ULTRASONIC ENERGY AS A CLEANING AGENT AND ITS INFLUENCE ON THE RESPIRATORY ACTIVITY AND LEAF ANATOMY OF *Brassica Oleraceae* var. *Acephala*

by

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INTRODUCTION

The need for improved methods of cleaning vegetables for processing and for fresh market to remove soil, insects, and pesticide residues is recognized by horticulturists and processors. Processors report that these contaminants remain a problem even after cleaning by the best methods available.

Removal of pesticide residues by improved washing techniques would eliminate the problem of pre-harvest application limitations and tolerances in the use of pesticides. Killing and removing insects from infested crops by improved washing techniques would also be beneficial.

The utilization of ultrasonic energy as a cleaning agent for vegetables involves the adaptation of a cleaning method used to clean many types of glass, metal, and electronic instrument parts. Neppiras (38) stated that the useful applications of high energy ultrasonics now touch almost every industry. Such materials as dust, oil, grease, blood, bits of tissue, radioactive soil, casting sand and many other types of foreign matter have been removed by the cavitations produced by ultrasonic energy in a liquid medium (1, 2, 10, 26).

No research has been reported on the influence of ultrasonic energy on the cleaning of any plant or plant parts to remove soil, insects, pesticide residues, or other

contaminants. Preliminary tests at V.P.I. of ultrasonic cleaning of leafy greens indicate that ultrasonic cleaning may make more effective the presently used washing techniques. Application of this method of cleaning vegetables necessitates a study of the removal of contaminants by ultrasonic energy, the effect of such treatments on the metabolic processes of certain plant parts, and its influence on the cellular structure of the plant parts utilized.

The purpose of this investigation was: a) to determine the effectiveness of ultrasonic energy in cleaning a chemical residue, thiodan, from Brassica oleraceae, var. acephala, and the influence of the cleaning treatments on the level of ascorbic acid and carotene in the leaf tissues, b) to investigate the influence of ultrasonic cleaning treatments on the metabolic processes of Brassica oleraceae, var. acephala, c) and to study the influence of these treatments on the histological changes within the tissues.

REVIEW OF LITERATURE

The Mechanisms Involved in Ultrasonic Cleaning

Ultrasonic energy is utilized as a cleaning agent to remove many types of foreign matter in industrial cleaning operations. According to Mattiat (35) high intensity ultrasonic irradiation in a cleaning liquid subjects dust particles adhering to small metal or glass parts to the explosive forces of cavitation. He states that these forces lift the adhering foreign particles from the surface and disperse them throughout the cleaning liquid. Tint (46) defines cavitation as the rapid formation and sudden collapse of countless thousands of microscopic bubbles in a liquid. The transmission of ultrasonic vibrations through the liquid generates tiny flaws or gas bubbles. These promote the occurance of cavities which Lehmann and Herrick (27) state disrupt the rarefaction of the liquid. The implosion or collapse of these bubbles during the compression phase of the cycle results in a powerful scrubbing action which pulls soil and other contaminants from the parts immersed in the liquid. Blake (6) indicated that the cohesive force of water is of the order of 1000 kg/cm^2 and that the amplitude of pressure in the ultrasonic waves is only about 5 kg/cm^2 at an intensity of 10 $watts/cm^2$. Rayleigh (40) found that when these cavities collapse during the compression phase of the cycle pressures of the order of 1000 atmos-

pheres are created. According to Hughes and Nyborg (21) alternations of pressure in the sound field cause bubbles to grow and take part in a complex and extremely energetic motion. Smith (43) stated that when gas bubbles grow to such a size that they oscillate in resonance with the frequency, great alternating accelerations are created. Putner (39) found that cavitation energies produced at lower frequencies greatly assisted in the removal of large particles from contaminated glass articles, while high cavitation energies damaged the surface of the article being cleaned. Neppiras (38) indicated that the limit of intensities in liquids is fixed by cavitation which sets in at low levels. Optimum range of frequencies for cavitation in liquid media are 5 to 40 kc. Applications in liquids include cell disruption, bactericidal sterilization, pasteurization, emulsification, dispersing solids and degreasing and cleaning solids.

Besides these mechanical effects cavitation is responsible for most of the chemical reactions created by ultrasonic energy. Sadayoski and Kosaku (42) inactivated pyrogenic substances in the culture filtrates of pyrogenic bacteria and fungi. Fitzgerald, Griffin and Sullivan (14) attributed the chemical effects directly produced by ultrasonic energy to thermal gas-phase reactions taking place within the gas bubble. Marinesco (33) indicated that the temperature of the bubbles may be raised considerably during the

collapse. In water solutions, the reaction is the production of OH radicals by thermal decomposition. Carlin (10) lists increases in speed of reaction, separation of certain chemicals, oxidation, decomposition, crystallization, and changes in boiling points as some of the common effects. Bol'shakov (8) studying the effect of ultrasonic vibrations on curing of hides found that high frequency sound waves increased the extraction of water and non-leather making proteins from the hides and intensified the removal of microorganisms from the hide surfaces. Kober (25) found that ultrasonic treatment during the silicic acid process in the manufacture of tobacco products resulted in a more effective denicotinization.

The Effects of Ultrasonic Energy on Biological Systems

Intense ultrasonic fields produce effects in biological systems that vary from cellular disruption to death of the organism. Spence (44) reported that cytological and histological aberrations in the root meristem of Pisum were caused by ultrasonic vibrations at a frequency of 500 kc. The nuclei of the meristem were greatly distorted and cell walls of the calyptral regions were disrupted. Sustained doses resulted in complete loss of cellular arrangement, loss of chromaticity, disintegration of cell walls, separation of tissues, and necrosis. Lepeschkin and Gold-

man (29) found that ultrasonic energy at frequencies of 400 to 1500 kc caused displacement of the nucleus and chloroplasts in plant cells. The chloroplasts fused and converted into droplets. The nucleus either dissolved or coagulated. The coagulated protoplasm and chloroplasts disintegrated to form a suspension of fine granules. The suspension was so fine that no granules were visible and the cells appeared empty. Lehmann, Herrick, and Krusen (28) studied the effects of ultrasonic energy at frequencies of 0.8 to 1.0 Mc on root tips of Allium, Narcissus, Vicia faba, Pisum and in shoot tips of Helianthus. They noted that sometimes the cells including the protoplasm, the cell membrane and all other structures were completely disintegrated. Cairns (9) working with ultrasonic energy at a frequency of 90,000 cps killed and disintegrated nematodes in less than one minute. He stated that seed plants show no injury from the nematode-lethal frequency range. The effects of ultrasound on nematodes followed a successive series of events from increased activity, to a slowing of locomotion, followed by spasmodic reactions and immobilization, then organ disruption, death and disintegration.

Respiratory Activity

The rate of oxygen utilization and carbon dioxide evolution of plant tissues varies over a wide range. Accord-

ing to Goddard and Meeuse (16) respiration involves the oxidation of cellular metabolites with the transfer of electrons through a series of cellular enzymes, coenzymes and biocatalysts to molecular oxygen. This results in the oxidation of hydrogen to water and the production of alpha keto acids. These acids undergo decarboxylation with the release of carbon dioxide. James and Das (23) studying the organization of respiration in chlorophyllous cells indicated the prime source of respirable carbon in leaves is the chloroplast which manufactures a complex of 3-chain to 6-chain carbon units in a wide variety of compounds. These appear commonly to range from starch to pyruvate. Stiles (45) stated that the respiratory quotient for aerobic respiration of carbohydrate substrates is unity. Any alteration of the respiratory quotient in this study may be expected to be due to the treatments involved rather than the oxidation of other substrates.

Müller and Meldgaard (37) indicated that ultrasonic waves at frequencies of 4kHe increased the respiratory activity of potatoes from 50 to 200 percent. Barker (5) reported that examining shrivelled potatoes to ascertain condition of the flesh immediately increased carbon dioxide output about 30 percent. Audus (4) stated that the different behavior of turgid and wilted tubers shown by Barker was probably due to cell deformation. He also reported

that cells in leaves are sufficiently deformable in a turgid condition to give a maximum response in respiratory activity and that variations in cell turgidity have no obvious effect on this response. Audus (3) and (4) found that mechanical deformation of cells in the lamina of starved foliage leaves greatly stimulated their oxidative respiration rate. This is followed by a slight depressing of the respiratory activity. He believed mechanical stimulation acted upon one or more of the stages of oxidative respiration subsequent to triosis. Lowered oxygen content of the surrounding atmosphere brought about a depressant effect on carbon dioxide output. Denny (12) noted that a decline in oxygen concentration in a range immediately below that of normal air resulted in a fall in oxygen absorption of about 5 percent while carbon dioxide production remained the same.

Ascorbic Acid and Carotene Studies

Collards are an excellent source of ascorbic acid and carotene. Constable (11) reported that ascorbic acid in green leaves is generally between 0.05 and 0.5 percent of the fresh weight. Since ascorbic acid is water soluble, washing treatments would be expected to reduce its level in leaf tissue. Mapson (30) stated that the inherent level of dehydroascorbic acid in non-photosynthetic tissues is dependant on the oxygen status of the tissue. A clearcut

association between ascorbic acid concentration in the leaves of turnip greens and solar radiation and temperature was noted by Whitacre, Brittingham and Grimes (49). Martin, et al (34) found that broccoli cooked from 1 to 5 minutes retained 80 to 91 percent of its ascorbic acid. Mapson (31) indicated that the chloroplasts were believed to be the site and chlorophyll the agent of ascorbic acid synthesis. Harris and Olliver (18) showed that loss of ascorbic acid is likely to be greater from chopped greenstuffs than from boiled ones due to heat-inactivation of enzymes in boiling. James (22) noted that damage leads to rapid oxidation.

Carotene is water insoluble but is sensitive to light, auto-oxidation and atmospheric oxygen. Martin, et al (34) found it fairly stable to heat and almost 100 percent retained in cooked broccoli. Significant losses of beta-carotene during the weathering of sorghum were noted by Blessin, Dimler, and Webster (7). Loss of nutrients due to changes in the structure of plant and animal tissues were reported by Watt and Wu Leung (48). They reported thirty times as much carotene and many times more ascorbic acid were contained in the interveinal area of collards as compared with the midrib and stems while the midrib and stems accounted for nearly half the weight.

MATERIALS AND METHODS

Collards grown in greenhouses and field plots were used in these investigations. Seed from the Vates variety were planted in flats containing a soil mix of 1 part peat to 1 part soil. When about 1-1/2 inches in height the seedlings were transplanted into 3-inch peat pots. After about five weeks the plants were set in the field. The plantings were made in a randomized complete block design consisting of three replicates per spray interval. Plants were spaced 3 feet apart in 30 foot rows, 5 feet apart. Greenhouse plantings were made in ground beds with plants spaced 18 inches in rows 2 feet apart.

When plants in the field plots reached a diameter of about 4 feet, Thiodan was applied at the rate of 1 lb per acre in 100 gallons of water using a two gallon garden-type sprayer with an adjustible nozzle. The nozzle orifice was adjusted to break the spray particles into a very fine mist. The spray was applied to the plots in a single application to permit harvesting at intervals of 0, 3, 7, 10, and 14 days. Two leaves were harvested at random from each plant for each cleaning treatment. The petiole was removed and the leaves cut into pieces about 8 to 10 cm wide and 16 to 20 cm long (plate 1). A 200 gm sample was used for each cleaning treatment.

Plate 1. Cleaning unit of the ultrasonic cleaning system. Collard leaf pieces show the size into which leaves were cut before cleaning and the effect of ultrasonic energy on the leaf tissue.

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I. Cleaning Studies

An ultrasonic cleaning system consisting of a generator and a cleaning unit with a 20 kilocycle frequency were used in these investigations (Plate 2). A powerstat on the generator could be adjusted to deliver any desired power output from 0 to 500 milliamperes (ma). Another powerstat permitted regulation of the polarization current to the cleaning unit. The cleaning unit consisted of a magnetostrictive transducer mounted to a three gallon tank. Five and seven-tenths liters of water were used as a cleaning medium. This permitted peak activity to be reached without exceeding the plate current rating of 450 ma. The polarization current was regulated to seven amperes throughout the investigation.

The collard leaves treated in the ultrasonic cleaning unit were submerged in the cleaning medium and agitated by stirring several times during the cleaning period. The leaves washed by hand were agitated throughout the cleaning period. The cleaning treatments used in the residue removal studies with Thiodan were as follows:

check - sprayed with Thiodan but unwashed

H-5 - 5-minute ultrasonic wash at 450 ma

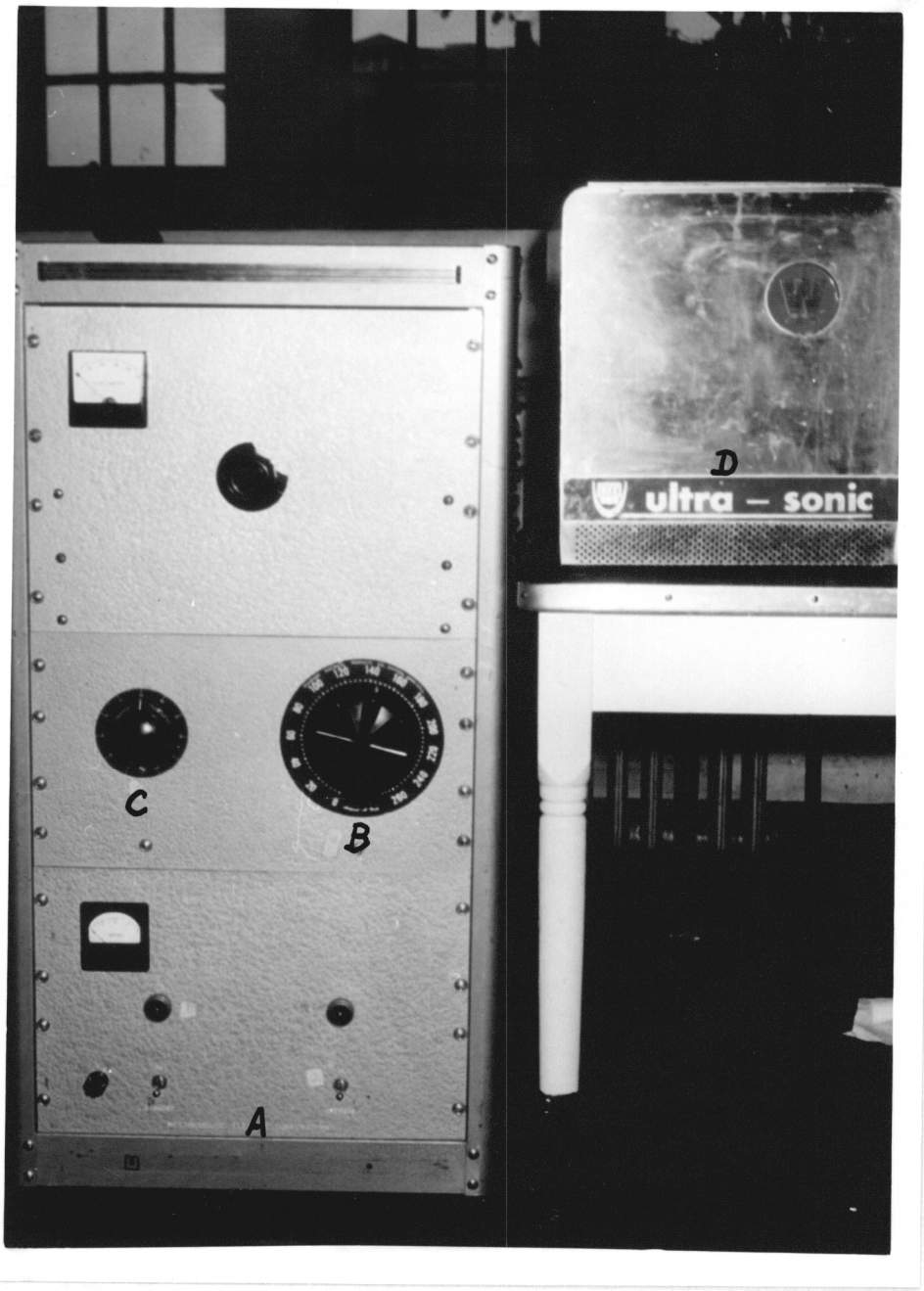
H-10 - 10-minute ultrasonic wash at 450 ma

H-20 - 20-minute ultrasonic wash at 450 ma

L-5 - 5-minute ultrasonic wash at 200 ma

Plate 2. The ultrasonic cleaning system used in these investigations

A. Entire generator
B. Powerstat controlling generator output
C. Powerstat controlling polarization current
D. Cleaning unit


D
ultra – sonic
C
B
A

L-10 - 10-minute ultrasonic wash at 200 ma

L-20 - 20-minute ultrasonic wash at 200 ma

0-5 - 5-minute hand wash

0-10 - 10-minute hand wash

0-20 - 20-minute hand wash

To prevent extreme variations in temperature water flowed at a constant rate through the cleaning unit. The temperature of the cleaning medium varied between 34 and 39°C. After washing, the samples were placed in plastic bags and frozen and stored at -20°C until analyses were carried out.

Residue Determination

Thiodan (endosulfan) (6, 7, 8, 9, 10 hexachloro-1, 5, 5a, 6, 9, 9a hexahydro - 6, 9 methano - 2, 3, 4 benzodioxathiepin 3 - oxide) was determined by the modified method of Maitlen, Walker, and Westlake (32). A one hundred gram sample of finely chopped leaf samples was weighed and placed in a quart mason jar to which was added 300 ml of a 2:1 n-hexane-isopropyl alcohol mixture. The extract was washed repeatedly to remove the alcohol and the washed extract dryed with anhydrous sodium sulfate.

Aliquots of the extract were made up to volumes varying from 20 to 60 ml depending on the amount of residue expected in the sample. To clear up the solution a carbon-magnesium oxide absorbant was added and the solution shaken in a wrist-action shaker. The extract was filtered through

a plug of acetone-extracted cotton overlayed with a layer of anhydrous sodium sulfate. The funnel and filter were washed 4 times with 15 ml of a 5:95 ethyl ether-distilled hexane mixture. The filtrate was evaporated to dryness in a 50°C water bath using a gentle stream of air. Five ml of methanolic sodium hydroxide-pyridine reagent were added to the test tubes containing the evaporated samples. The test tubes were stoppered and placed in a 100°C oil bath for four minutes. Then the stoppers were loosened and the samples placed in ice for one minute. The solution was filtered to remove fats and waxes that would interfere with transmittance and the absorbance determined on a Beckman D. U. spectrophotometer. Residue was determined from a standard curve that had been prepared from a standard solutions containing 0-100 mg of Thiodan.

Ascorbic Acid Determination

Ascorbic acid was determined by the Heinze-Kanapaux method (19). One hundred gm samples of collards were treated in the ultrasonic cleaning unit as described in the determination of Thiodan residues. Twenty five gm of each sample were ground in a Waring Blendor to which 100 ml of 3 percent metaphosphoric acid was added to extract the ascorbic acid. The extract was filtered through Whatman number 12 filter paper. One ml aliquots of the filtrate were pipetted into 50 ml volumetric flasks. Enough sodium

citrate buffer was added to bring the pH to 3.6. The aliquots were then diluted to volume with a phosphate-citrate buffer solution.

Readings were made on an Evelyn photoelectric colorimeter using a wavelength of 520 mu. Transmission was set at 100 percent and tubes containing 5 ml of phosphate-citrate buffer, and 5 ml of dye plus a few crystals of ascorbic acid for complete decolorization were read to insure correct setting. The dye solution was prepared by dissolving 34.4 mg of 2, 6-dichlorophenol indophenol dye in hot water. Blank readings were made in triplicate, using 5 ml of phosphate-citrate buffer in 5 ml of dye and reading immediately. Sample readings were made by adding 5 ml of the buffered extract to 5 ml of dye plus a few crystals of ascorbic acid. Ascorbic acid was determined against a series of standards made up from a standard solution composed of 50 mg of U.S. Reference Ascorbic Acid dissolved in 500 ml of phosphate-citrate buffer.

Carotene Determinations

Carotene was determined by the method of Moore and Ely (36). Five-gram samples of the ultrasonically treated collard leaves from which the petiole and midrib had been removed were weighed and placed in 275 ml bottles to which was added 100 ml of redistilled petroleum ether. The samples were placed in a boiling water bath for several

minutes to destroy enzyme activity. After cooling, the samples were blended in a Waring Blendor with 150 ml of 95 percent ethanol to make a foaming mixture in the blendor. The blended mixture was transferred to beakers and filtered into separatory funnels using 95 percent ethanol to rinse the blendor jar. The alcohol and petroleum ether phases were separated with distilled water and the alcohol phase drained into a second funnel. The residue was extracted with petroleum ether three times for complete separation of the carotene. The ether extract was washed with water to remove the ethanol and concentrated under vacuum to about 25 ml. The extract was then passed through a column of dicalcium phosphate which had been wetted with petroleum ether. The column and flasks were rinsed with petroleum ether until all the carotene had passed into the receiving flask. The filtrate was made to volume with petroleum ether and read on an Evelyn photoelectric colorimeter at 450 mu, set at 100 percent transmission with petroleum ether. The carotene content of the unknown extracts was determined against a curve from standard solutions containing 15 to 100 ug carotene per 100 ml of solution.

II. Respiration Studies

Following the procedure of Umbreit, Burris, and Stauffer (47) the Warburg Respirometer was used to study respiratory activity. For the monometric investigations,

15 ml Warburg flasks with a single sidearm and alkali well were used. For the determination of carbon dioxide evolved, 1 gm samples of collards were placed in each vessel. Two and eight-tenths ml of distilled water were added and the vessels placed in a constant-temperature water bath at 37.5°C. The vessels, for the determination of oxygen utilization, were prepared in the same manner as for carbon dioxide evolution except that 0.4 ml of a 20 percent potassium hydroxide solution was placed in the well of each vessel to absorb the carbon dioxide. A 2 cm^2 piece of Whatman No. 4 filter paper was placed in the well to increase the effectiveness of carbon dioxide absorption. The ultrasonic treatments for the respiratory activity were the same as those used in Thiodan residue removal studies. Leaf disc samples from the interveinal areas of the treated leaves were taken with a No. 4 stainless steel cork borer. The rates of carbon dioxide evolved and oxygen utilized were determined on a dry weight basis as microliters per milligram per hour.

III. Histological Study

For the histological study of the effect of the ultrasonic treatments on collard leaf tissue, 100 gm samples were treated as previously described in the ultrasonic cleaning system. Samples were taken from the interveinal areas of the treated leaves with a No. 4 stainless steel

borer. These samples were killed and fixed in a formal-acetic-alcohol solution of the following composition: 5 parts 40 percent formalin, to 5 parts glacial acetic acid, to 90 parts 50 percent ethanol. The material was then dehydrated with a butyl alcohol and an ethyl alcohol series. The dehydrated material was embedded in paraffin and sectioned with a rotary microtome and affixed to glass slides with Haupts adhesive. Then the material was stained with safranin and fast green as described by Johansen (24). The prepared slides were studied under a Reichert Zetopan research microscope and photomicrographs taken with a Leica 35 mm camera.

EXPERIMENTAL RESULTS

I. Cleaning Study

Persistence data for Thiodan residue after cleaning collards are given in Table 1. A tolerance of 2 ppm is allowable on the crop with a seven day restriction on application prior to harvest (20). Residue on the leaves harvested and washed for 20 minutes at 450 ma reached 2 ppm the day the plants were sprayed. Other cleaning treatments, with the exception of the hand wash for 5 minutes, reduced the residue to 2 ppm three days after spraying. Seven days after the spray was applied residue on the leaves harvested and washed by hand for 5 minutes were reduced to an average of 1.57 ppm. The level of Thiodan on the unwashed leaves reached the tolerance level of 2 ppm ten days after the spray was applied. Residue levels after all cleaning treatments were below 1 ppm at both 10- and 14-day spray intervals.

Table 2 compares the percent of spray residue retained by the different treatments after cleaning. The percent retained is based on the content on the unwashed treatment for each spray interval. All cleaning treatments, except for the 5 and 20 minute hand-washed treatments 10 days after spray application, were lower than the unwashed treatment. Data on the effect of the cleaning treatments for the 0-, 3-, 7-, 10- and 14-day spray intervals are given in Tables 3 to 7. The data were analyzed by the analysis

Table 1. Thiodan residue on collard leaves after cleaning. Data cited are the means of three replications.

Days After Spraying	Cleaning Treatment[a]									
	0-5	0-10	0-20	L-5	L-10	L-20	H-5	H-10	H-20	Check
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
0	6.25	5.94	5.49	2.49	2.97	2.25	3.89	3.88	2.00	10.63
3	3.26	1.94	1.32	1.86	1.58	1.92	1.75	1.40	1.40	3.64
7	1.57	1.05	1.33	1.53	1.50	1.28	1.39	1.04	0.79	7.06
10	0.78	0.48	0.87	0.37	0.33	0.61	0.47	0.47	0.26	0.65
14	0.56	0.63	0.46	0.46	0.54	0.23	0.44	0.50	0.34	1.08

[a]0-5, 0-10, and 0-20 were hand washed for 5, 10, and 20 minutes; L-5, L-10, and L-20 were ultrasonically washed at 200 ma for 5, 10, and 20 minutes; H-5, H-10 and H-20 were ultrasonically washed at 450 ma for 5, 10, and 20 minutes; check, was sprayed with Thiodan but unwashed.

Table 2. Persistence of Thiodan on collard leaves after cleaning.

Cleaning Treatment[b]	Residue[a] after cleaning Harvest Interval				
	0 Days	3 Days	7 Days	10Days	14 Days
	Percent	Percent	Percent	Percent	Percent
H-20	18.81	38.46	11.19	40.00	31.48
H-10	36.50	38.46	14.73	72.31	46.30
H-5	36.59	48.08	19.69	72.31	40.74
L-20	21.17	52.75	18.13	93.85	21.30
L-10	27.94	43.41	21.24	50.77	50.00
L-5	23.42	51.10	21.67	56.92	42.59
0-20	51.65	36.26	18.84	133.85	42.59
0-10	55.88	53.30	14.87	73.85	58.33
0-5	58.80	89.56	22.24	120.00	51.85

[a]Percent residue remaining is on the basis of residue contained on unwashed sample at each harvest date.

[b]Cleaning treatments are given in the footnote of Table 1 on page 27.

of variance and Duncan's (13) multiple range test was used to test for significant differences.

As shown in Table 3, the Thiodan retained at the 0-day spray interval ranged from 10.63 ppm on the unwashed collard leaves to 2.00 ppm on the ultrasonic wash for 20 minutes at 450 ma. This was a reduction of 81 percent in the residue level. The ultrasonic wash for 20 minutes at 200 ma reduced the residue level 79 percent to 2.25 ppm. The hand wash for 20 minutes decreased the residue level 48 percent to 5.49 ppm. The ultrasonic wash for 10 minutes at 200 ma reduced the residue level 72 percent to 2.97 ppm. The hand wash for 5 minutes reduced the Thiodan 41 percent to 6.25 ppm. At the 0-day spray interval, the differences between the residue remaining on the leaves cleaned by each cleaning treatment and the leaves which were sprayed but unwashed were significant at the 1 percent level.

When compared with the hand wash for 5 minutes, the ultrasonic wash for 20 minutes at 450 ma reduced the residue level 68 percent or 4.25 ppm. The ultrasonic wash for 20 minutes at 200 ma reduced the residue level 64 percent or 4.00 ppm. The ultrasonic wash for 5 minutes at 200 ma decreased the residue level 60 percent or 3.76 ppm. When compared with the hand wash for 10 minutes the ultrasonic wash for 20 minutes at 200 ma reduced the residue 62 percent or 3.69 ppm. The ultrasonic wash for 20 minutes at 450 ma

Table 3. The effect of cleaning treatments[a] on residue removal the same day Thiodan was applied.

Treatment	Mean ppm Thiodan	Difference Between the Means Mean ppm Thiodan									
		2.00	2.25	2.49	2.97	3.88	3.89	5.49	5.94	6.25	10.63
H-20	2.00	----	0.25	0.49	0.97	1.88*	1.89*	3.49**	3.94**	4.25**	8.63**
L-20	2.25		----	0.24	0.72	1.63	1.64	3.24**	3.69**	4.00**	8.38**
L-5	2.49			----	0.48	1.39	1.40	3.00**	3.45**	3.76**	8.14**
L-10	2.97				----	0.91	0.92	2.52**	2.97**	3.28**	7.66**
H-10	3.88					----	0.01	1.61	2.06*	2.37**	6.75**
H-5	3.89						----	1.60	2.05*	2.36**	6.74**
0-20	5.49							----	0.45	0.76	5.14**
0-10	5.94								----	0.31	4.69**
0-5	6.25									----	4.38**
Check	10.63										----

[a]Cleaning treatments are given in the footnote on Table 1 on page 27.
*Significant at the 5% level (Based on Duncan's multiple range test).
**Significant at the 1% level.

decreased the residue 66 percent or 3.94 ppm. The differences between these treatments and the 10 minute hand wash were significant at the 1 percent level.

When compared with the hand wash for 20 minutes the ultrasonic wash for 20 minutes at 200 ma reduced the residue 59 percent or 3.24 ppm. The ultrasonic wash for 20 minutes at 450 ma reduced the level 64 percent or 3.49 ppm. These differences were significant at the 1 percent level of probability.

The differences in levels of Thiodan after the cleaning treatments of collard leaves from the 3-day spray interval are given in Table 4. The residue retained varied from 1.32 ppm for the 20 minute hand wash, to 3.64 ppm for the unwashed leaves. This was a difference of 64 percent. The ultrasonic washes for both 10 and 20 minutes at 450 ma reduced the residue level 62 percent to 1.40 ppm. The ultrasonic wash for 10 minutes at 200 ma decreased the level of spray residue 57 percent to 1.58 ppm. The ultrasonic treatment for 5 minutes at 450 ma reduced the residue level 52 percent to 1.75 ppm. However, the 10 percent reduction of the residue by the hand wash for 5 minutes was not significant. The hand wash for 20 minutes reduced the residue level 59 percent or 1.94 ppm more than the hand wash for 5 minutes. The ultrasonic washes for both 10 and 20 minutes at 450 ma reduced the residue level 57 percent

Table 4. The effect of cleaning treatments on residue removal from collard leaves 3 days after Thiodan application.

Treatment[a]	Mean ppm Thiodan	Difference Between the Means Mean ppm Thiodan									
		1.32	1.40	1.40	1.58	1.75	1.86	1.92	1.94	3.26	3.64
0-20	1.32	----	0.08	0.08	0.26	0.43	0.54*	0.60*	0.62*	1.94**	2.32**
H-20	1.40		----	0.00	0.18	0.35	0.46	0.52	0.54*	1.86**	2.24**
H-10	1.40			----	0.18	0.35	0.46	0.52	0.54*	1.86**	2.24**
L-10	1.58				----	0.17	0.28	0.34	0.36	1.68**	2.06**
H-5	1.75					----	0.11	0.17	0.19	1.51**	1.89**
L-5	1.86						----	0.06	0.08	1.40**	1.78**
L-20	1.92							----	0.02	1.34**	1.72**
0-10	1.94								----	1.32**	1.70**
0-5	3.26									----	0.38
Check	3.64										----

[a]Cleaning treatments are given in the footnote of Table 1 on page 27.
*Significant at the 5% level (Based on Duncan's multiple range test).
**Significant at the 1% level.

or 1.86 ppm. These differences were significant at the 1 percent level.

The residue retained at the 7-day spray interval is shown in Table 5. It ranged from 7.06 ppm on the unwashed leaves to 0.79 ppm on the leaves washed for 20 minutes at 450 ma. This was a difference of 89 percent. The ultrasonic wash for 10 minutes at 450 ma reduced the residue level 85 percent to 1.04 ppm. The hand wash for 10 minutes reduced the residue level 85 percent to 1.05 ppm. The ultrasonic wash for 20 minutes at 200 ma reduced the residue level 82 percent to 1.28 ppm. The hand wash for 20 minutes reduced the residue level 81 percent to 1.33 ppm. The ultrasonic wash for 5 minutes at 450 ma reduced the residue level 78 percent to 1.53 ppm. The hand wash for 5 minutes reduced the residue level 78 percent to 1.57 ppm. The differences in residue levels between these cleaning treatments and the unwashed leaves were significant at the 1 percent level. The differences between the various cleaning treatments at the 7-day spray interval were not significant.

The effect of the cleaning treatments on residue removal 10 days after spray application are given in Table 6. No significant differences were shown between the treatments. However, considerable variations exist in the level of residue retained. The ultrasonic wash for 20 minutes at 450 ma reduced the residue level 0.52 ppm more than the

Table 5. The effect of cleaning treatments on residue removal 7 days after Thiodan application.

Treatment[a]	Mean ppm Thiodan	Difference Between the Means Mean ppm Thiodan 0.79	1.04	1.05	1.28	1.33	1.39	1.50	1.53	1.57	7.06
H-20	0.79	----	0.25	0.26	0.49	0.54	0.60	0.71	0.74	0.78	6.27**
H-10	1.04		----	0.01	0.24	0.29	0.35	0.46	0.49	0.53	6.02**
O-10	1.05			----	0.23	0.28	0.34	0.45	0.48	0.52	6.01**
L-20	1.28				----	0.05	0.11	0.22	0.25	0.29	5.78**
O-20	1.33					----	0.06	0.17	0.20	0.24	5.73**
H-5	1.39						----	0.11	0.14	0.18	5.67**
L-10	1.50							----	0.03	0.07	5.56**
L-5	1.53								----	0.04	5.53**
O-5	1.57									----	5.49**
Check	7.06										----

[a]Cleaning treatments are given in the footnote of Table 1 on page 27.
**Significant at the 1% level (Based on Duncan's multiple range test).

Table 6. The effect of cleaning treatments on residue removal 10 days after Thiodan application.

Treatment[a]	Mean ppm Thiodan	Difference Between the Means Mean ppm Thiodan									
		0.26	0.33	0.37	0.47	0.47	0.48	0.61	0.65	0.78	0.87
H-20	0.26	----	0.07	0.11	0.21	0.21	0.22	0.35	0.39	0.52	0.61
L-10	0.33		----	0.04	0.14	0.14	0.15	0.28	0.32	0.45	0.54
L-5	0.37			----	0.10	0.10	0.11	0.24	0.28	0.41	0.50
H-10	0.47				----	0.00	0.01	0.14	0.18	0.31	0.40
H-5	0.47					----	0.01	0.14	0.18	0.31	0.40
0-10	0.48						----	0.13	0.17	0.30	0.39
L-20	0.61							----	0.04	0.17	0.26
Check	0.65								----	0.13	0.22
0-5	0.78									----	0.09
0-20	0.87										----

[a]Cleaning treatments are given in the footnote of Table 1 on page 27.

hand wash for 5 minutes, a reduction of 67 percent. It reduced the residue level 70 percent lower than the 20 minute hand wash. The ultrasonic treatment for 20 minutes at 200 ma reduced the residue level 22 percent more than the hand wash for 5 minutes, and 30 percent lower than the hand wash for 20 minutes.

The effects of the cleaning treatments on residue removal for the 14-day spray interval is shown in Table 7. The residue level ranged from 1.08 ppm on the unwashed collard leaves to 0.23 ppm on the leaves washed in the ultrasonic cleaning unit for 20 minutes at 200 ma. This was a difference of 79 percent. The ultrasonic wash for 20 minutes at 450 ma reduced the residue level 69 percent to 0.34 ppm. The hand wash for 5 minutes reduced the residue level 48 percent. The 10 minute hand wash reduced it 42 percent to 0.63 ppm. These treatments, when compared with the residue level on unwashed leaves, were significant at the 1 percent level.

When compared with the hand wash for 10 minutes the ultrasonic wash for 20 minutes at 200 ma reduced the residue level 63 percent to 0.40 ppm. This was significant at the 1 percent level. In comparison with the hand wash for 5 minutes the ultrasonic wash for 20 minutes at 200 ma reduced the residue level 59 percent. When compared with the hand wash for 10 minutes the ultrasonic wash for 20

Table 7. The effect of cleaning treatments on residue removal 14 days after Thiodan application.

Treatment[a]	Mean ppm Thiodan	Difference Between the Means Mean ppm Thiodan									
		0.23	0.34	0.44	0.46	0.46	0.50	0.54	0.56	0.63	1.08
L-20	0.23	----	0.11	0.21*	0.23*	0.23*	0.27**	0.31**	0.33**	0.40**	0.85**
H-20	0.34		----	0.10	0.12	0.12	0.16	0.20*	0.22*	0.29**	0.74**
H-5	0.44			----	0.02	0.02	0.06	0.10	0.12	0.19	0.64**
L-5	0.46				----	0.00	0.04	0.08	0.10	0.17	0.62**
0-20	0.46					----	0.04	0.08	0.10	0.17	0.62**
H-10	0.50						----	0.04	0.06	0.13	0.58**
L-10	0.54							----	0.02	0.09	0.54**
0-5	0.56								----	0.07	0.52**
0-10	0.63									----	0.45**
Check	1.08										----

[a]Cleaning treatments are given in the footnote of Table 1 on page 27.
*Significant at the 5% level (Based on Duncan's multiple range test).
**Significant at the 1% level.

minutes at 450 ma reduced the residue level 0.29 ppm. This was a difference of 54 percent. These comparisons were significant at the 1 percent level.

Table 8 gives the analysis of the accumulated residue levels retained after cleaning treatments. The analysis of variance indicated that there were significant differences between treatments, replications, and days. The variation in the amount of residue retained on the collard leaves after the cleaning treatments ranged from 0.96 ppm for the ultrasonic treatments for 20 minutes at 450 ma to 4.61 ppm for the unwashed leaves. This was a 79 percent reduction in the residue level by the ultrasonic cleaning treatments 20 minutes at 450 ma. The hand wash for 5 minutes reduced the residue level 44 percent to 2.58 ppm. The hand wash for 10 minutes reduced it 56 percent to 2.01 ppm. The ultrasonic wash for 20 minutes at 200 ma reduced it 73 percent to 1.26 ppm. The reduction in residue level by the 5 minute hand wash was significant at the 5 percent level of probability, and all other cleaning treatments at the one percent level when compared with the unwashed leaves. The difference between the 5 minute hand wash and the ultrasonic wash for 20 minutes at 450 ma was significant at the 1 percent level. Differences among the other cleaning treatments were insignificant.

Table 8. The effect of cleaning treatments on total Thiodan residue on collards.

Analysis of Variance

Variation due to	D.F.	S.S.	M.S.S.	F.
Replications	2	12.22	6.11	7.91**
Treatments	9	150.31	16.70	21.63**
Error	18	13.91	0.772	
Days	4	337.00	84.25	78.01**
Days x Replications	8	21.12	2.64	2.44
Days x Treatments	36	150.97	4.19	3.87
Days x Replications x Treatments	72	77.93	1.08	

**Significant at the 1% level.

Duncan's multiple range test:

H-20 ppm	L-20 ppm	L-5 ppm	L-10 ppm	H-10 ppm	H-5 ppm	0-20 ppm	0-10 ppm	0-5 ppm	Check
0.96	1.26	1.34	1.38	1.46	1.58	1.92	2.01	2.58	4.61

Any two means not underscored by the same line are statistically different. Any two means underscored by the same line are not significantly different.

Ascorbic Acid Content

Data on the content of ascorbic acid in collards after treating in the ultrasonic cleaning unit are given in Table 9. Analysis of variance was determined for the data and significance between the treatments was tested by Duncan's multiple range test. The ascorbic acid ranged between 37 mg/100 gm for the ultrasonic treatment for 10 minutes at 200 ma and 55 mg/100 gm for the untreated leaves. This was a difference of 33 percent. The loss of cellular contents into the cleaning medium can be observed in Plate 3. The ultrasonic treatment for 20 minutes at 450 ma reduced the ascorbic acid content 31 percent to 38 mg/100 gm. The ultrasonic treatment for 20 minutes at 200 ma reduced the ascorbic acid content 29 percent to 39 mg/100 gm. The ultrasonic treatment for 10 minutes at 450 ma decreased the ascorbic acid content 27 percent to 40 mg/100 gm. These differences were significant at the 1 percent level. The ultrasonic treatment for 5 minutes at 450 ma reduced the ascorbic acid content 14 mg/100 gm. The 5 minute ultrasonic treatment at 200 ma reduced the ascorbic acid content 13 mg/100 gm, a 24 percent reduction. The reduction in ascorbic acid content by the two 5 minute treatments were significant at the 5 percent level. Variations in the ascorbic acid content among the ultrasonic treatments were not significant.

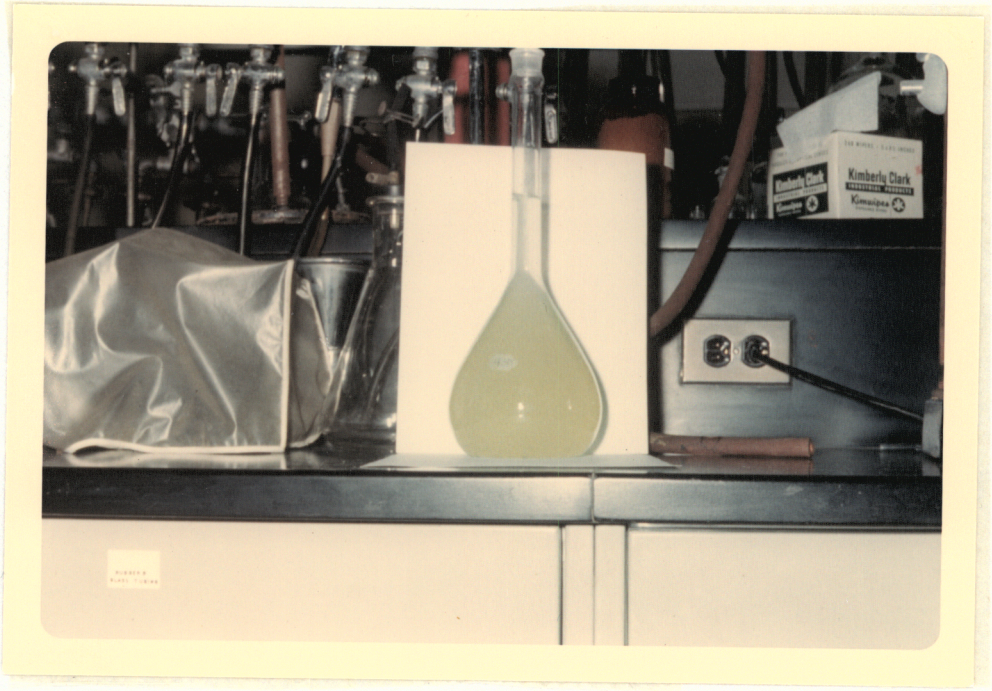
Table 9. Ascorbic acid content of collards after cleaning in an ultrasonic cleaning unit.

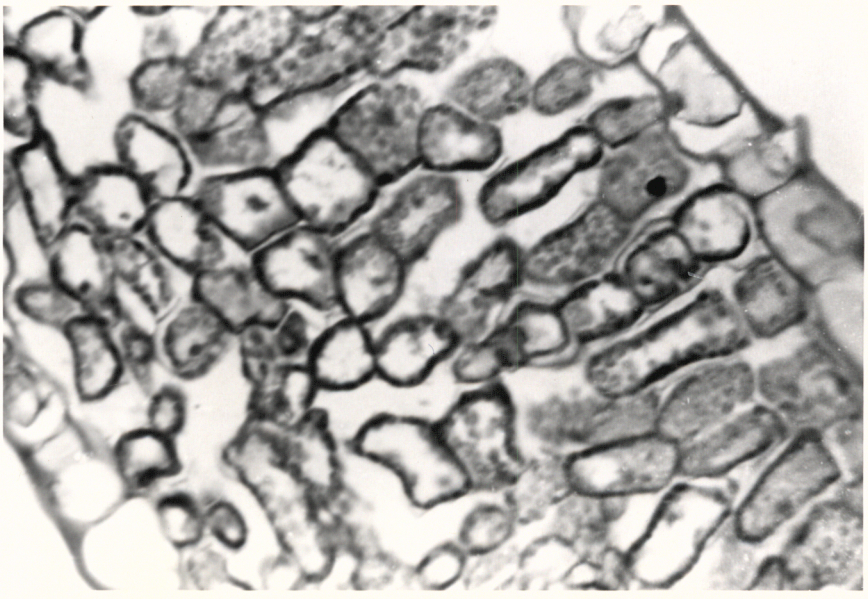
Treatment[a]	Mean mg Ascorbic acid /100 gms	Difference Between the Means Mean mg Ascorbic Acid/100 gm 37	38	39	40	41	42	55
L-10	37	--	1	2	3	4	5	18**
H-20	38		--	1	2	3	4	17**
L-20	39			--	1	2	3	16**
H-10	40				--	1	2	15**
H-5	41					--	1	14*
L-5	42						--	13*
Check	55							--

[a]Cleaning treatments are given in the footnote of Table 1 on page 27.
*Significant at 5% level (Based on Duncan's multiple range test).
**Significant at 1% level.

Plate 3. Sample of cleaning solution taken from cleaning unit after washing collards at 450 ma.

Plate 4. Transverse section from an untreated collard leaf showing arrangement of cells and amount of intercellular space. (X250).


Kimberly Clark
Kimwipes



Carotene Content

Table 10 gives the data on the carotene content of collard leaves after the ultrasonic cleaning treatments. The reduction in the carotene content is attributed to the losses of cellular contents due to the ultrasonic treatments. The carotene content ranged between 4940 ug/100 gm in the untreated leaves and 2520 ug/100 gm in the leaves washed for 10 minutes at 450 ma, a reduction of 49 percent. The ultrasonic treatment for 20 minutes at 450 ma reduced the carotene content 37 percent to 3100 ug/100 gm. The ultrasonic treatment for 20 minutes at 200 ma reduced it 34 percent to 3275 ug/100 gm. These decreases were significant at the 1 percent level.

The ultrasonic treatment for 10 minutes at 200 ma reduced the carotene content to 3355 ug/100 gm. This reduction of 32 percent was significant at the 5 percent level. The ultrasonic treatment for 10 minutes at 450 ma, when compared to the ultrasonic treatment for 5 minutes at 450 ma reduced the carotene content 1830 ug/100 gm, a reduction of 42 percent. This reduction was significant at the 1 percent level. The ultrasonic treatment for 10 minutes at 450 ma when compared to the ultrasonic treatment for 5 minutes at 200 ma reduced the carotene content 1640 ug/100 gm, a reduction of 39 percent. These differences were significant at the 5 percent level.

Table 10. Carotene content in collards after cleaning in an ultrasonic cleaning unit.

Treatment[a]	Mean ug carotene/ 100 gm	Difference Between the Means Mean ug/100 gm 2520	3100	3275	3355	4160	4350	4940
H-10	2520	----	580	755	835	1640*	1830**	2420**
H-20	3100		----	175	255	1060*	1250**	1840**
L-20	3275			----	80	885	1075*	1665**
L-10	3355				----	805	995	1585*
L-5	4160					----	190	780
H-5	4350						----	590
Check	4940							----

[a]Cleaning treatments are given in the footnote of Table 1 on page 27.
*Significant at the 5% level (Based on Duncan's multiple range test).
**Significant at the 1% level.

II. Respiration Studies

The effects of ultrasonic treatment on the respiratory activity of collard leaves are given in Tables 11 through 14. As shown in Table 11, oxygen was utilized by the untreated leaves at the rate of 4.9 ul/mg of leaf tissue per hour. Carbon dioxide was released by the untreated leaves at the rate of 4.3 ul/mg/hr. The rate of oxygen utilized by ultrasonically treated collard leaves from greenhouse grown collards, ranged between 5.1 ul/mg/hr after treatment for 20 minutes at 450 ma to 3.2 ul/mg/hr after treatment for 5 minutes at 200 ma. This was a difference of 37 percent. The carbon dioxide evolved ranged from 4.7 ul/mg/hr after the 20 minute treatment at 450 ma to 3.2 ul/mg/hr after the 5 minute treatment at 200 ma. This difference was 26 percent.

After ultrasonic treatment for 20 minutes at 200 ma oxygen was used at the rate of 3.9 ul/mg/hr, a 20 percent slower rate than the untreated leaves, while the carbon dioxide evolved was at a 5 percent slower rate. The 5 minute treatment at 200 ma reduced the utilization of oxygen 35 percent, and the production of carbon dioxide 26 percent. After this treatment oxygen was utilized by the leaf tissues at the same rate that carbon dioxide was produced.

Table 11. The effect of ultrasonic treatment on the respiratory activity of leaf discs from the interveinal area of greenhouse grown collards.

Treatment[1]	Oxygen utilized[2] Mean ul/mg/hr	Carbon dioxide evolved Mean ul/mg/hr
H-20	5.1 a	4.7 e
Check	4.9 ab	4.3 ef
L-20	3.9 c	4.1 fg
H-5	3.5 cd	3.7 gh
L-10	3.5 cd	3.4 h
H-10	3.3 cd	3.4 h
L-5	3.2 d	3.2 h

[1]Ultrasonic treatments are given in footnote of TAble 1 on page 27.

[2]For each time period any means not having letters in common are significantly different at the 5% level by Duncan's multiple range test.

When compared with the rate from untreated leaves, all treatments except the one for 20 minutes at 450 ma significantly reduced the rate of oxygen utilized. The other ultrasonic treatments were significantly different from 20-minute treatment at 450 ma. Neither the 20-minute treatment at 450 ma nor the 20-minute treatment at 200 ma significantly differed from the rate of carbon dioxide evolved by untreated leaves. The treatments for 5 and 10 minutes at both 200 and 450 ma, when compared with the untreated leaves, significantly reduced the rate of carbon dioxide produced.

Table 12 gives the results from a second investigation in which collard leaves were harvested and one ultrasonic treatment a day was applied and the respiratory rate determined and compared with the rate from untreated leaves. Oxygen was utilized by the untreated leaves at the rate of 4.6 ul/mg/hr. After ultrasonic treatment for 20 minutes at 450 ma oxygen was used at the same rate as by the untreated leaves. The carbon dioxide was evolved at a 10 percent greater rate than by the untreated leaves.

After treatment for 20 minutes at 200 ma oxygen uptake was reduced to 4.0 ul/mg/hr, a reduction of 13 percent, while the carbon dioxide evolved was reduced 16 percent.

The treatment for 5 minutes at 200 ma reduced the oxygen utilization 22 percent to 3.6 ul/mg/hr. The reduction

Table 12. The utilization of oxygen and the production of carbon dioxide by collard leaves when the rate of one ultrasonically treated sample was compared with that from untreated leaves on a given day.

Treatment[1]	Oxygen utilized[2] Mean ul/mg/hr	Carbon dioxide evolved Mean ul/mg/hr
H-20	4.6 a	4.9 e
Check	4.6 a	4.4 ef
H-10	4.3 ab	4.0 fg
L-20	4.0 b	3.7 fg
L-5	3.6 c	3.5 g
L-10	3.4 c	3.5 g
H-5	2.6 d	2.8 h

[1]Ultrasonic treatments are given in the footnote of Table 1 on page 27.

[2]For each time period, any means not having letters in common are significantly different by Duncan's multiple range test.

of carbon dioxide evolved was 20 percent to 3.5 ul/mg/hr. After treatment for 10 minutes at 200 ma 3.4 ul of oxygen/mg/hr was utilized, and 3.5 ul of carbon dioxide/mg/hr was evolved. These reductions were at the rate of 26 percent for oxygen uptake and 20 percent for carbon dioxide production.

After the treatment for 5 minutes at 450 ma the utilization of oxygen was reduced to the rate of 2.6 ul/mg/hr, a reduction of 43 percent, while the carbon dioxide production was lowered to 2.8 ul/mg/hr. This was a 36 percent reduction. The treatments for 5, 10, and 20 minutes at 200 ma and the 5 minute treatment at 450 ma significantly reduced the rate of oxygen utilized when compared with the rate from untreated leaves, or from leaves treated for 20 minutes at 450 milliamperes. The rate of carbon dioxide evolution, when compared with untreated leaves, was significantly decreased after treatment for 5 minutes at both 200 and 450 ma and after treatment for 10 minutes at 200 ma.

The influence of ultrasonic treatment on the utilization of oxygen 3 days after treating is shown in Table 13. The oxygen utilized by the untreated leaves increased 25 percent, from 4.4 ul/mg/hr to 5.5 ul/mg/hr. Initially the oxygen was utilized 14 percent more rapidly after the leaves were treated 20 minutes at 450 milliamperes. After

Table 13. The influence of ultrasonic energy on the utilization of oxygen as determined 1 and 3 days after treating collard leaves.

Treatment[1]	Oxygen Utilized[2] Mean ul/mg/hr 1 Day	3 Days
H-20	5.1 a	5.2 e
Check	4.4 b	5.5 e
H-5	4.0 bc	3.2 gh
L-20	3.7 cd	4.2 f
L-5	3.4 cd	3.0 h
L-10	3.4 cd	3.7 fg
H-10	3.3 d	3.3 gh

[1]Ultrasonic treatments are given in the footnote of Table 1 on page 27.

[2]For each time period, any means not having letters in common are significantly different at the 5% level by Duncan's multiple range test.

3 days oxygen was used 5 percent more slowly than by the untreated leaves. At the end of 3 days it had decreased to 3.2 ul/mg/hr. Compared to the untreated leaves this was a reduction of 42 percent. Initially, the treatment for 10 minutes at 200 ma utilized oxygen at the same rate as the 5 minute treatment at 200 ma. When it was compared with the rate of oxygen utilized by untreated leaves, the decrease was 33 percent. After treatment for 10 minutes at 450 ma the rate of oxygen utilization was reduced 25 percent to 3.3 ul/mg/hr. Three days later the rate was the same. However, when compared with the rate of the untreated leaves, there was a reduction of 40 percent.

Table 14 shows the effect of ultrasonic treatment on the production of carbon dioxide 3 days after treating the leaves. The treatment for 5 minutes at 450 ma reduced the rate 5 percent to 4.0 ul/mg/hr. Three days later it had decreased to 3.3 ul/mg/hr. This was a 17 percent reduction. In comparison with the untreated leaves, after 3 days the rate had decreased 25 percent. The treatment for 5 minutes at 200 ma decreased the rate to 3.3 ul/mg/hr. This was a reduction of 21 percent. After 3 days the rate had dropped to 3.0 ul/mg/hr. When compared with the untreated leaves, this was a decrease of 32 percent. After treatment for 10 minutes at 200 ma, the rate was reduced 17 percent to 3.5 ul/mg/hr. Three days after treatment

Table 14. The influence of ultrasonic energy on the evolution of carbon dioxide as determined 1 and 3 days after treating collard leaves.

Treatment[1]	Carbon Dioxide Evolved[2] Mean ul/mg/hr 1 Day	3 Days
H-20	4.7 a	4.8 e
Check	4.2 abc	4.4 ef
L-20	4.1 bc	4.1 f
H-5	4.0 cd	3.3 g
H-10	3.7 d	3.1 g
L-10	3.5 d	3.3 g
L-5	3.3 d	3.0 g

[1]Ultrasonic treatments are given in the footnote of Table 1 on page 27.

[2]For each time period, any means not having letters in common are significantly different at the 5% level by Duncan's multiple range test.

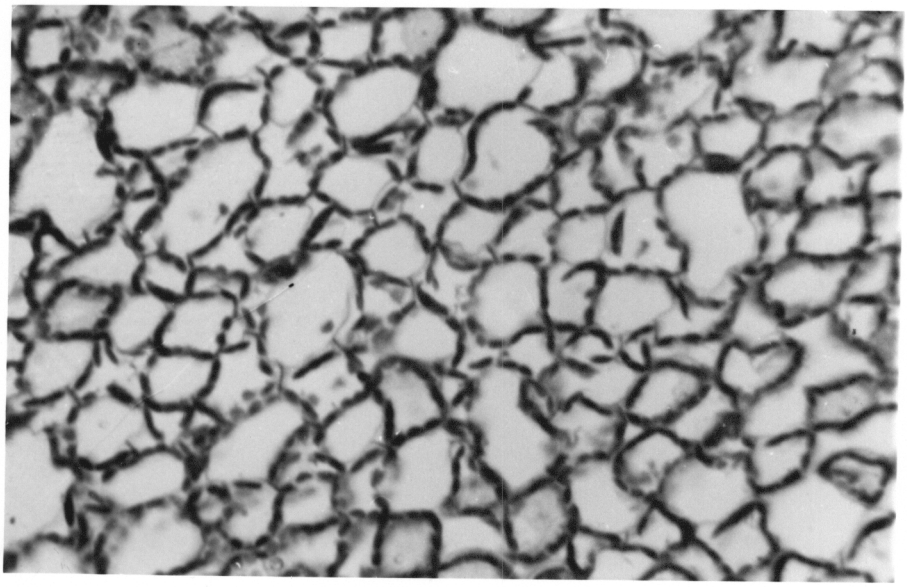
the rate had reduced 24 percent to 3.3 ul/mg/hr. The treatment for 10 minutes at 450 ma reduced the rate 22 percent to 3.7 ul/mg/hr. After 3 days the rate had reduced to 3.1 ul/mg/hr. This was a difference of 16 percent. When compared with the untreated leaves, the rate had decreased 30 percent. The rate, after treating for 20 minutes at 450 ma, was significantly different from all other treatments except the untreated leaves. The rates of carbon dioxide produced after treating for 5 minutes at both 200 and 450 ma and for 10 minutes at both 200 and 450 ma differed significantly from that of untreated leaves.

III. Histological Study

Microscopic examination of sectioned and stained leaf discs of collards, treated in the ultrasonic cleaning unit, revealed histological aberrations when compared with sections from untreated leaves. The palisade and spongy mesophyll tissues (Plate 4) are composed of loosely arranged parenchyma cells with large areas of intercellular airspace. Tangential sections (Plates 5 and 6) indicate the size and amount of intercellular space in the untreated tissue. Abnormalities within the leaf tissues of ultrasonically treated leaves (Plates 7, 8, 9, 10 and 11) were due to the mechanical forces produced by cavitation. Intercellular atmospheres were decreased resulting in smaller intercellular spaces within the tissues of the palisade and

Plate 5. Tangential section through the palisade tissue of an untreated leaf. Showing arrangement of chloroplasts within the cell and size and amount of intercellular space. (X250).

Plate 6. Tangential section through the spongy mesophyll of an untreated leaf. Showing size and amount of intercellular space and number of chloroplasts within the cells. (X250).



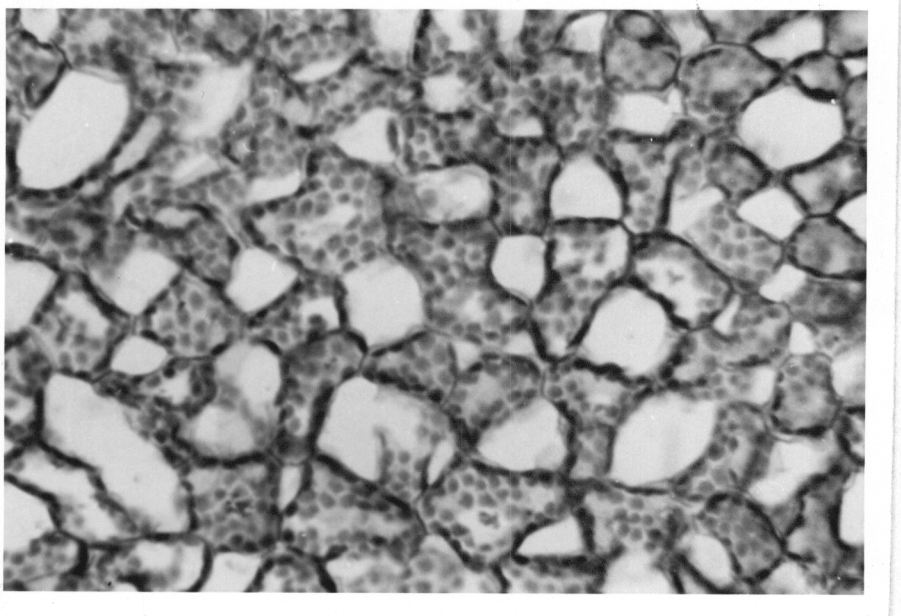
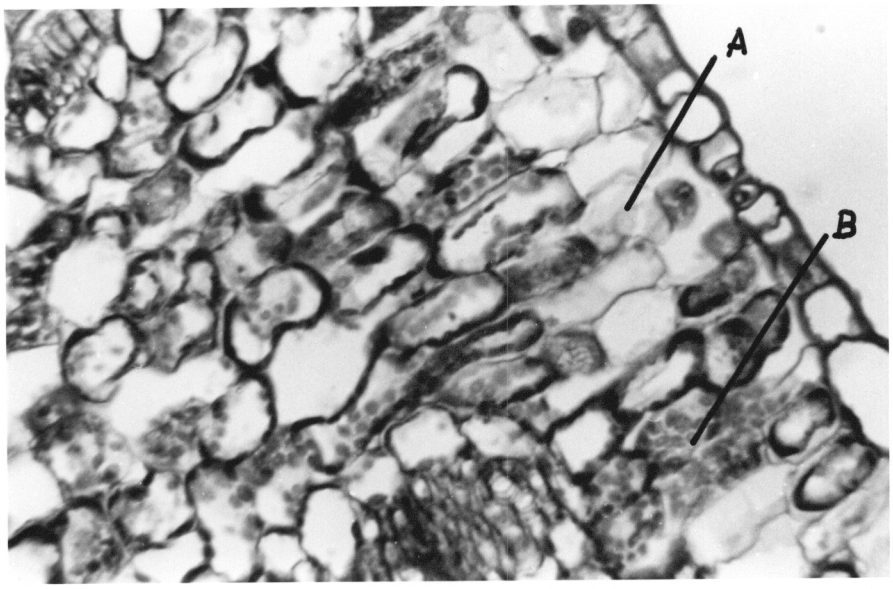


Plate 9. Tangential section through a leaf treated 20 minutes at 450 ma showing the compacting effect of degassing in the palisade tissue. With dispersion of the contents from disrupted cells. (X250).

Plate 10. Tangential section through a leaf treated 20 minutes at 450 ma showing an area of cells from which the contents have been expelled. (X125).


A
B

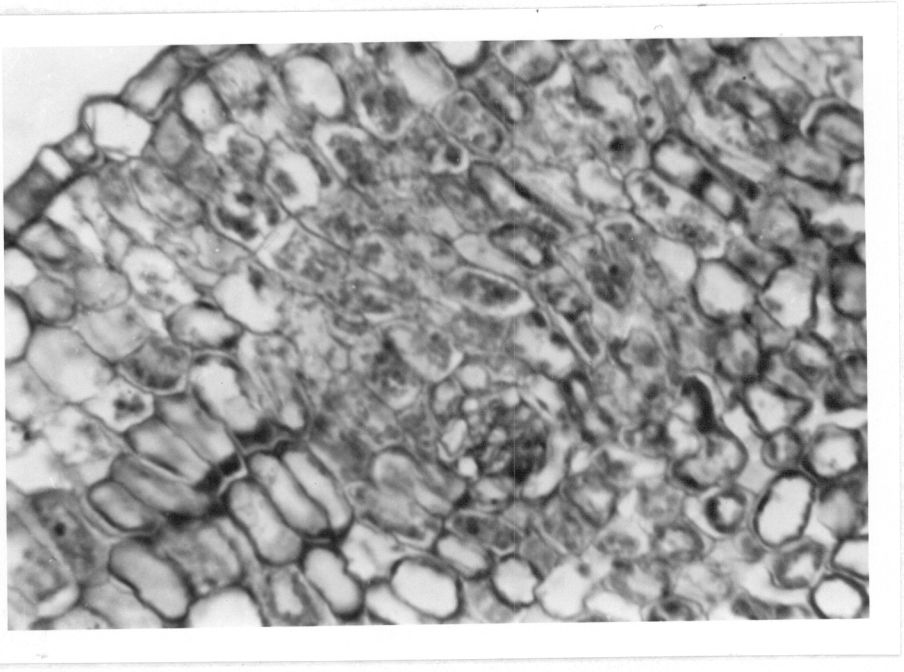
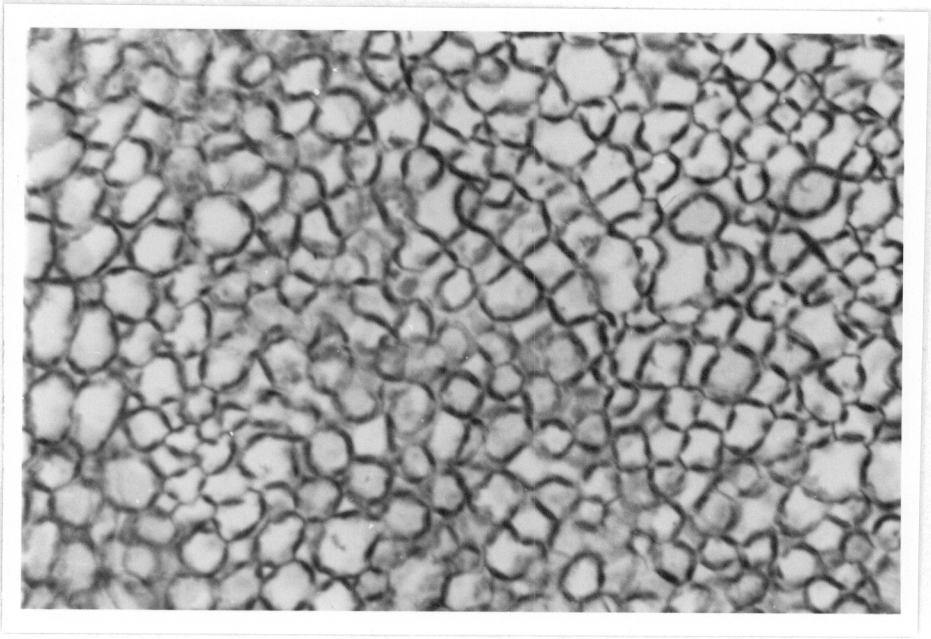


Plate 7. Transverse section through a leaf treated 5 minutes at 200 ma

A. Cells from which the contents have been dissipated.

B. Cells showing chloroplasts that are coagulated and fragmented. (X250).

Plate 8. Transverse section through a leaf treated 10 minutes at 450 ma showing the degree of compacting in the palisade tissue and spongy mesophyll. Fragmentation of chloroplasts within the cells can be observed.



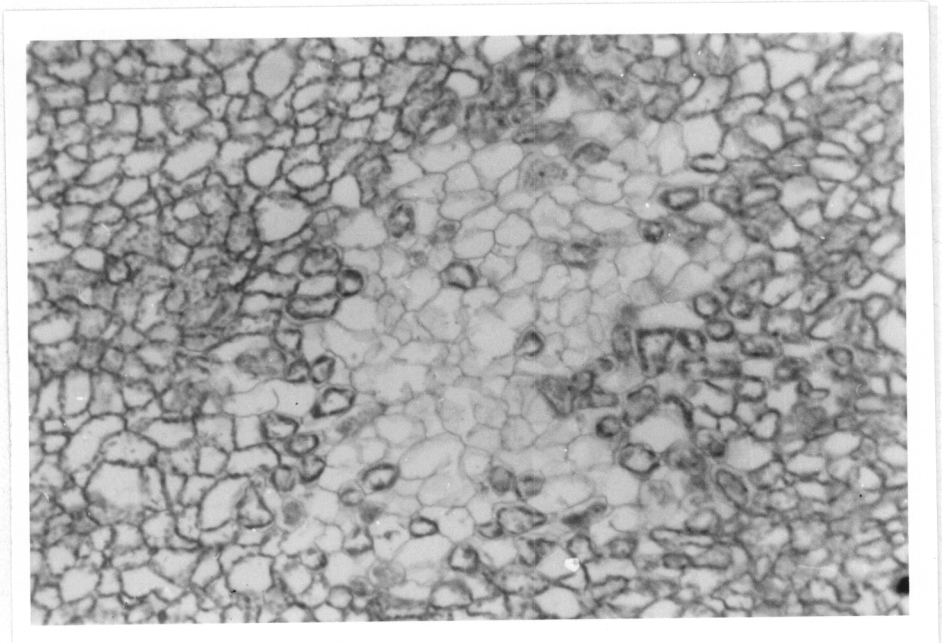
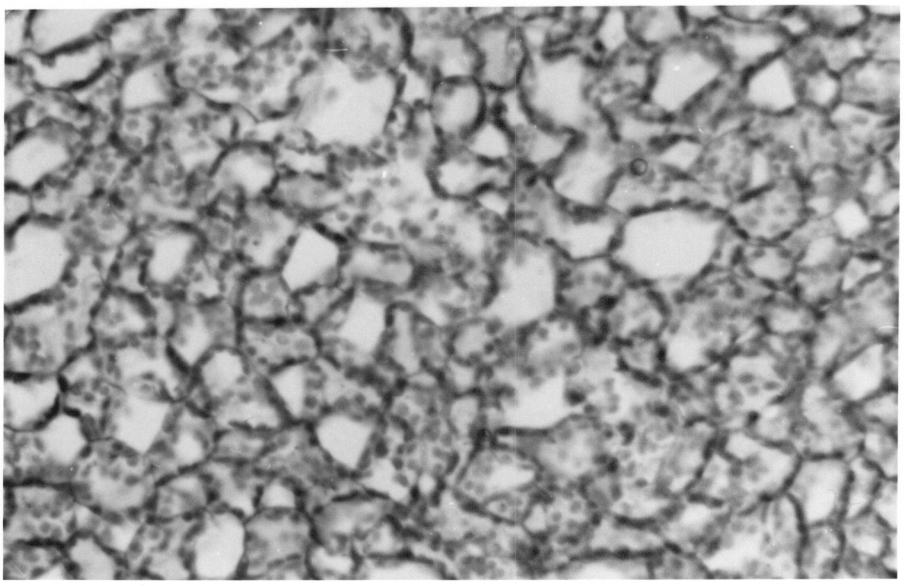
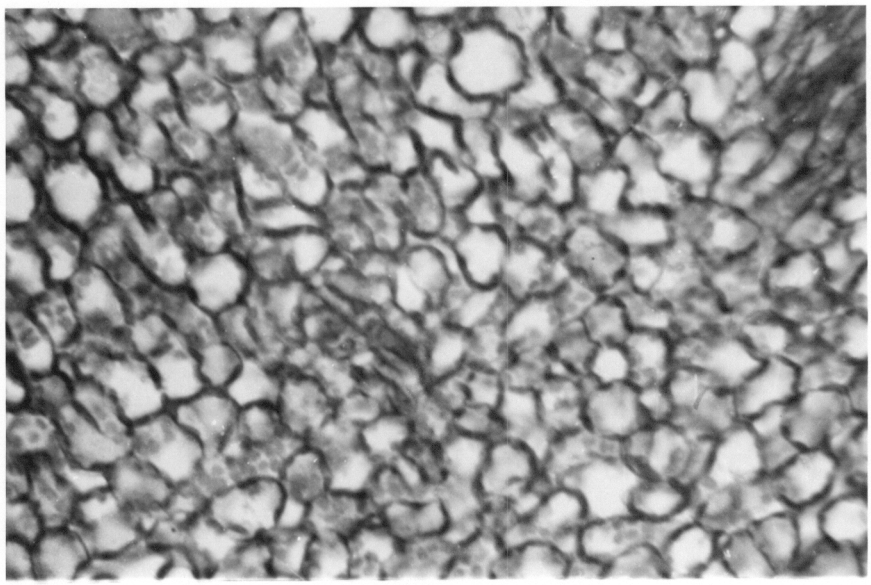


Plate 11. Tangential section through a leaf treated 20 minutes at 450 ma showing the effects of degassing on tissues of the spongy mesophyll. (X250).

Plate 12. Transverse section through a leaf treated for 10 minutes at 200 ma displaying the degree of degassing and compacting of the tissues of the palisade and spongy mesophylls. (X250).





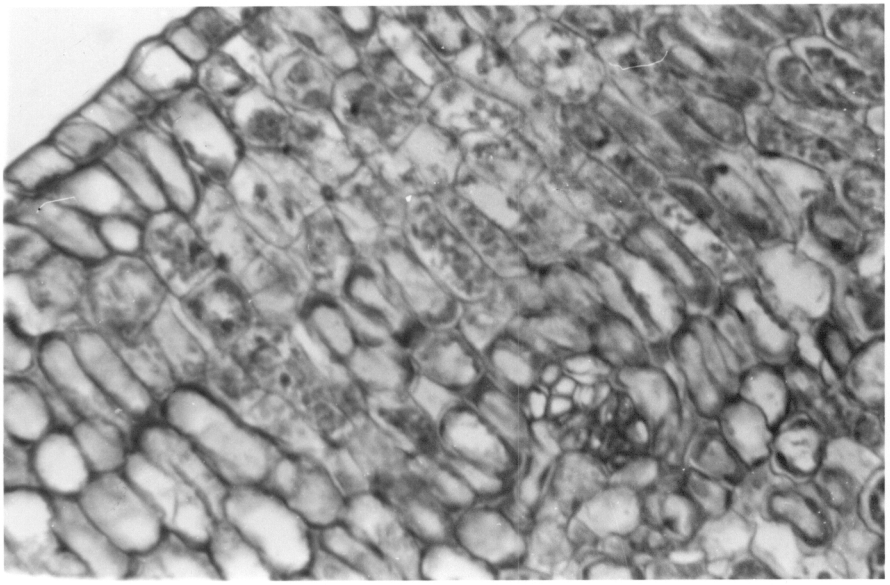
spongy mesophyll. The amount of degassing varied with the length of ultrasonic treatment, and with some treatments, caused the cells of the palisade and spongy parenchyma tissues to be very densely packed. The 10-minute treatments at 200 and 450 ma showed the greatest degree of cellular compaction (Plates 12 and 13). The 20-minute treatments (Plates 14 and 15) displayed more intercellular space than the 10-minute treatments. Treating for 5 minutes (Plate 16) caused a slight reduction of intercellular space.

Within the cells the cavitational forces apparently displaced the nuclei and chloroplasts, fused and fragmented them to form suspensions of fine granules, and expelled them from the cells (Plates 12, 13, 16, 17, 18, 19 and 20). All ultrasonic treatments used in this investigation produced these effects. Cells within the palisade and spongy mesophyll tissues in samples treated for 5 minutes at 200 ma displayed chloroplasts that appeared to be coagulated and fragmented, along with cells in which the contents appeared undisturbed (Plate 19). Contents of other cells were shown as suspensions of fine granules indicating fragmentation of the chloroplasts (Plate 17). Other areas displayed cells that appeared devoid of cellular constituents (Plate 21). Sections from samples treated 10 minutes at 200 ma revealed no indication of coagulation or fragmentation of the chloroplasts (Plates 12 and 18). Samples treated 20 minutes

Plate 13. Transverse section through a leaf treated for 10 minutes at 450 ma showing the degree of cellular compaction and fragmentation and disarrangement of chloroplasts within the cells. (X250).

Plate 14. Transverse section through a leaf treated 20 minutes at 450 ma displaying the compacting effects on cells of the palisade and spongy mesophyll

A. Palisade tissue
B. Spongy Mesophyll
C. Area of cells showing coagulated chloroplasts



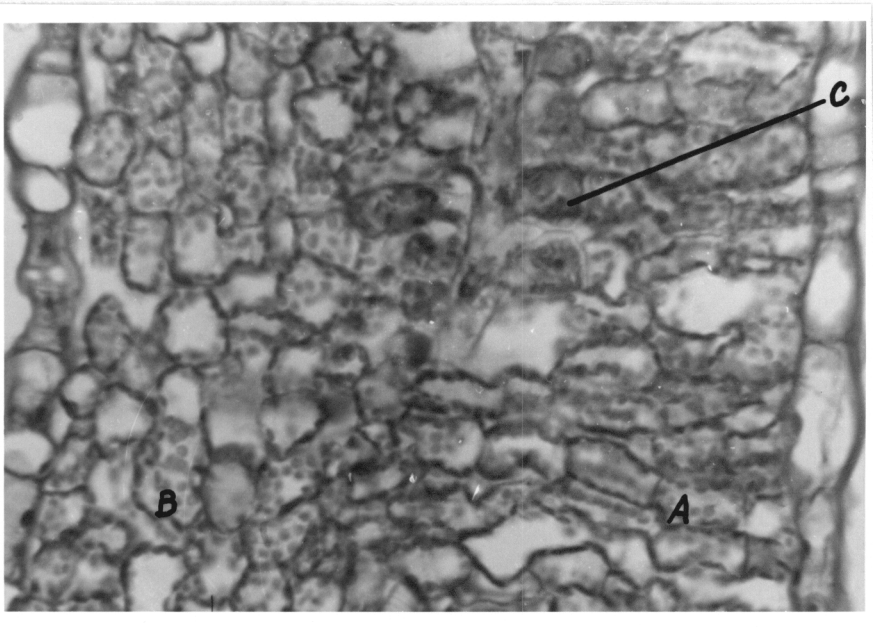
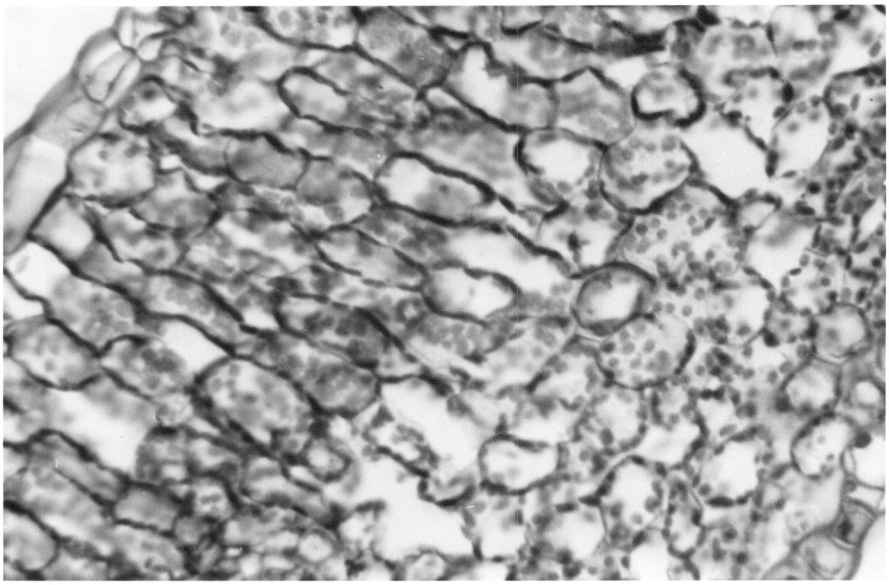

C
B
A

Plate 15. Transverse section through a leaf treated at 200 ma for 20 minutes showing the degree of compacting of the leaf tissues from this treatment. (X250).

Plate 16. Transverse section through a leaf treated for 5 minutes at 450 ma showing the degree of compacting in the tissues of the palisade and spongy mesophylls. (X250).



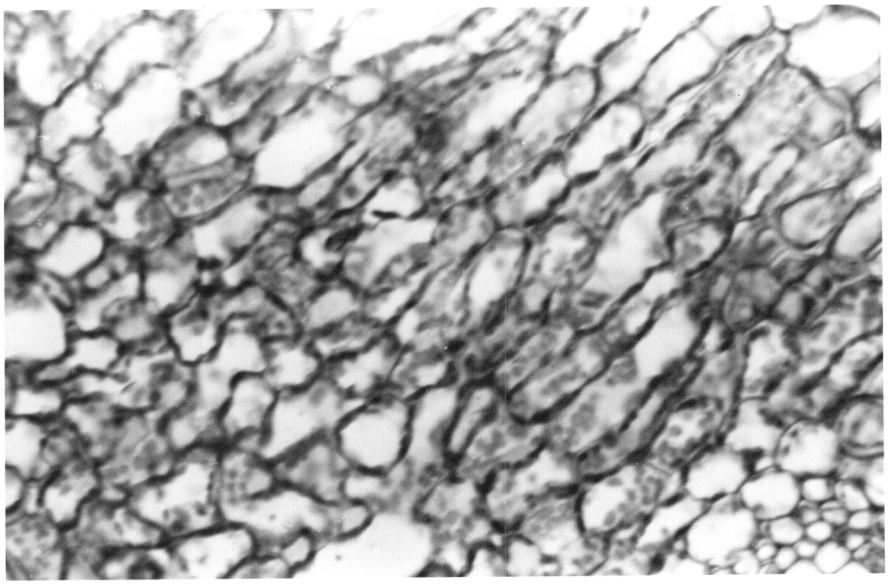
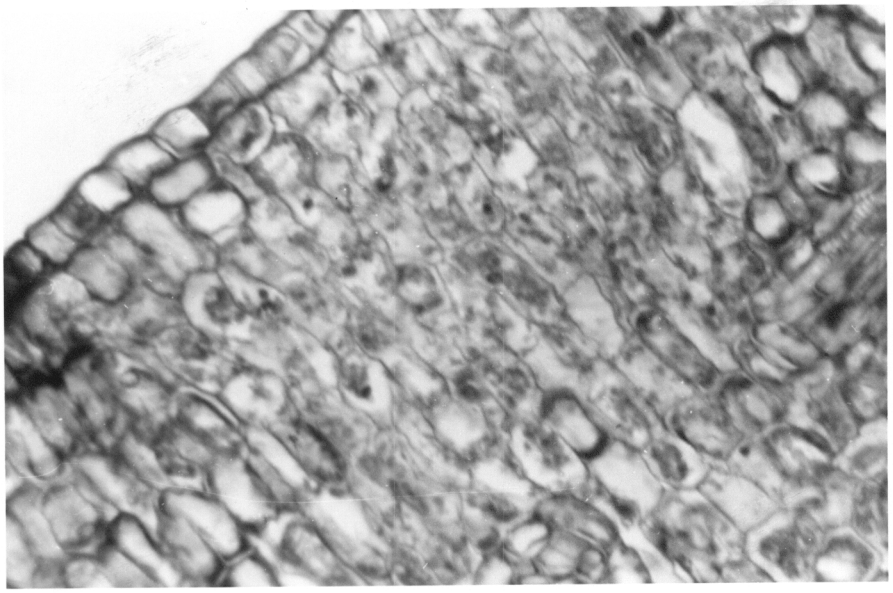


Plate 17. Transverse section through a leaf treated at 450 ma for 10 minutes showing fragmented and dispersed contents within the cells. (X250).

Plate 18. Transverse section through a leaf treated for 10 minutes at 200 ma showing degassing effects on cell compaction but only minor disarrangement of the contents within the cells. (X250).



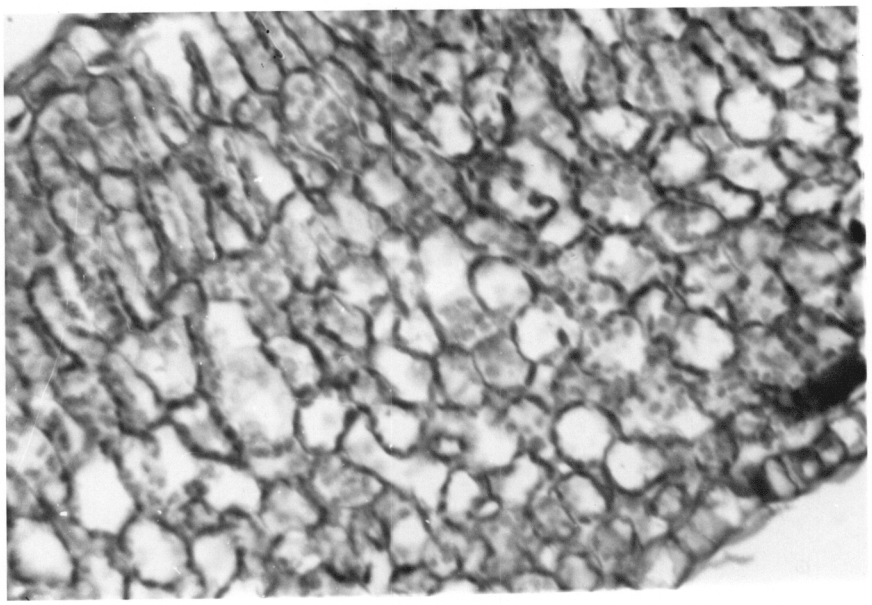
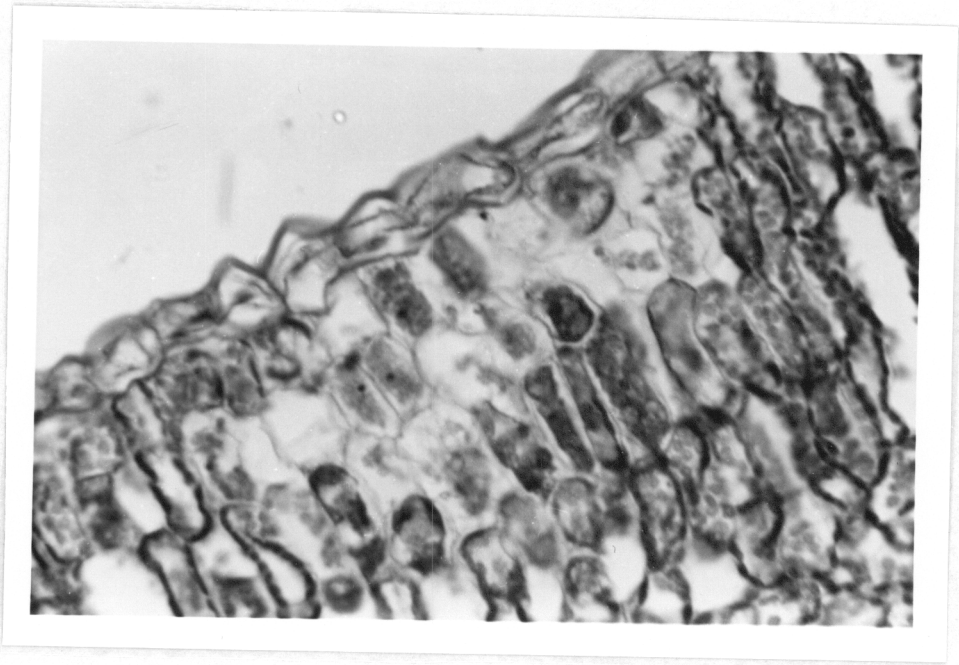


Plate 19. Transverse section through a leaf treated for 20 minutes at 200 ma showing cells in an injured area with varying degrees of coagulation, fragmentation, and dispersion of cellular contents. (X250).

Plate 20. Transverse section through a leaf treated for 5 minutes at 200 ma showing the extent of fragmentation, disarrangement and dispersion of chloroplasts. (X250).



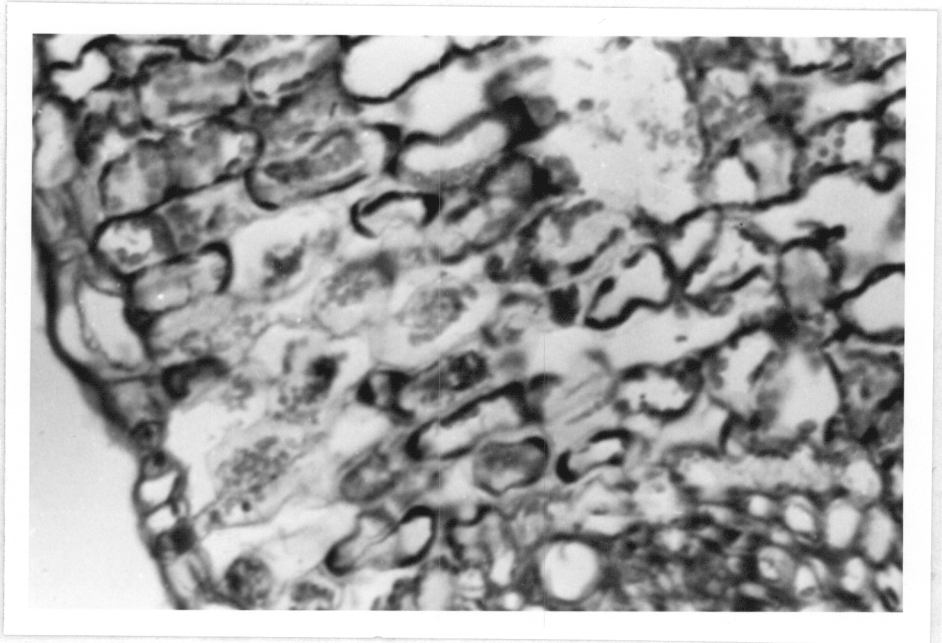
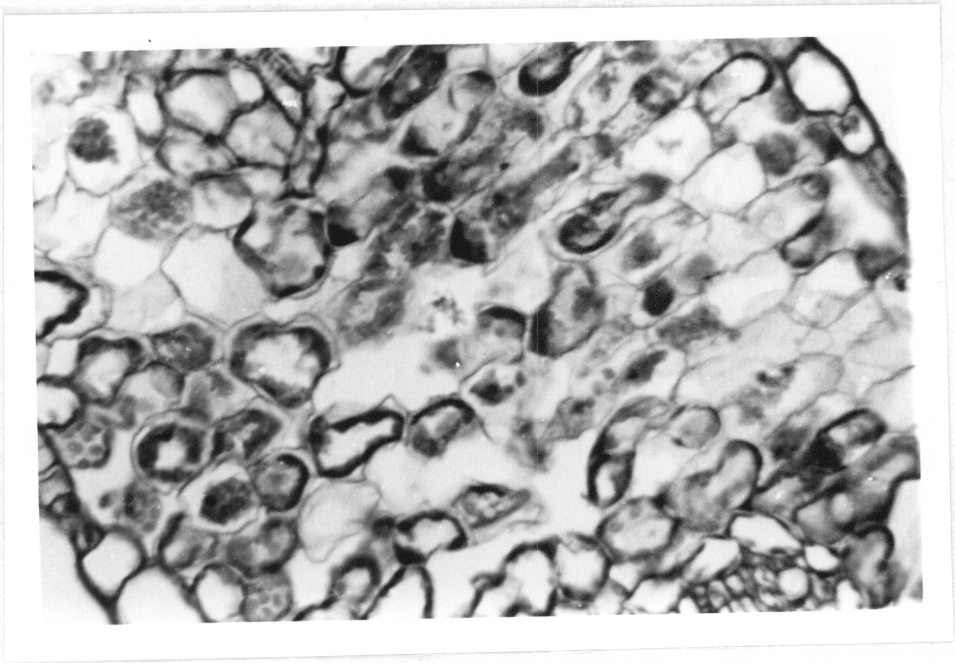
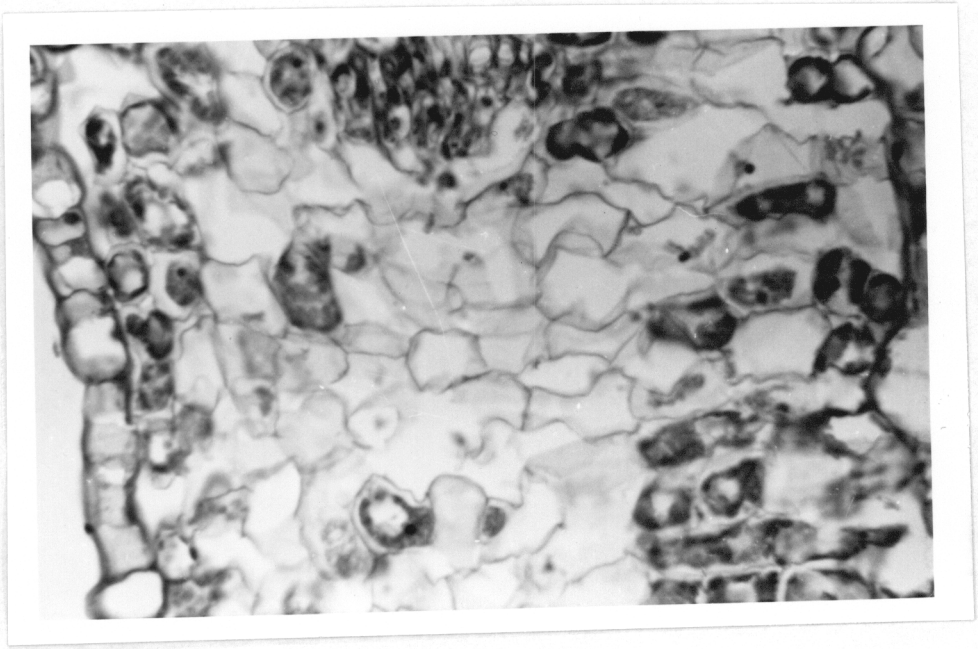


Plate 21. Transverse section through a leaf treated for 5 minutes at 200 ma showing an area with more extensive damage and loss of cellular contents. (X250).

Plate 22. Transverse section through a leaf treated 20 minutes at 450 ma showing an area where the cell walls have disintegrated. (X250).





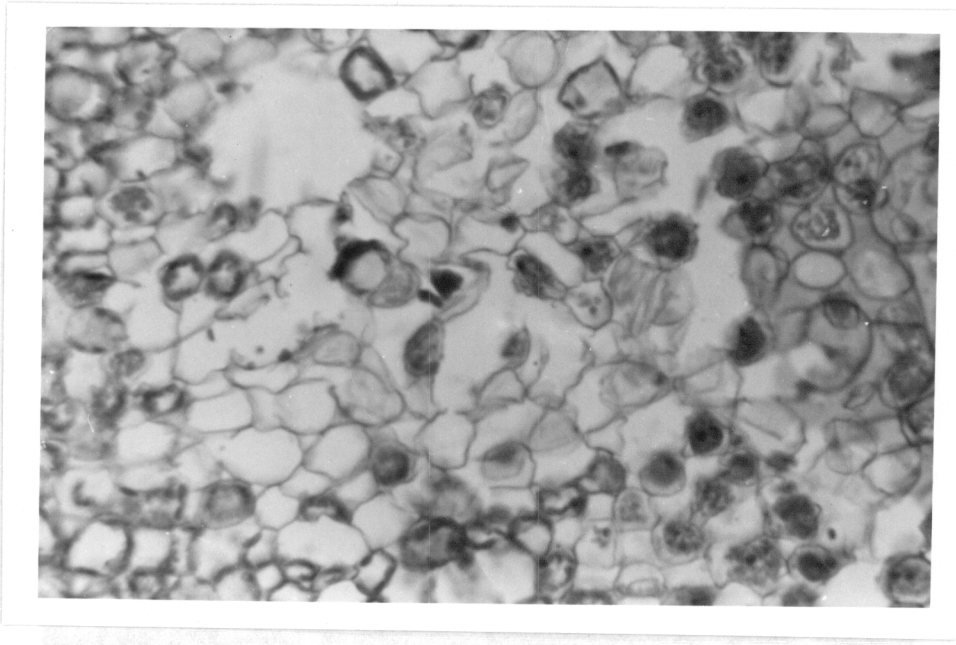
generally exhibit coagulation and fragmentation of chloroplasts (Plates 19, 22 and 23) with cells in some areas losing constituents and others appearing empty. This is similar to the findings of Hughes and Nyborg (21) who found that components within yeast cells may be released before major damage has been caused to the cell wall.

Sections from samples treated at 450 ma for periods of 5, 10 and 20 minutes exhibited extensive damage to the leaf tissues. Chloroplasts of some cells were coagulated, fragmented, and suspended as fine granules in the protoplasm. Cell walls were ruptured and the contents dispersed. Cellular constituents were expelled from some areas. In areas where the damage was most severe cell walls were destroyed. Similar effects on root meristems of peas were noted by Spence (44).

Tissues treated for 5 minutes had reduced intercellular spaces. Some cells in the damaged areas (Plates 24 and 21) appeared empty while others were filled with a suspension of fine granules. Cells in the palisade tissue from the 10 minute treatment (Plates 15, 17 and 18) were rectangular, and cells of the spongy mesophyll were polyhedral in shape. Almost a complete absence of intercellular space was noted. In damaged areas (Plates 25 and 26) loss of cellular constituents is evident, while many cells are filled with a homogenous suspension of fine granules.

Plate 23. Tangential section through a leaf treated 20 minutes at 450 ma showing ruptured and disintegrated cells of the spongy mesophyll and cells displaying varying degrees of coagulation and fragmentation of the cellular contents. (X250).

Plate 24. Transverse section through a leaf treated for 5 minutes at 200 ma showing coagulation, fragmentation and dispersion of cellular constituents, and an area of cells from which the contents have been dispelled. (X250).



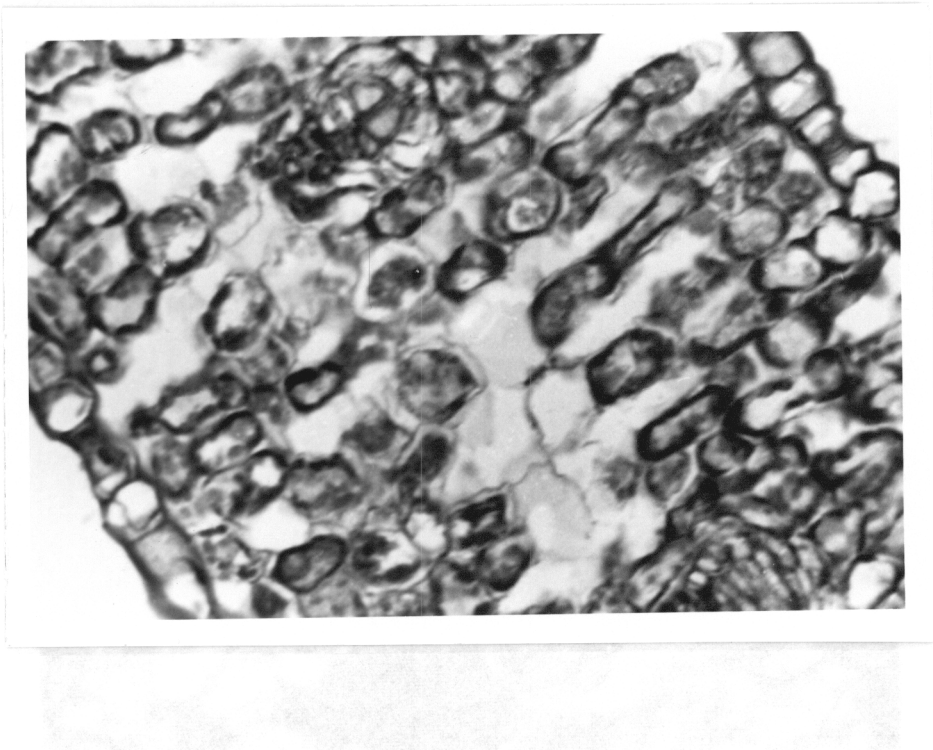
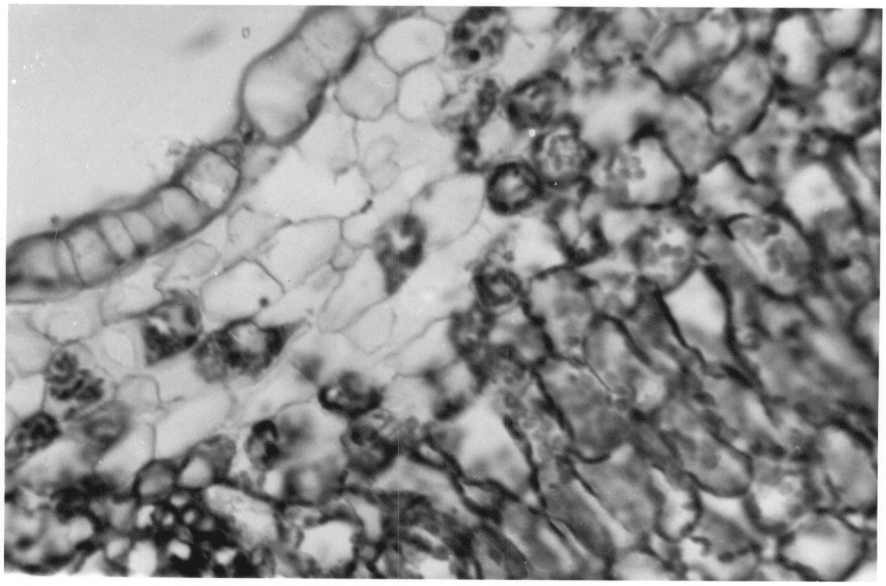
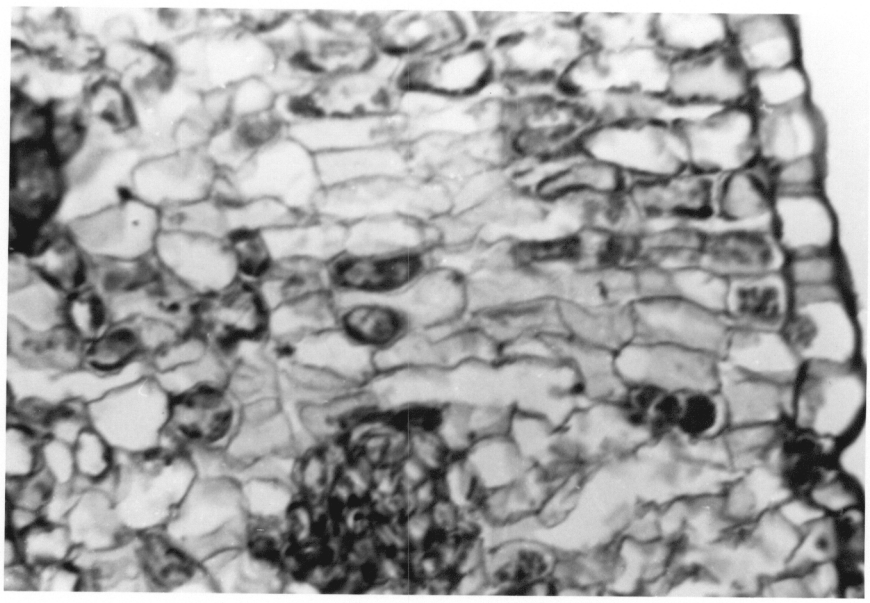


Plate 25. Transverse section through a leaf treated 20 minutes at 200 ma showing loss of cellular constituents from the damaged area and disarrangement of cellular contents in the surrounding cells. (X250).

Plate 26. Transverse section through a leaf treated 5 minutes at 450 ma showing the dispersion of cellular contents with occasional cells indicating only coagulation and fragmentation of chloroplasts. (X250).

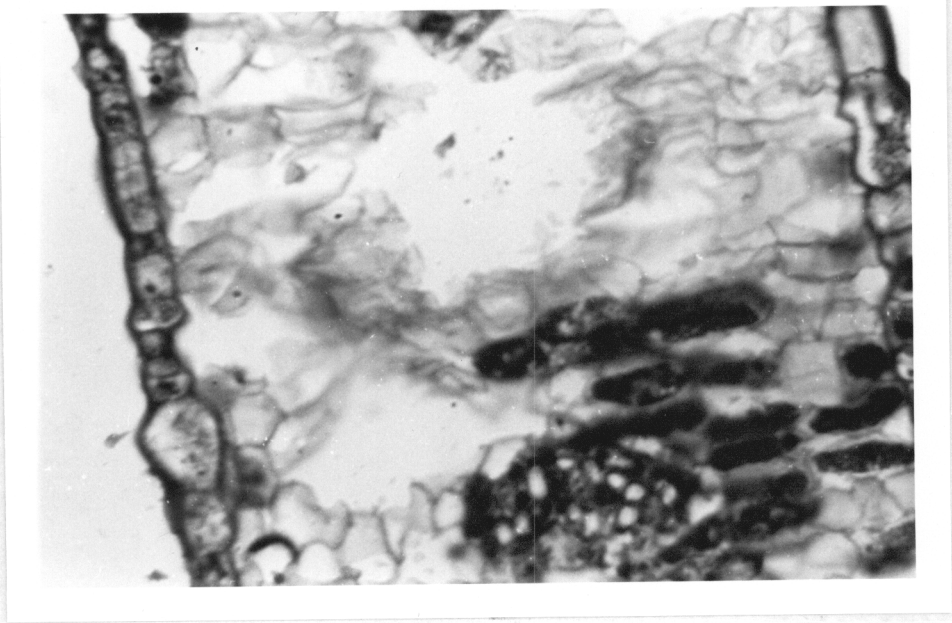




The 20 minute treatment caused most extensive damage and loss of cellular constituents (Plates 22, 23 and 27). Cell walls were disrupted (Plate 28) and in some areas the structure was completely destroyed (Plates 27, 28 and 29). Tangential sections through the leaf tissue indicate that extensive injury and loss of cell components were confined to small areas when compared with the total surface area observed (Plate 10). These results concur with the findings of Lepeschkin and Goldman (29) concerning the displacement, coagulation, and dispersion of the contents of plant cells radiated in an ultrasonic field.

Plate 27. Transverse section through a leaf treated for 20 minutes at 450 ma showing an area of extensive damage and loss of cellular contents. (X250).

Plate 28. Transverse section through a leaf treated for 20 minutes at 450 ma showing an area where cells are ruptured and an adjacent area where little damage to the cell and its contents are shown. (X250).



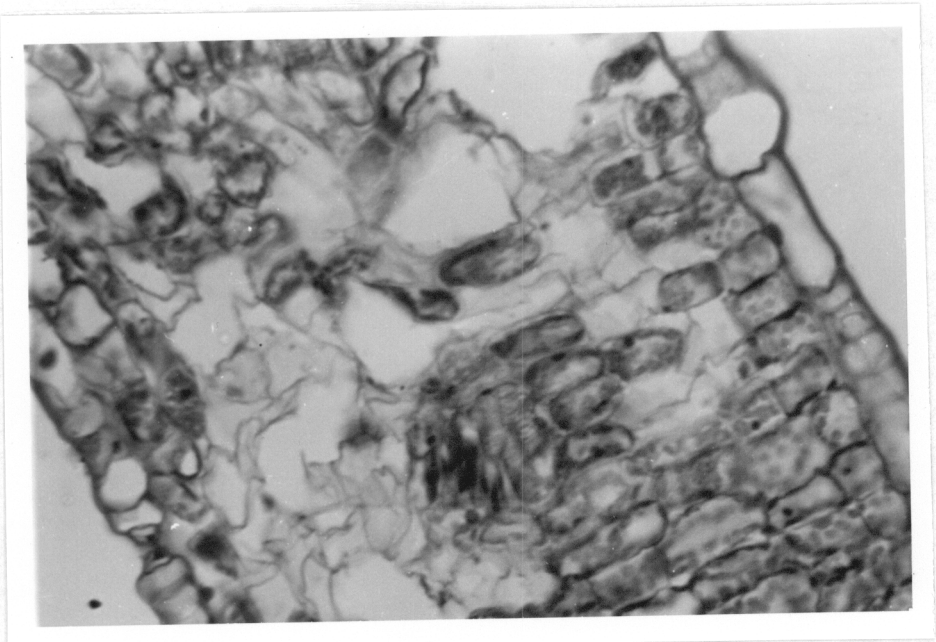
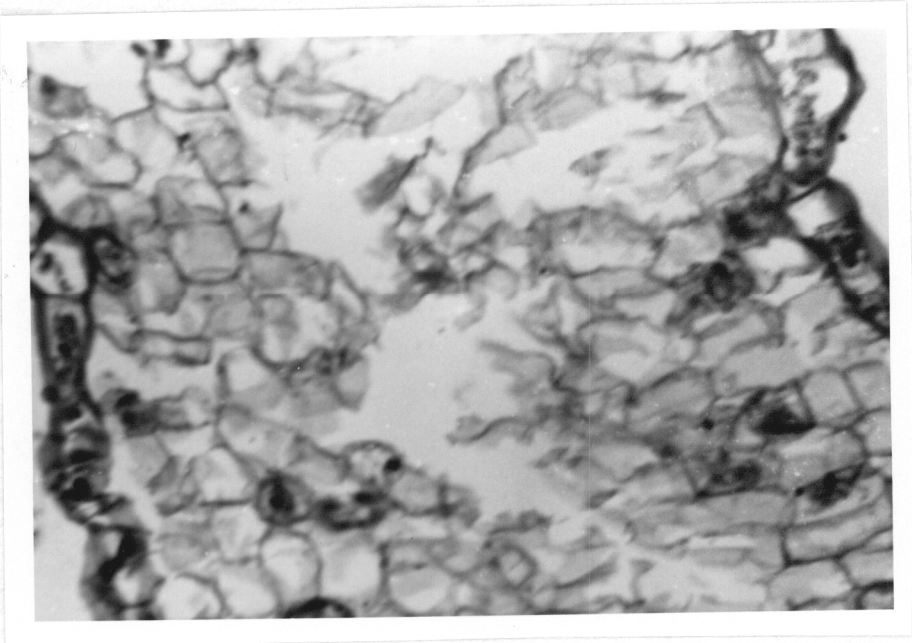


Plate 29. Transverse section through a leaf showing an area in which the ultrasonic energy ruptured the cell walls, disintegrated the tissue and dispersed the cellular contents. (X250).



DISCUSSION OF RESULTS

I. Cleaning Study

Ultrasonic energy is being used commercially to clean many types of contaminants from metal, glass and precision instrument parts. However, no research has been reported on the use of ultrasonic cleaning techniques to remove soil, insects, pesticide residues and other similar contaminants from plants or plant parts. Preliminary tests at V.P.I. with the ultrasonic cleaning of collard, kale and spinach leaves indicate that ultrasonic energy might improve the removal of these contaminants. The effectiveness of ultrasonic energy as an aid in cleaning collard leaves is shown in Plates 30 and 31. Differences in the amount of residue retained between the treatments can be observed.

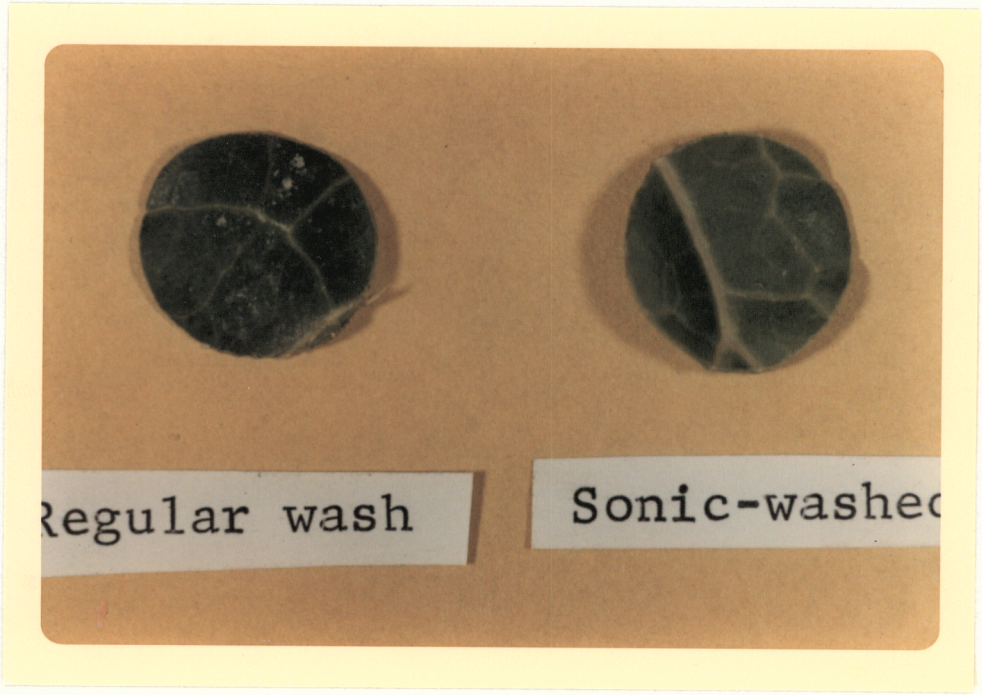
On the day of application 10.63 ppm Thiodan were found on the unwashed leaves. Seven days after application the level had decreased to 7.00 ppm. At the end of 14 days the residue level had reduced to 1.08 ppm. The reduction was attributed to chemical breakdown and to losses of the Thiodan in solution when moisture which condensed on the leaf surfaces dripped from them.

The removal of Thiodan residue from collard leaves by the application of ultrasonic energy to the cleaning medium was superior to the cleaning treatments in which ultrasonic energy was not used. Several workers (26),

Plate 30. Leaf discs removed from unwashed collards to show the degree of contamination.

Plate 31. Leaf discs from sample of collards after washing by hand and after ultrasonic washing. Compares the degree of cleaning of the two methods.


Unwashed


Regular wash
Sonic-washed

(35), and (39) reported that the removal of dust, grease, and other similar contaminants was improved by ultrasonic cleaning when compared with conventional cleaning methods such as pressure washing, agitation, vapor degreasing, and manual brushing for metal, glass, or fiber parts. The forces of cavitation involved in the cleaning of collard leaves was the same as for glass of metal parts. The particles of residue are subjected to the explosive forces of cavitation in which, according to Tint (46), cavitation-formed bubbles collapse during the compression phase of the cycle. This creates a powerful scrubbing action which excedes the tension by which the particles are held to the leaf surface. The forces of cavitation lift the particles from the leaf surfaces and disperse them throughout the cleaning medium.

Pressures exerted on the cell walls ruptured them dissipating the contents of the cells. The losses of cellular contents due to disruption of the cell walls and dispersion of the constituents of the cells was extensive enough to significantly decrease the content of ascorbic acid and carotene in the ultrasonically washed collards. Evidence that these losses might be significant can be observed in Plate 3.

Although sections of the 10-minute ultrasonically treated leaves showed little evidence of cell disruption,

losses of components tend to support the findings of Hughes and Nyborg (21) that components of cells of microorganisms might be released before major damage has been caused to the cell wall.

II. Respiration Study

The respiratory rate of collard leaves was predominately inhibited by the ultrasonic treatments. Except for the ultrasonic treatment for 20 minutes at 450 ma, all ultrasonic treatments reduced the rate of oxygen utilization and carbon dioxide evolution. The ultrasonic treatment for 20 minutes at 450 ma increased both the utilization of oxygen and the evolution of carbon dioxide. This is similar to results obtained by several workers (3), (5) and (17) that mechanical stimulation of cherry laurel leaves and cell deformation of potato tuber tissues increased the respiratory activity. The studies, on the effect of the ultrasonic treatments on the influence on cellular structure, indicated that the treatment for 20 minutes at 450 ma injured the cells and disturbed and disrupted the chloroplasts more extensively than the other treatments. These effects are brought about by the high pressures and accelerations produced by cavitation-formed bubbles which set the contents of the cell in motion (10). These effects coupled with the increase in temperature due to the cavitation increased the rate of the chemical processes involved in

oxidative respiration, the breakdown of sugars with the release of carbon dioxide, to increase the respiratory activity. This is in agreement with Carlin (10) concerning the biological and chemical effects of ultrasonic energy. It further agrees with the report of Audus (4) that stimulation of cherry laurel leaves acted on one or more of the stages of oxidative respiration subsequent to the breakdown of metabolite into 3-chain carbon compound and further to carbon dioxide in a later stage. Deformation of the tissues by the ultrasonic treatment at 450 ma for 20 minutes was apparently extensive enough to increase the respiration but not great enough to curtail it.

The ultrasonic treatments for 5 and 10 minutes at 450 ma, and 5, 10 and 20 minutes at 200 ma decreased the respiratory rate of the collard leaves. This response may be due either to the differences in the injury to the tissues or to the "degassing" effect on the tissue which alters the levels of oxygen and carbon dioxide present. Inhibition of the respiratory activity due to a decrease in oxygen concentration within the intercellular spaces is in agreement with the observations of Goddard and Bonner that low levels of oxygen decrease cellular respiration (15), and Denny (12) that low levels of oxygen markedly inhibits respiration of potatoes, and roots of beets, radish, and turnips. Injury to the tissues was prevalent on the treatments at 450 ma.

This increased their respiration in comparison with the rate of the treatments at 200 ma which showed less injury and less intercellular space.

SUMMARY

These investigations of the removal of endosulfan (Thiodan) residues from collard leaves by using ultrasonic cleaning treatments, and the influence of such treatments on the ascorbic acid and carotene content, respiratory rates, and histological changes within the tissues were conducted to evaluate the feasibility of applying ultrasonic cleaning to vegetable crops. Samples of collard leaves, to which Thiodan had been applied, were washed for periods of 5, 10 or 20 minutes each at 200 or 450 milliamperes (ma) in an ultrasonic cleaning unit. For comparison, leaves were washed by hand for the same periods of time.

The treatments at 450 ma most extensively injured the leaf tissues. Cell walls were ruptured and in some areas the structure of the walls was destroyed. Chloroplasts and nuclei in the injured areas were coagulated or fragmented forming a suspension of fine particles. Cleaning for 20 minutes at 450 ma reduced the residue level an average of 79 percent. This was significantly different when compared with the level retained on the unwashed leaves, or leaves washed by hand for 5 minutes. The reduction was 12 percent greater than either the 5 or the 10 minute sonic wash, and 27 percent greater than the average of the hand washes. The utilization of oxygen and production of carbon dioxide was increased, indicating a more

rapid breakdown of metabolites. Losses of 31 percent of the ascorbic acid and 37 percent of the carotene contained were brought about by the ultrasonic wash. The reductions were less after treating for 5 and 10 minutes. Carbon dioxide evolution was reduced 18 percent and oxygen utilization by 25 and 21 percent. This indicated a probable lack of oxygen within the treated leaves and supported the theory that cavitation produced a degassing effect within the tissues. Reduction of intercellular space and compacting of the cells during treatment further supported the degassing theory.

The treatments at 200 ma did less damage to the cells and tissues than those at 450 ma. The principle effects were those of degassing of the cells and dissipating of the cellular contents. These effects are supported by a 28 percent average loss in ascorbic acid content, a 27 percent average loss in carotene content, a reduction up to 22 percent in oxygen utilized and up to a 26 percent decrease in carbon dioxide evolved. Sectioned leaf tissues indicated few cell walls were ruptured but extensive coagulation and fragmentation of chloroplasts and nuclei were evident. The ultrasonic treatments at 200 ma reduced the Thiodan residue level an average of 71 percent. This was an average of 20 percent greater decrease than by the hand washes. When compared with the Thiodan level on unwashed leaves,

all treatments at 200 ma significantly reduced the residue level.

In general, increasing the duration and power output of the ultrasonic treatments decreased the residue level and the content of ascorbic acid and carotene, and increased the rate of oxygen utilized and carbon dioxide produced by the tissues. Increasing the power output and the duration of the ultrasonic treatment increased the injury to the tissues and cells and coagulated and fragmented chloroplasts and nuclei more extensively, forming a suspension of very fine grained particles within the cells.

CONCLUSION

It may be concluded from this investigation that ultrasonic energy at the frequency, amplitudes and duration of treatments used in these experiments reduced Thiodan residue to significantly lower levels than the check and also significantly lower than cleaning treatments without ultrasonic energy. Injury, produced by the forces of cavitation, ruptured cell walls and dissipated the cellular contents. The ascorbic acid and carotene was significantly reduced in leaves washed in the ultrasonic cleaning unit. Cells in the most extensively injured areas were empty.

Ultrasonic cleaning of collard leaves caused a significant amount of damage to the tissues at the energy levels and for the durations used, thus it is felt that such cleaning is not commercially feasible.

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VITA

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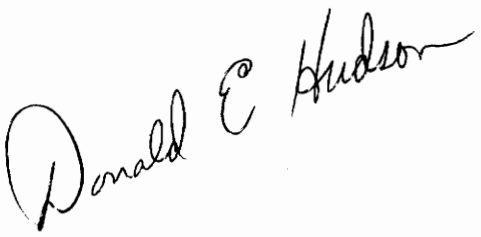
He obtained his B.S. degree in Horticulture from North Carolina State College in 1957. From 1957 to 1959 he worked as an assistant county agricultural agent in Bladen County, North Carolina. He attended Clemson University from 1959-1961 where he was a Graduate Research Assistant and obtained his M.S. degree in Horticulture in 1961. In 1961 he received a graduate research assistantship to V.P.I. to work on his Ph.D in Horticulture. In 1963 and 1964 he filled the temporary position of Assistant Extension Horticulturist for the Department of Horticulture.



ULTRASONIC ENERGY AS A CLEANING AGENT AND ITS INFLUENCE ON THE RESPIRATORY ACTIVITY AND LEAF ANATOMY OF *Brassica Oleraceae* var. *Acephala*

by

Donald Elmer Hudson

This investigation was undertaken to determine the effectiveness of ultrasonic energy in removing spray residue from collards, to determine its effect on the content of ascorbic acid and carotene levels in the treated tissues, and to investigate the influence of ultrasonic energy on the respiratory activity and the histological changes of the tissues involved.

Samples of collards sprayed with Thiodan at the rate of 1 lb per acre were harvested at 0, 3, 7, 10 and 14 day intervals after spraying and washed in an ultrasonic cleaning unit at 200 and 450 milliamperes (ma) power output for periods of 5, 10 and 20 minutes. Samples washed by hand for the same length of time were used as comparisons. The samples for residue determination were extracted with a n-hexane-isopropyl alcohol mixture, the extracts cleared up with a carbon-magnesium oxide absorbant, evaporated in a 50°C water bath and reacted with methanolic sodium hydroxide and pyridine. The ultrasonic wash for 20 minutes at 450 ma reduced the residue level 81 percent from 10.63 ppm to 2.00

ppm, the official tolerance level established for the crop, the day Thiodan was applied. All other treatments except the hand wash for 5 minutes reached the tolerance level 3 days after application of Thiodan. The hand wash for 5 minutes and the unwashed leaves did not reach the tolerance level until 7 days after the spray was applied. The ultrasonic cleaning treatments differed significantly, when compared with unwashed leaves and the most severe ultrasonic wash had a significantly lower overall Thiodan residue level than the 5-minute hand wash, but most differences between the ultrasonic washes were insignificant.

Aliquots, of ascorbic acid extracted with metaphosphoric acid collard leaves were determined colorimetrically using 2, 6 dichlorophenol indophenol as an indicator dye. Compared to the untreated samples, all ultrasonic treatments significantly decreased the ascorbic acid content. Thirty-three percent of the ascorbic acid was removed from the leaves by the 10-minute ultrasonic treatment at 200 ma and 31 percent was removed from the leaves by the 20-minute ultrasonic treatment at 450 ma.

Petroleum ether extracted carotene, filtered through columns of dicalcium phosphate, was determined colorimetrically. When compared with the content of untreated leaves, a 49 percent reduction in carotene was caused by washing for 10 minutes at 450 ma. When compared to the 5-

minute treatments at both 200 and 450 ma, the 10-minute treatment reduced the content by 42 percent and 39 percent respectively.

Discs from collard leaves were analyzed manometrically, in a Warburg Respirometer, to determine the effect of ultrasonic cleaning treatments on the metabolic processes of oxygen utilization and carbon dioxide production. Except for the treatment for 20 minutes at 450 ma which increased the respiratory activity, the general effect of the ultrasonic treatments was to decrease the respiratory activity as the duration decreased. Evidence of two causes of inhibition of respiratory activity existed. One was the degassing effect of cavitation which appeared to greatly reduce gases in the intercellular spaces. The second cause was injury to the tissue. The increase in injury apparently caused the respiratory activity to accelerate.

Standard methods of microtechnique were used to prepare sections from collard leaves to study the effect of ultrasonic energy on cells and tissues. Abnormalities and aberrations within the tissues were caused by cavitation. Degassing of the tissues reduced intercellular space. Chloroplasts and nuclei were displaced, coagulated and often fragmented to form a suspension of fine grained particles. In areas of most extensive injury, cell walls were ruptured and the cellular contents dissipated, with cells in some

area disintegrating.

Although ultrasonic energy at the frequency, and duration of treatments used in these experiments reduced Thiodan residue on collard leaves to lower levels than other cleaning treatments, it is felt that injury to the leaf tissues and subsequent losses of cellular contents by such a method of cleaning makes it commercially infeasible.