlenmeyer flash and vigorously shaken for one hour on a Eberbach variable speed shaker, Following agitation the solutions were diluted to 250 ml and filtered, discarding the first few ml of filtrate. Two ml of the filtrate were diluted to 100 ml, and three 2 ml aliquots were used for analysis.

The assaying procedure was as follows: Twelve ml of concentrated sulfuric acid were measured into a 25 x 170 mm test tube. The tube and contents were cooled to about 3°C in an ice bath, and a 2 ml aliquot of the pectic material was added to the solution, and mixed. The tube was replaced in the ice bath and the contents cooled below 5°C. After heating for 10 minutes in a boiling water bath, cooling to approximately 20°C, 1.0 ml of 0.15 percent carbozole reagent (reagent grade carbazole in 100 percent ethyl alcohol) was added to the sample. The color was allowed to develop for 20 to 30 minutes, at room temperature. The optical density of the samples was determined using a Beckman DU spectrophotometer at a wavelength of 520 millimicrons. Samples were analyzed in sequence so that the time and temperature between addition of carbozole and color development were the same. A standard curve was run on samples containing .01, .05, .10 and 1.5 mg/100 ml of D-alpha-galacturonic acid. The results were expressed as mg galacturonic acid per g of fruit.

Determination of Titratable Acidity of Fruit -- Twenty wedges were removed from the same fruit, used for determining firmness, including the peel and the flesh, but not the core. Two 100 gm samples were weighed, placed in appropriately labeled polyethylene bags, and rapidly frozen to avoid excessive browning of the tissue. For analysis each tissue sample was placed in a 400 ml beaker. Two hundred ml of distilled deionized water were added, and boiled for three minutes. After cooling, the sample was macerated in a Waring blender for three minutes. The suspension was transferred to a Buchner funnel containing a milk filter pad and filtered under suction from a water aspirator. Two 50 ml aliquots of the filtrate were titrated to a pH of 8.1 with 0.10 N NaOH. The titratable acidity was expressed as meq per 100 g of fruit.

Water Soluble Protein Determination in Fruit -- Twenty-five g of tissue from fruit harvested at orchards D and G and stored for eight to nine months, were ground with 20 ml of 1.0 M phosphate buffer at pH 9.0 in an electric mortar grinder. After centrifugation in a Sorvall GLC-1 at 6000 R.P.M. for 20 minutes, a 3 ml sample of the supernatent solution was precipitated with one ml of 40 percent trichloroacetic acid. The precipitate was washed twice with 10 ml of 10 percent trichloroacetic acid, centrifuged, and the supernatent solution discarded. The pellet was dried in an air stream, and solubilized in 2 ml of 1N NaOH. Protein assay was made in the same manner as described under protein determination on apple buds.

Respiration Studies -- Respiration rates of whole fruit samples harvested in 1965 and 1966 were monitored utilizing an open, or gas flow, system. Fruit from each treatment were monitored in seperate containers, in a room held at 20°C. Respiratory rates were measured for the following time periods. Orchard A, 8/21/65 to 9/8/65, B and C 11/4/65 to 11/15/65. Orchards D and G, from 11/14/66 to 11/21/66. The analysis of carbon dioxide was achieved with a Beckman IR-115 infrared analyzer. Oxygen levels were determined with a Beckman Model G-2 oxygen analyzer. The respiration rate was expressed as ml of O_2 consumed, or CO_2 produced per kilogram of fruit per hour at standard conditions (23).

Statistical Analysis

Data were subjected to analysis of variance. Where mean comparisons were necessary, Duncan's Multiple Range Test was employed. Respiration data were analyzed using the Control Data Corporation 3600 Computer, installed in the Computer Laboratory at Michigan State University. An analysis of variance routine was programmed to analyze the differences in the respiratory rate for each 12 hours cycle during the monitoring process.

RESULTS AND DISCUSSION

Tests Conducted on Tree Portions

Nitrogen Analysis -- Simazine applications to the soil, around the base of fruit trees, caused no increase in the foliage nitrogen level in any orchard treated in 1965 (Tables 1, 2, 3). In 1966, the simazine treated trees in orchards G (Table 4) and J (Table 5), had higher nitrogen levels than those where no herbicide was applied. A significant increase in leaf nitrogen, not related to herbicide application, occurred at the highest nitrogen level in orchard F. Similar effects were not evident in other orchards treated that year Table 5, 6, 7).

The lack of increase in nitrogen content, due to simazine applications, in the majority of the orchards may be related to several factors. Examination of this data, and prior weed control studies where simazine was applied, indicate a trend for increased nitrogen content appearing after two or three consecutive years application of herbicide. This may be related to the low solubility of simazine and associated minimal leaching to the root zone where absorption takes place. In both orchards G and J simazine was applied by the grower, or weed control researchers, prior to the year of the increased nitrogen. Climatological conditions in 1966 add support to this postulate. The temperature for the five months from April through

Table 1. Pear tree leaf nitrogen content and qualitative characteristics of fruit in relation to treatments in orchard A.

Weed Control	Leaf N (%)	Firmness (lbs)	Soluble Solids (%)	Titratable Acidity (meq)
None	1.49[1/]	19.8[1/]	12.2[2/]	6.64[1/]
Plastic mulch	1.35	18.6	11.1	6.16
Simazine	1.63	20.8	11.0	6.67
Simazine + amitrole-T	1.54	18.6	11.5	5.78

[1/] F value for treatments not significant at 5% level.

[2/] F value for comparison of no weed control vs weed control significant at 5% level.

Table 2. Apple tree leaf nitrogen content and qualitative characteristics of fruit in relation to treatments in orchard B.

Treatment		Leaf N (%)	Firmness (lb)	Soluble solids (%)	Titratable acidity (meq)
Weed Control	Area (sq ft)				
None		2.04[1/]	9.3[1/]	12.3[2/]	6.5[1/]
Simazine	16	2.04	9.1	11.5	6.0
Simazine	64	2.05	8.9	11.8	6.0
Simazine	144	2.08	9.1	11.5	5.3
Simazine + amitrole-T	16	1.87	9.3	11.3	6.1
Simazine + amitrole-T	64	2.15	9.0	11.5	5.6
Simazine + amitrole-T	144	2.01	8.8	11.7	5.6

[1/] F value for treatments not significant at 5% level.

[2/] F value for comparison no weed control vs weed control significant at 1% level.

Table 3. Apple tree leaf nitrogen content and qualitative characteristics of fruit in relation to treatment in orchard C.[1/]

Treatment						
Weed control	Rate (lb/A)	Suppl. nitrogen (lb/tree)	Leaf N (%)	Firmness (lb)	Soluble solids (%)	Titratable acidity (meq)
None	0	0	1.82	9.8	11.7	5.0
Simazine + amitrole-T	4+2	0	1.89	10.0	11.0	5.2
Simazine + amitrole-T	8+2	0	1.53	10.2	11.9	5.7
None		4	1.91	10.3	11.3	4.0
Simazine + amitrole-T	4+2	4	2.39	10.3	11.5	4.6
Simazine + amitrole-T	8+2	4	1.93	10.7	11.8	6.0

[1/] F value for treatments not significant at 5% level.

Table 4. Apple tree leaf nitrogen content and qualitative characteristics of fruit in relation to treatments in orchard G.[1/]

Treatment			Leaf N	Firmness	Soluble solids	Titratable acidity
Weed Control	Nitrogen (lb/trees)	Source	(%)	(lb)	(%)	(meq)
None	0		1.74c	16.4	12.5	8.3
Simazine + amitrole-T	0		2.04b	16.3	12.2	10.1
None	1	$Ca(NO_3)_2$	1.73c	16.5	12.0	10.0
Simazine + amitrole-T	1	$Ca(NO_3)_2$	2.01b	16.0	11.7	9.8
None	0		1.57c	16.3	12.0	11.4
Simazine + amitrole-T	0		1.94b	16.3	11.7	8.5
None	1	$(NH_4)_2SO_4$	1.74c	16.1	12.2	10.2
Simazine + amitrole-T	1	$(NH_4)_2SO_4$	2.21a	15.9	11.5	8.9

[1/] Means followed by unlike letters significantly different at 1 percent level.

[1/] Means not followed by letters not significantly different at 5 percent level.

Table 5. The nitrogen content of the foliage from trees in orchards E and J in relation to weed control treatments.

Weed Control	Apple Orchard E	Pear Orchard J
None	1.59[1/]	1.38[2/]
Plastic mulch	--	1.55
Simazine	1.68	1.72
Simazine + amitrole-T	1.65	--

[1/] F value for treatments not significant at 5% level.

[2/] F value for comparison of weed control vs weed control significant at 5% level.

Table 6. Apple tree leaf nitrogen content and qualitative characteristics of fruit in relation to treatments in orchard D.[1/]

Treatment		Leaf N	Firmness	Soluble solids	Titratable acidity
Weed Control	Area (sq ft)	(%)	(lb)	(%)	(meq)
None		1.66	15.3	11.2	8.2
Simazine	16	1.74	15.1	11.7	8.7
Simazine	64	1.77	15.1	11.0	9.2
Simazine	144	1.66	15.7	11.5	9.6
Simazine + amitrole-T	16	1.67	15.1	11.3	9.2
Simazine + amitrole-T	64	1.79	14.8	11.2	9.1
Simazine + amitrole-T	144	1.80	15.1	11.2	8.5

[1/] F value for treatments not significant at 5% level.

Table 7. The nitrogen content of the foliage from trees in orchard H, and F in relation to herbicide and nitrogen treatments.

Treatment			Nitrogen levels	
Weed Control	Nitrogen (lb/tree)	Source	Apple Orchard F (%)	Apple Orchard H (%)
None	0		1.91[2/]	1.78[1/]
Simazine + amitrole-T	0		1.92	1.76
None	1/2	$(Ca(NO_3)_2$	2.07	1.94
Simazine + amitrole-T	1/2	$(Ca(NO_3)_2$	1.96	1.75
None	1	$(Ca(NO_3)_2$	1.98	1.72
Simazine + amitrole-T	1	$(Ca(NO_3)_2$	2.06	2.07
None	0		1.62	1.74
Simazine + amitrole-T	0		1.71	1.76
None	1/2	$(NH_4)_2SO_4$	1.99	1.75
Simazine + amitrole-T	1/2	$(NH_4)_2SO_4$	1.90	1.87
None	1	$(NH_4)_2SO_4$	2.04	1.92
Simazine + amitrole-T	1	$(NH_4)_2SO_4$	2.21	1.87

[1/] F value for herbicide treatment within fertilizer level not significant at 5% level.

[2/] F value for average 1 l/btree of N vs average of others significant at 5% level.

August did not vary appreciably between experimental sites. The orchard G area, however, received approximately two and four inches more rain than the area around orchards H and F, respectively. The greatest difference occurred in the critical growth month of June, when the G, H, and F areas received 1.23, .66 and .87 inches, respectively (84, 85). The orchard J area received considerably more rain than either of these three, with the month of June accounting for a large part of the difference.

The optimum time of fertilizer application, in relation to maximum availability, absorption and utilization of nitrogen by perennial fruit trees, is not agreed upon among research workers. Much of the literature relating to this topic is reviewed by Boynton and Oberly (12), who believe apple trees take up appreciable amounts of nitrogen during the winter months. However, little of this is translocated upward until the spring. The presence of sub-optimal temperatures and a low level of nitrate, conditions under which simazine appears to increase either nitrate absorption, nitrate assimilation, or both (82), could exist at this time. The necessity of having simazine in the root absorption zone at this time, or in the late fall or early spring, when the previously listed conditions could exist, would be of obvious importance in relation to consecutive herbicide applications.

Summarizing, it appears that successive annual applications of simazine, adequate rainfall to leach the chemical into the root zone,

and the time of nitrogen absorption in relation to environmental factors (i.e. nitrogen availability and temperature), are important in accounting for the lack of nitrogen response in the majority of the treated orchards.

Nitrogen analyses were used as a guide to select apple orchards for the quality and composition studies. Two orchards were chosen: G, where simazine caused a marked increase in the percent leaf nitrogen; and F because it did not show an increase in nitrogen due to simazine. However, orchard F had a history of several years of herbicide application on the same plots.

Nitrate Reductase -- Assay of leaf and root tissue from orchard G disclosed no nitrate reductase activity in either plant portions. Attempts by other workers to detect this enzyme in apple tissue resulted in similar findings (71). Recent studies by Gasmanis and Nicholas (39), disclosed the presence of inhibitor(s) of nitrate reductase in extracts of apple roots. By dialzying the extracts against phosphate buffer for 42 hours, or by passing them through Sephadex G-25, the inhibitor(s) were removed and nitrate reductase was detected. Since neither of these latter two procedures were used in the method employed in this study, this may account for the lack of detection of nitrate reductase activity in root extracts.

Nitrate nitrogen normally is not found in apple leaves or other tissue of the tree above the fibrous roots (12). Since nitrate reductase is an inducible enzyme requiring the presence of nitrate (8), this could account for the lack of detection of enzyme activity in apple leaves. The possibility also exists that the best conditions for extraction and detection of enzyme activity were not utilized.

Protein Determination - Apple Buds -- The marked increase in leaf nitrogen found in orchard G was not reflected by a higher protein level in the lateral buds (Table 8). This may be due to the incorporation of this nitrogen in other nitrogenous compounds. Arginine, asparagine, aspartic and glutamic acids have been found to account for up to 90 percent of the nitrogen reserves of apple twig, bark, bud, root bark, and thin root tissue (13).

Tests Conducted on Fruit

Quality of Fruit at Harvest -- Fruit harvested in both 1965 and 1966 had an exterior appearance of high quality, with no evident differences in color.

Firmness -- Neither simazine, nor the increase in foliage nitrogen due to simazine, affected apple or pear fruit firmness (Table 1-5). No detectable residues of the herbicide have been found in the fruits (30), which could directly affect respiration and alter ripening and softening processes.

Table 8. Protein content of lateral buds of twigs from McIntosh apple trees in relation to treatments in orhcard G.[1/]

Treatment			Protein (mg/g fr wt)
Weed Control	Nitrogen (lb/tree)	Source	
None	0		25.8
Simazine + amitrole-T	0		23.4
None	1	$Ca(NO_3)_2$	26.8
Simazine + amitrole-T	1	$Ca(NO_3)_2$	30.5
None	0		24.2
Simazine + amitrole-T	0		24.5
None	1	$(NH_4)_2SO_4$	23.9
Simazine + amitrole-T	1	$(NH_4)_2SO_4$	25.9

[1/] F value for treatments not significant at 5% level.

Positive correlations between nitrogen, cell volume and higher internal breakdown have been found by Letham (52) and Martin et al. (55). The possibility exists that if a critical threshold value for nitrogen were exceeded due to simazine, it could result in a greater cell volume and its associated effect on firmness as reported by these workers.

Soluble Solids -- Except for orchards A and B (Tables 1, 2), simazine did not alter the soluble solids (Tables 3, 4, and 6). The average decrease in these two orchards of approximately 6 percent would not be of practical importance. To attribute this effect to the herbicide without further research seems questionable, since there was no apparent effect on the other fruit quality parameters, or on the nitrogen content of the foliage.

Large applications of nitrogen have been found to cause a trend of decreasing soluble solids (76), however, the increase in nitrogen content of orchard G was not accompanied by any alteration of the soluble solids content. Since more leaves per fruit usually results in a greater percent sugar, up to a certain point, the possibility exists that increased nitrogen content, causing a more vigorous tree, could result in greater leaf/fruit ratios, and thereby increase sugars.

Total Pectic Material -- The total pectic material of apples was not altered by herbicide treatment in any of the plots (Tables 9, 10). The amount of total pectic substances remains fairly constant following harvest until the fruit becomes overripe, at which time a decrease occurs. During this time, soluble pectic materials are being transformed into nonpectic materials (77). Since this test was conducted after considerable storage, any hastening of breakdown, to the point where there was a loss in pectic material should have been detected. Further contemplation discloses several factors that could be considered in relation to firmness, pectic materials and nitrogen. Sharples[1] expresses the opinion that the effect of excess nitrogen may be that of causing an imbalance of nutrient levels that are necessary for quality fruit. Calcium would be an important element in this relationship, since it is involved in binding the middle lamella in the form of calcium pectates. An increase in nitrogen, due to simazine, without a readily available source of calcium could result in growth and cell enlargement without normal strengthening, possibly related to the aforementioned calcium pectates.

Titratable Acidity -- As stated in the literature review, acidity has been shown to increase as the number of leaves per fruit increased.

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[1] R. Sharples. Personal Communication. East Malling Research Station. East Malling, England

Table 9. Protein and total pectic material content of the flesh of McIntosh apples in relation to treatments in orchard G.[1/]

Treatment			Protein	Total pectic
Weed Control	Nitrogen (lb/tree)	Source	(mg/g fr wt)	material (mg/g)
None	0		.123	7.07
Simazine + amitrole-T	0		.121	6.83
None	1	$Ca(NO_3)_2$	.126	6.07
Simazine + amitrole-T	1	$Ca(NO_3)_2$	.145	6.33
None	0		.126	6.37
Simazine + amitrole-T	0		.124	6.08
None	1	$(NH_4)_2SO_4$	.098	6.23
Simazine + amitrole-T	1	$(NH_4)_2SO_4$	.146	5.83

[1/] F value for treatments not significant at 5% level.

Table 10. Protein and total pectic material content of the flesh of McIntosh apples from orchard D as influenced by herbicides and their area of application.[1/]

Treatment		Protein (mg/g fr wt)	Total pectic material (mg/g)
Weed Control	Area (sq ft)		
None		.173	6.37
Simazine	16	.131	6.22
Simazine	64	.109	6.30
Simazine	144	.126	6.78
Simazine + amitrole-T	16	.149	6.42
Simazine + amitrole-T	64	.119	6.28
Simazine + amitrole-T	144	.139	6.45

[1/] F value for treatments not significant at 5% level.

The longer terminal growth reported by Ries, et al. (67), associated with increases in nitrogen due to simazine application, would be reflected by higher leaf to fruit ratios. No effect was noted on titratable acidity among the fruit tested, including those harvested from orchard G, having an increased leaf nitrogen (Tables 1-5). It should be noted that no terminal growth measurements were made to determine if the increase in nitrogen was reflected by longer terminal growth in this orchard.

Water Soluble Protein Determination - Fruit -- There was no difference in water extractable protein of apple flesh due to simazine or its effect on nitrogen content of foliage (Tables 9, 10). Recent work by Chmiel[1] in assaying for protein, has disclosed that the most dramatic simazine induced protein increases occur in plants that normally synthesize high levels of protein. Apples are characteristically low in protein.

In a study carried out on Jonathan apples, it was found that only a diminishing proportion of a noted increase in nitrogen was incorporated into the protein fraction of the fruit (55). Similarly, Hill (45) found that a 19 percent increase in leaf nitrogen was accompanied by 176 percent increase in soluble nitrogen of the fruit, but only a 16 percent increase occurred in fruit protein.

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[1] H. Chmiel. Personal communication. Dept. of Horticulture, Michigan State University. East Lansing, Michigan

The relationship between fruit respiration rates and proteins, or vice versa, has resulted in many hypotheses attempting to relate protein to fruit climacterics and senescence. The necessity for formation of high energy compounds utilized in DNA replication, transcription and RNA biosynthesis, and translation, all steps involved in protein formation, make respiration of obvious importance in the overall process of protein biosynthesis. Though respiration is necessary for protein synthesis to occur, synthesis may not be strictly dependent on the respiratory rate. To relate the lack of increase in proteins to the fact that there was no significant increase in the respiration rate of fruit from either orchard D or G (to be discussed in next section), would be extremely presumptuous.

Respiration Studies

Quantitative analysis of carbon dioxide, and oxygen utilized, at each separate cycle of the respiratory monitoring process, indicated that there was no significant differences in respiration of apples from orchards C, D, F, and G or pears from orchard A, due to any of the treatments. The quantity of carbon dioxide evolved and oxygen utilized at the time of the pre- and post climacteric mimimums, and the climacteric peak in respiration are presented in Tables 11-15.

Table 11. Carbon dioxide evolved and oxygen utilized by apple fruit from orchard B at the time of the preclimacteric minimum and climacteric maximum of respiration.[1]

Treatment		Preclimacteric minimum				Climacteric maximum			
Weed Control	Area (sq ft)	CO_2 evolved (ml/kg/hr)	Time (hr)	O_2 utilized (ml/kg/hr)	Time (hr)	CO_2 evolved (ml/kg/hr)	Time (hr)	O_2 utilized (ml/kg/hr)	Time (hr)
None		11.8	12	12.1	12	14.2	24	13.9	36
Simazine	16	11.1	12	11.4	12	13.2	48	13.3	36
Simazine	64	12.2	12	11.7	12	13.8	24	14.1	36
Simazine	144	11.0	12	11.2	12	12.8	48	13.0	36
Simazine + amitrole-T	16	12.6	12	13.1	12	14.4	48	15.1	36
Simazine + amitrole-T	64	11.7	12	12.1	12	13.4	48	13.8	24
Simazine + amitrole-T	144	12.3	12	12.3	12	13.7	24	14.0	24

[1] F value for treatment effects on these and all other cycles not significant at 5% level

Table 12. Carbon dioxide evolved and oxygen utilized by apple fruit from orchard D at the time of the preclimacteric minimum and climacteric maximum of repiration.[1]

Treatment		Preclimacteric minimum				Climacteric maximum			
Weed Control	Area (sq ft)	CO_2 evolved (ml/kg/hr)	Time (hr)	O_2 utilized (ml/kg/hr)	Time (hr)	CO_2 evolved (ml/kg/hr)	Time (hr)	O_2 utilized (ml/kg/hr)	Time (hr)
None		14.2	12	13.4	12	16.1	48	16.1	36
Simazine	16	14.6	12	13.5	12	15.9	36	16.0	36
Simazine	64	13.2	12	12.2	12	14.7	36	14.9	36
Simazine	144	13.2	12	12.6	12	15.6	48	15.5	36
Simazine + amitrole-T	16	15.0	12	14.2	12	16.9	36	17.3	36
Simazine + amitrole-T	64	14.6	12	13.5	12	16.3	36	16.3	36
Simazine + amitrole-T	144	13.3	12	12.3	12	15.1	48	15.4	36

[1] F value for treatment effects on these and all other 12 hr. cycles not significant at 5% level

Table 13. Carbon dioxide evolved and oxygen utilized by apple fruit from orchard B at the time of the preclimacteric minimum and climacteric maximum of respiration.[1]

Treatment			Preclimacteric minimum				Climacteric maximum			
Weed Control	Rate (lb/A)	Suppl. nitrogen (lb/tree)	CO_2 evolved (ml/kg/hr)	Time (hr)	O_2 utilized (ml/kg/hr)	Time (hr)	CO_2 evolved (ml/kg/hr)	Time (hr)	O_2 utilized (ml/kg/hr)	Time (hr)
None	0	0	9.9	12	10.2	12	13.3	48	14.4	48
Simazine + amitrole-T	4+2	0	9.2	12	10.3	12	12.8	48	13.7	36
Simazine + amitrole-T	8+2	0	9.6	12	11.6	12	13.4	60	14.6	24
None	0	4	10.4	12	11.0	12	13.1	48	14.0	36
Simazine + amitrole-T	4+2	4	11.6	12	12.9	12	15.1	48	16.1	36
Simazine + amitrole-T	8+2	4	11.0	12	11.5	12	14.1	48	14.3	48

[1]F value for treatment effects on these and all other cycles not significant at 5% level

Table 14. Carbon dioxide evolved and oxygen utilized by apple fruit from orchard G at the time of the preclimacteric minimum and climacteric maximym of respiration.[1]

Weed Control	Nitrogen (lb/tree)	Source	Preclimacteric minimum				Climacteric maximum			
			CO_2 evolved (ml/kg/hr)	Time (hr)	O_2 utilized (ml/kg/hr)	Time (hr)	CO_2 evolved (ml/kg/hr)	Time (hr)	O_2 utilized (ml/kg/hr)	Time (hr)
None	0		10.9	12	11.3	12	15.5	24	15.3	36
Simazine + amitrole-T	0		10.9	12.	104	12	15.1	24	15.1	36
None	1	$Ca(NO_3)_2$	11.0	12	11.3	12	15.5	24	15.1	36
Simazine + amitrole-T	1	$Ca(NO_3)_2$	10.4	12	10.9	12	15.1	24	15.0	36
None	0		11.2	12	11.2	12	15.7	24	15.5	36
Simazine + amitrole-T	0		10.5	12	10.9	12	14.9	24	14.7	36
None	1	$(NH_4)_2SO_4$	10.8	12	12.0	12	15.6	24	15.3	36
Simazine + amitrole T	1	$(NH_4)_2SO_4$	11.8	12	12.2	12	16.1	24	15.5	36

[1] F value for treatment effects on these and all other 12 hr. cycles not significant at 5% level.

Table 15. Carbon dioxide evolved and oxygen utilized by pear fruit from orchard A at the time of the preclimacteric minimum and climacteric maximum of respiration.[1]

Weed Control	Preclimacteric minimum				Climacteric maximum			
	CO_2 evolved (ml/kg/hr)	Time (hr)	O_2 utilized (ml/kg/hr)	Time (hr)	CO_2 evolved (ml/kg/hr)	Time (hr)	O_2 utilized (ml/kg/hr)	Time (hr)
None	8.6	84	9.9	84	28.4	374	26.2	348
Black plastic	8.1	84	9.2	96	25.3	372	24.0	348
Simazine	8.2	84	9.9	84	27.0	336	26.7	324
Simazine + amitrole-T	7.3	84	8.3	84	29.9	372	26.9	360

[1] F value for treatment effects on these and other cycles not significant at 5% level.

SUMMARY

The possibility of a detrimental effect on pome fruit orchards from consecutive annual applications of herbicides suggested a study of the influence of simazine on fruit quality. The triazine herbicide, simazine, is widely employed for orchard weed control. Simazine increases the nitrogen content of several crops including fruit trees. This is considered to be of critical importance, since there is an accepted correlation between nitrogen content and fruit quality.

Simazine was applied, either alone or in combination with amitrole-T, to four pome fruit orchards in 1965, and five in 1966, throughout Michigan. Supplemental nitrogen applications up to 4 lb/tree were also applied at six experimental sites.

Kjeldahl analysis of foliage detected an increase in nitrogen, associated with herbicide, in only one pear and one apple orchard out of the total number treated. This foliar increase was not reflected by an increment in water soluble protein in apple tree buds. Enzyme inhibitor(s) may have interfered with the detection of nitrate reductase activity, which has been associated with the effect of simazine on increasing nitrogen content. This increase in foliar nitrogen of apples had no effect on the firmness, total pectic material, soluble solids, titratable acidity, protein content or the respiration rate of the fruit.

One pound of supplemental nitrogen per tree, increased the leaf nitrogen, as compared with none and one-half pound in a single experiment.

Where no increment in foliage nitrogen occurred, herbicide application had no influence on any of the quality characteristics, except for a 6 percent decrease in soluble solids in pear and apple fruit from individual orchards treated in 1965. Because of the lack of effect on any other quality parameter, or recurrence of the condition in 1966, this decrease was not considered significant.

This research indicates that there are no direct effects of simazine on pome fruit quality. Furthermore, simazine's indirect effect of increasing foliar nitrogen should not alter quality, provided the grower annually considers the nitrogen status of the fruit trees.

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APPENDIX

Table 1. Sites of experiments and additional pertinent information for the years 1965 and 1966.[1/]

Orchard	Fruit Type	Cultivar	Location	Year of herbicide Treatments	Approx. age of trees (yrs)
A	Pears	Bartlett	Pearl Grange, Mich	1964, 1965	10
B	Apples	McIntosh	Belding, Mich.	1964, 1965	12
C	Apples	McIntosh	Sparta, Mich.	1965	20
D	Apples	McIntosh	Belding, Mich.	1965, 1966	13
E	Apples	McIntosh	Sparta, Mich.	1965, 1966	21
F	Apples	McIntosh	Traverse City,Mich.	1966	9
G	Apples	McIntosh	Ludington, Mich.	1965, 1966	20
H	Apples	Red Delicious	Shelby, Mich.	1966	9-12
J	Pears	Bartlett	Pearl Grange, Mich.	1964, 1965, 1966	11

[1/] Orchards A and J, B and D, and C and E: same orchards receiving treatments in consecutive years.

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[1/] Orchards A and J, B and D, and C and E: same orchards receiving treatments in consecutive years.

Table 2. Chemical and cultural treatments utilized at experimental sites

Orchard	Weed Control	Rate (lb/A)	Supplemental nitrogen	Fertilizer applied by grower
A	None	--	None	None Applied
	Black plastic mulch	--	None	
	Simazine + amitrole-T	4+2	None	
	Simazine	4	None	
	None	--	1 lb NH_4NO_3/tree	
	Black plastic mulch	--	None	
	Simazine + amitrole-T	4+2	None	
	None	--	2 lb NH_4NO_3/tree	
	Black plastic mulch	--	None	
	Simazine + amitrole-T	4+2	None	
	Simazine	4	None	
C	None	--	None	None Applied
	Simazine + amitrole-T	4+2	None	
	Simazine + amitrole-T	8+2	None	
	None	--	4 lb NH_4NO_3/tree	
	Simazine + amitrole-T	4+2	None	
	Simazine + amitrole-T	8+2	None	

Table 2, continued

Orchard	Weed Control	Trt. area (sq.ft)	Rate (lb/A)	Supplemental nitrogen	Fertilizer applied by grower
B	None	--	--	None	
	Simazine + amitrole-T	16	4+2	None	
	Simazine + amitrole-T	64	4+2	None	Approx. 1-1/2 lb. NH_4NO_3/ tree on all plots
	Simazine + amitrole-T	144	4+2	None	
	Simazine	16	8	None	
	Simazine	64	8	None	
	Simazine	144	8	None	
D	None	--	--	None	
	Simazine + amitrole-T	16	4+2	None	
	Simazine + amitrole-T	64	4+2	None	
	Simazine + amitrole-T	144	4+2	None	1-1/2 lb NH_4NO_3/tree on all plots
	Simazine	16	4	None	
	Simazine	64	4	None	
	Simazine	144	4	None	

Table 2, continued

Orchard	Weed Control	Rate (lb/A)	Supplemental nitrogen	Fertilizer applied by grower
E	None	--	None	Approx. 1-1/2 lb. NH_4NO_3/ tree on all plots
	Simazine + amitrole-T	4+2	None	
	Simazine	4	None	
F	None	--	None	
	Simazine + amitrole-T	4+2	None	
	None	--	1/2 lb $ca(NO_3)_2$/tree	
	Simazine + amitrole-T	4+2	1/2 lb $ca(NO_3)_2$/tree	None Applied
	None	--	1 lb $Ca(NO_3)_2$/tree	
	Simazine + amitrole-T	4+2	1 lb $Ca(NO_3)_2$/tree	
	None	--	None	
	Simazine + amitrole-T	4+2	None	
	None	--	1/2 lb $(NH_4)_2SO_4$/tree	
	Simazine + amitrole-T	4+2	1/2 lb $(NH_4)_2SO_4$/tree	
	None	--	1 lb $(NH_4)_2SO_4$/tree	
	Simazine + amitrole-T	4+2	1 lb $(NH_4)_2SO_4$/tree	

Table 2, continued

Orchard	Weed Control	Rate (lb/A)	Supplemental nitrogen	Fertilizer applied by grower
G	None	--	None	None
	Simazine + amitrole-T	4+2	None	None
	None	--	1 lb $Ca(NO_3)_2$/tree	None
	Simazine + amitrole-T	4+2	1 lb $Ca(NO_3)_2$/tree	None
	None	--	None	None
	Simazine + amitrole-T	4+2	None	None
	None	--	1 lb $(NH_4)_2$/tree	None
	Simazine + amitrole-T	4+2	1 lb $(NH_4)_2$/tree	None
J	None	--	None	
	Black plastic mulch	--	None	Approx. 2 lbs. 15-5-15/ tree on all plots
	Simazine + amitrole-T	4+2	None	
H	All treatments, rates, supplemental nitrogen similar to orchard F			