

at some location other than in the field. It is estimated that each mechanical harvester will replace 20 to 30 field laborers.

Although mechanical lettuce harvesting is nearing realization, it is doubtful that many machines will be commercially available before Fall, 1966.

Biochemical Studies of Rib Discoloration and Pink Rib of Lettuce
(G. C. Sharples)

Introduction

Rib discoloration and pink rib, serious nonparasitic diseases of head lettuce, are thought to be the result of adverse climatic conditions or direct physical action of various agents, including growth stresses and strains to which leaves of tightly formed lettuce heads may be subjected.

The resulting injury to specific cells of the leaves releases the contents of the vacuoles, allowing intermixing with enzymes associated with other cell structures. Phenol oxidase is such an enzyme and causes the oxidation of various polyphenols (tannin-like substances) normally found in the cell vacuole. Oxidation of polyphenols, followed by vaguely understood polymerization reactions, result in the formation of a variety of red or brown colored pigments that are then deposited at the site of the damaged cells or on the walls of surrounding cells.

The following information was obtained from experiments devised to investigate (1) changes in phenol oxidase activity in relation to seasonal growing temperature, (2) the development of rib discoloration in relation to seasonal growing temperature and (3) the identification and analyses of polyphenolic substances in lettuce leaves, the oxidation of which leads to the formation of colored pigments.

Methods

Colorimetric assays of phenol oxidase activity were made on Great Lakes and Imperial types of lettuce leaves harvested from plantings made at intervals from August through February, 1959-60 and 1960-61. Similarly, rib discoloration data was obtained from separate variety trials planted at intervals so that mature heads could be harvested under a variety of temperature conditions.

Paper chromatography and ultraviolet spectrophotometry were employed as methods for the identification of polyphenolic substances found in alcoholic extracts of the leaves.

Colorimetric methods involving the use of the Folin-Denis reagent for total polyphenols and of the Arnow reagent, specific for orthodihydroxy phenols, were used in the analysis of leaves of varying age from similar plants and of similar leaves from different lettuce cultivars.

Methods for the study of pink rib have not yet been worked out completely, but some interesting observations have been made and are discussed at the conclusion of this report.

Results and Discussion

Phenol Oxidase and Rib Discoloration in Relation to Seasonal Growing Temperature:

Phenol oxidase activities measured in cap leaf midribs from mature heads harvested from various fall, winter, and spring plantings showed good positive correlations with the mean minimum temperature of the 7-day period just preceding harvest. The relation is shown in Figure 1, where each value is the mean of 10 heads assayed individually.

Each 10-head sample was obtained from a different planting, each with a different harvest date.

Figure 2 shows that the percentage of heads with rib discoloration is likewise correlated with the mean minimum temperature of the 7-day period just preceding harvest.

Maximum temperatures were correlated with phenol oxidase activity ($r = 0.665$) and with per cent rib discoloration ($r = 0.766$), but with less efficiency. Maximum and minimum temperatures for 4-day and 10-day periods preceding harvest were tested, but all gave lower correlation coefficients than when the 7-day period was used.

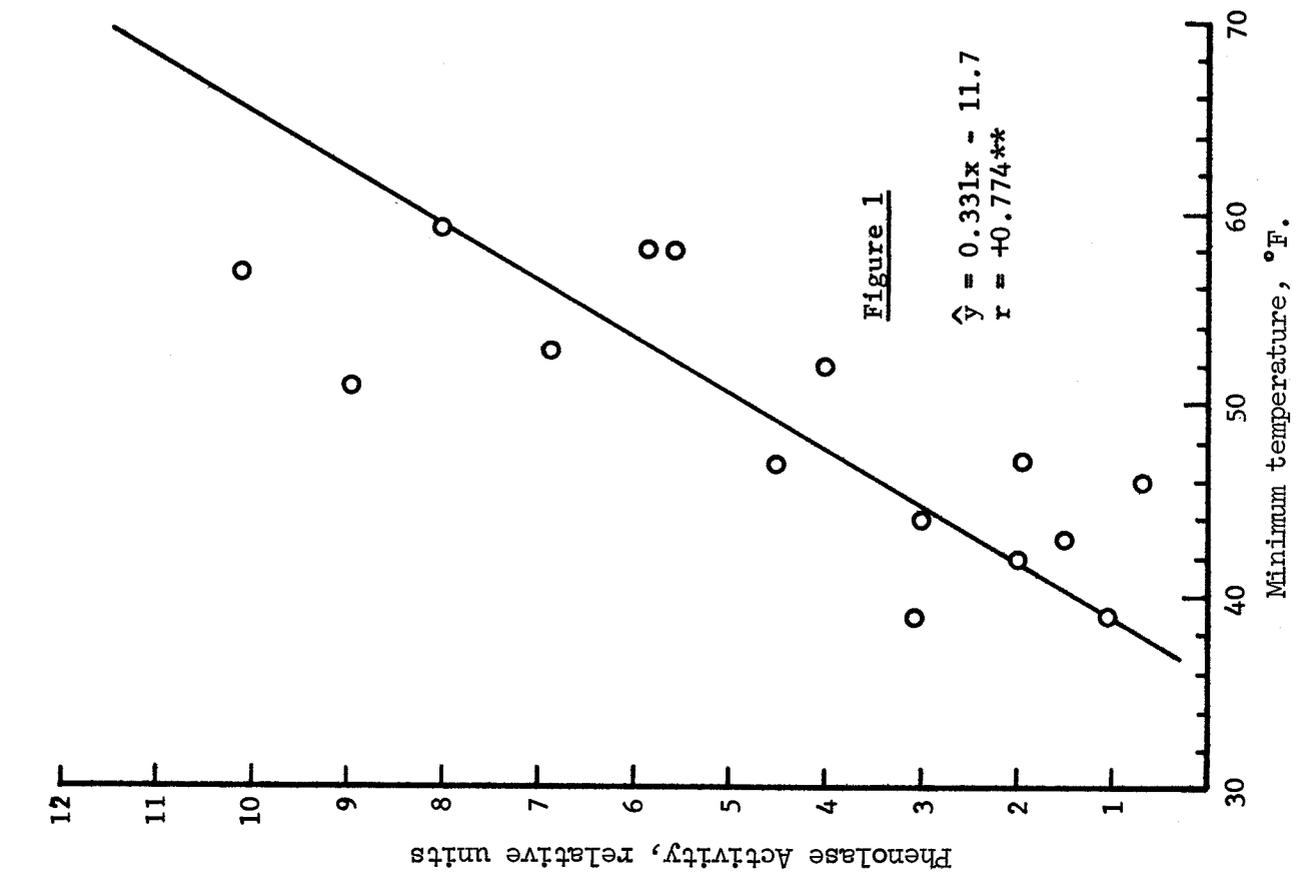
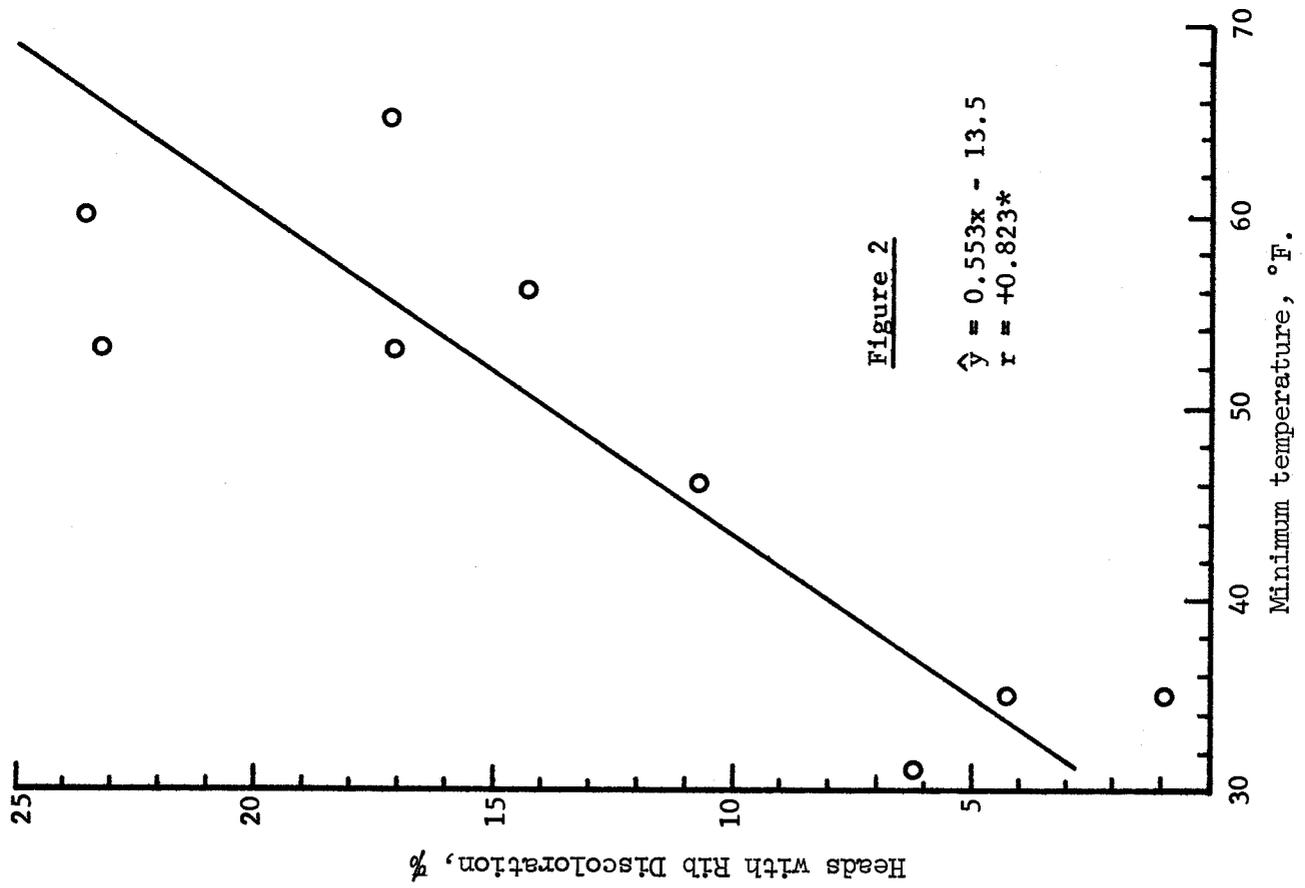
In other words, both rib discoloration and the concentration of phenol oxidase in the leaves are similarly correlated with minimum temperature at a definite time. This suggests that night temperature conditions during a specific period of head formation are critical for the appearance of rib discoloration and for the accumulation of an enzyme known to be involved in tissue browning reactions.

Identification of Polyphenolic Substances in Lettuce Leaves:

The naturally occurring flavonoids and related hydroxycinnamic acids are widely distributed in plants and are important because they are acted upon by phenol oxidase to produce red and brown colored pigments in injured cells.

Analysis of alcoholic extracts of lettuce leaves show that at least six different members of this group are present:

1. Chlorogenic acid (both stereoisomers).
2. Isochlorogenic acid (both stereoisomers).
3. Caffeic acid (both stereoisomers).
4. Quercetin (glycoside form).
5. Kaempferol (glycoside form).
6. Unidentified (probably a flavone).



Relation of phenolase activity (Fig. 1) and % of heads with rib discoloration (Fig. 2) to mean minimum temperature of 7-day period preceding harvest.

Concentration of Polyphenols in Lettuce Leaves in Relation to Rib Discoloration:

Most of the polyphenolic substances were found to be located in the green portion of fully mature outer (wrapper) leaves of lettuce plants. Inner head leaves contained only about one-sixth as much. It has been shown that this is probably due to differences in exposure of the leaves to sunlight. The inner head leaves receive little, if any, light, while wrapper leaves are directly exposed.

Analyses of the polyphenols in mature outer leaves of several commercial lettuce cultivars are shown in Table 1. Within this group the cultivar Climax consistently shows the greatest amount of rib discoloration. Val Verde and Imperial T show the least. There is no evidence here to indicate that polyphenolic concentration is related to rib discoloration susceptibility, and there seems to be no major differences between Great Lakes and Imperial types.

Observations on Pink Rib of Lettuce:

This defect of lettuce is characterized by a diffuse pink or reddish discoloration of cell walls of midrib parenchyma. Affected cells often are adjacent to latex ducts. Infrequently, the walls of conducting vessels only are involved, the surrounding parenchyma being normal.

The discoloration can be initiated by post-harvest mechanical damage, but it is occasionally observed in the field before harvest, especially in the cold winter months. This could be the result of freezing injury.

Attempts have been made to isolate the red pigment from affected tissue without success. When thin slices of normal midrib tissue are placed on moist filter paper in a closed chamber at room temperature for 12-16 hours, the paper beneath the slices becomes stained with a bright red pigment.

Presumably, the red pigment is derived from one or more substances which diffuse from the tissue slices into the paper. Observations suggest that at least one of the substances originates from the general area of the vascular bundles.

The presence of cellulose appears to be essential for pigment formation, since the pigment fails to appear when tissue slices are held in contact with other materials such as fiber glass or asbestos mats and polyethylene sheets. This is consistent with histological findings that only cell walls are stained red in tissue affected with pink rib.

When a drop of latex obtained from a cut lettuce stem is placed on moist filter paper under the same conditions as before, no pigment is formed. Therefore, if latex is involved in red pigment formation, another substance must also be present, perhaps an enzyme freed from cells that are injured upon slicing the tissue. The fact that pinking is inhibited by 0.1 M ascorbic acid and by steaming the freshly cut tissue for 2 minutes constitutes further evidence that the reaction is enzymically controlled.

Table 1. Total and o-Dihydroxy phenols in fully mature outer head leaves of six lettuce cultivars

Cultivar	Polyphenols, mg/100 g Fresh Weight ^a			
	Total (Folin-Denis)		o-Dihydroxy (Arnow)	
	1961	1962	1961	1962
Great Lakes				
Arizona Sunbright	387 ± 37	406 ± 66	187 ± 16	176 ± 68
Climax	368 ± 37	321 ± 41	145 ± 23	170 ± 51
407 P	410 ± 62	402 ± 62	213 ± 40	186 ± 36
Valverde ^b	375 ± 49	402 ± 49	174 ± 35	170 ± 33
Imperial				
847	417 ± 68	351 ± 62	154 ± 22	189 ± 50
T	396 ± 53	333 ± 35	128 ± 17	166 ± 22

^aCalculated as chlorogenic acid. Variabilities shown are standard deviations.

^bValverde is intermediate in appearance and growth character between Great Lakes and Imperial types.

Paper chromatography and ultraviolet examination of neutral and alkaline extracts of the pigmented filter paper suggest that chlorogenic acid and another, as yet unidentified, polyphenol may be involved in the red pigment formation.

Characteristics of Harvested Lettuce Heads

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Abstract: A study was made of the characteristics of harvested lettuce heads in Arizona during the 1964-65 season. Information on size, weight, firmness and number of wrapper leaves of each head sampled was collected and set up for analysis. No results were available at the time of this progress report.

Introduction

To develop background information on the lettuce being packed and marketed in Arizona, a survey was made of the characteristics of the heads being harvested in the different areas and seasons. This information is to be used in developing new containers and in improving handling techniques for the Western lettuce industry. This is a progress report of work done up to June 1965.