PRELIMINARY STUDIES RELATED TO INSECT CONTROL IN HARVESTED LETTUCE[1,2]

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I. OVERALL SUMMARY AND CONCLUSIONS

Summary: Fumigation of lettuce with concentrations of methyl bromide or hydrocyanic acid, effective for 100% mortality of aphids, corn earworms and cabbage loopers, proved injurious to the lettuce. Pre-transit fumigation was more detrimental to the lettuce than post-transit fumigation with both fumigants. Presently used controlled atmospheres during transit, including added carbon monoxide, are not effective in insect control. For a seven-day transit period, low temperature alone cannot be depended on for control. Although various degrees of mortality are found in both controlled atmosphere and low temperature treatments, they do not give dependable or predictable results.

Effect of HCN on Quality: It is our understanding that the use of HCN at the rate of 1.8 mg/l (1.8 gm/m^3) for ½ hour is the accepted treatment when fumigation is required for lettuce imported to Japan. Our results indicate that this level of HCN can be damaging to lettuce - Fig. 1. The symptoms include a yellowing of cut surfaces including mechanical or insect damaged areas. At this rate, damage from the fumigant does not extend to the center of the head but leaves internal to the surface may develop a yellowish appearance especially on the midrib and veins. In severe cases pale yellow, diffused lesions are found on both surfaces of the leaf. Concentrations of HCN greater than 3 mg/l result in a water-soaked, decayed, appearance along with a "cyanide odor" which persists for several days. To observe full extent of HCN damage, it is necessary to tear the leaves apart since the damage may be greater within the head.

Effect of Methyl Bromide on Quality: Since this is a commonly used fumigant, we investigated its effects on lettuce quality and found it is not tolerated. Rates as low as ¼ lb/1000 ft^3 for 2 hours caused damage to the lettuce and did not provide insect control. Damage symptoms range from small oblong, sunken lesions resembling russet spotting to complete discoloration, softening and destruction of the tissue. Moderate to severe damage is accompanied by droplets of yellow exudate from the butt and midrib areas. Figure 2 shows a gradient of the type of external damage that can be expected. Methyl bromide penetrates the tissues rapidly; therefore, the total head is generally affected with severe damage to the heart leaves.

Effect of Fumigants on Lettuce Insects: The effectiveness of various HCN and methyl bromide treatments were evaluated. In general, applied dosages of HCN and methyl bromide at either 5° or 20°C effective for insect control were detrimental to lettuce. At concentrations of HCN which caused minimal damage to lettuce, green peach aphids (*Myzus persicae*) were more susceptible than corn earworm (*Heliothis zea*). However since HCN is highly sorbed by the lettuce, an applied dosage of 2 to 3 times the effective insect control dosage would be required, resulting in damage to lettuce. All dosages of methyl bromide which resulted in 100% mortality of the insects tested caused extensive damage to lettuce.

Effect of Transit Conditions on Insects: Controlled atmosphere conditions, including addition of carbon monoxide were not effective in killing insects that may be found on lettuce (green peach aphids, cabbage loopers, and corn earworms). Exposure to low temperatures (0°, 2.5°, and 5° C) for a 7-day duration (simulated transit conditions) did not result in effective or predictable insect control. Some corn earworm control was achieved by these conditions but appears to be highly dependent on specific characteristics (instar, general condition, etc.) of the worm at time of transit.

Conclusions: This report is not all-inclusive and many avenues of investigation are still open for exploration if there exists a need for postharvest fumigation of lettuce. The area of sublethal, non-phytoxic dosages of fumigants, followed by holding under cold temperatures merits investigation, using perhaps other materials than were tested here.

One of the basic questions that remains is the question of the need for quarantine fumigation of lettuce. It has been our experience over the past several years of examining some 8-10 thousand heads of lettuce, individually, that insects in or on the head at harvest are not generally a problem. Granting that peaks of insect activity do occur, an alternative to fumigation might be the development of specific standards of insect level for export lettuce. Lettuce which meets such standards would then not be subject to quarantine fumigation.

II. BACKGROUND

This study was initiated after conversation with representatives of Western Growers Association and the Sealand Transportation Company in the Spring of 1973. The need for some "quick" answers pertaining to fumigation of head lettuce was evident, since shipment of lettuce to Japan was planned for July, 1973. A letter, received from the Ministry of Agriculture and Forestry, Japanese Government, on April 24, 1973, stated that when aphids and other injurious insects are found in imported lettuce, the general procedure is to fumigate with hydrocyanic acid (HCN) at the rate of 1.8 gr/m^3 for 30 minutes. Apparently this is done in special warehouses, requiring the removal of lettuce from the desirable storage temperature, treating at a higher temperature, repackaging the lettuce and placing it back into a more desirable storage temperature. If an export market is to be developed for lettuce, it is imperative that the consumer receive the best quality possible. The basic questions were: (1) what is the effect of fumigation on quality, and (2) are there alternative methods of insect control in a quarantine situation that would maintain lettuce quality? Unquestionably, time was of major importance, and therefore only certain types of experiments could be run, the chief goal being to develop a general idea of what might be expected under certain fumigation practices.

A preliminary report was submitted in mid-July, 1973, indicating our findings on what to expect as a result of HCN fumigation, as well as a summary of other tests. It was hoped that the preliminary report would serve as a guide for evaluation of lettuce, as well as a basis for discussions in Japan while W.G.A. representatives were there for the Trade Fair. This report is a more complete summary of tests conducted.

III. INTRODUCTION

Postharvest fumigation of head lettuce is not a common practice, hence the literature concerning recommendations, tolerances, etc., for this commodity is limited. The use of hydrocyanic acid (HCN) is not generally recommended for fumigation of fresh fruits and vegetables (Monro, 1969), but since this was the fumigant that would be used, if necessary, on the current shipment, it was necessary to evaluate its effect on lettuce, as well as its effectiveness on insect mortality. Methyl bromide has been used successfully on numerous vegetables, and data are available concerning relative phytotoxic sensitivity of the commodity; however, lettuce has not been evaluated. Looking for a possible substitute for the HCN treatment necessitated evaluating methyl bromide as a fumigant for lettuce. The effects of carbon monoxide (CO) on lettuce tissue has been studied (Kader *et al.*, 1973) and it is known that lettuce tissue will tolerate relatively high levels of carbon monoxide under prescribed atmospheric conditions. CO is also known to be extremely toxic to man and warm blooded animals at levels far below those that cause damage to lettuce tissue. A search of the literature revealed little information concerning CO effects on insects; therefore, it was included in the experiments. The possibility also existed that a 7-day transit period under controlled atmosphere at the desirable lettuce storage temperature may in itself be sufficient to kill insects, thus eliminating the need for fumigation.

The selection of a fumigation treatment cannot be based alone on its ability to eliminate target insects, but must include such factors as residue and commodity responses. The responses of interest with reference to lettuce would include 1) the formation of visible lesions, 2) internal injury, 3) shortening of storage life, 4) stimulation of storage disorders. The occurrence of any one of these reactions to any degree would have a direct bearing on either the choice of a particular fumigant, or as to whether fumigation is practical at all. The residue factor was not included in these tests since any adverse reactions of the commodity, or lack of insect control, would preclude the value of residue data.

Another factor which plays an important role in the selection of a fumigant, and affects the reactions mentioned above, is "sorption" of the fumigant. This terms refers to the total uptake of the gas resulting from the attraction and retention of the fumigant by any solid material present in the system, including containers, internal surfaces of the chamber and the commodity. Sorption, under a given set of conditions, determines the dosage to be applied, because the amount of fumigant used must be sufficient, both to satisfy the total sorption during treatment, and also leave enough free gas to kill the insect. Physical sorption increases as temperature decreases. This fact has important practical applications in that it is one of the reasons why dosages have to be progressively increased as the temperature of fumigation is lowered; but temperatures above 5°C are certainly not conducive to extension of storage life of lettuce. Sorption is also greater in high moisture commodities, therefore creating more of a problem with commodities like lettuce. Fumigants with higher boiling points such as HCN tend to be more highly sorbed than the more volatile compounds such as methyl bromide. This factor, coupled with the higher water solubility factor of HCN tends to make fumigation of lettuce with HCN questionable from the start. The conclusion that can be drawn is that the specific physical reaction between a given gas and a given commodity cannot be accurately predicted from known laws and generalizations. Usually a certain fumigant must be tested with each material concerned before a recommendation for treatment can be drawn up.

Many factors determine the effect of a particular fumigation treatment on insect mortality as well as the effect on commodity responses. Factors such as insect species, stage of development, pre- and post-fumigation temperatures, as well as temperature during fumigation, affect insect mortality. The scope of this investigation did not allow for examination of all these factors. Both the source and supply of all insects likely to infest lettuce, and all the stages of growth in which they may be found, were limited. All tests included the green peach aphids, *Myzus persicae* (Sulzev), and corn earworm, *Heliothis zea* (Boddie); and for some of the initial tests the cabbage looper, *Trichoplusia ni* (Hübner), was available.

Experiments with various fumigants and controlled atmospheres were conducted using lettuce alone, insects alone, and combined fumigants where the insects were present with the lettuce during treatment. The results presented in this report represent a summary of some 85 various combinations of treatments. It is stressed that the major purpose of this work was to determine the effect of the anticipated HCN fumigation on the lettuce, and to explore for alternative methods of insect control on harvested lettuce.

IV. GENERAL MATERIALS AND METHODS

Lettuce (*Lactuca sativa* L. cv. Calmar) for all tests was obtained from Bruce Church Inc., Salinas, California. In all cases the lettuce was harvested in the morning, vacuum cooled and transported to the Mann Laboratory, University of California at Davis, the same day. Lettuce temperatures on arrival varied from 37°F to 42°F. Wrapper leaves were removed and the best quality heads were selected for the experiments. The initial test compared heads with and without wrapper leaves and there was no difference in the effect of fumigation; therefore, only trimmed heads were used thereafter as selection for uniform quality was facilitated. Depending on the test, 4, 6 or 8 heads were used per treatment. A 7-day simulated transit period at 2.5°C (36°F) was followed by a 4-day simulated marketing period at 10°C (50°F). A constant and continuous flow of the desired atmosphere was maintained throughout the test by use of flowmeters. Evaluation of quality was done at the end of the simulated market period. Fumigants were either applied before the simulated transit period ("pre-transit") or after the transit period ("post-transit"). The latter would simulate fumigation after shipment to Japan, the former fumigation before shipment. Levels of oxygen (O_2), carbon dioxide (CO_2), and carbon monoxide (CO), were monitored, using gas chromatography.

All procurement, rearing, maintaining and evaluation of the insects were done by the Entomology Department, University of California at Davis. Aphids, *Myzus persicae* (Sulzev), used were from local colonies, while corn earworms, *Heliothis zea* (Boddie), and cabbage loopers, *Trichoplusia ni* (Hübner), were obtained from Shell Chemical Corp., Modesto, California. Loopers were available only for some of the initial tests. Corn earworm larvae varied from 3rd to 5th instar, but were generally constant for any one test. Insect mortality was evaluated after a minimum of 24 hours following removal from test conditions to allow for recovery of the insects from a sub-lethal fumigation treatment.

Fumigation temperatures used were 20°C (68°F) and 5°C (41°F). Test containers were sealed metal cans, approximately 1 ft^3 in volume. Specific methods concerning a particular treatment will be given as each treatment is discussed.

V. FUMIGATION STUDIES WITH HYDROCYANIC ACID (HCN)

Introduction: HCN is a volatile, colorless liquid, boiling point 26°C and is comparatively soluble in water. Although it is one of the most toxic of insect fumigants, the fact that it is very soluble in water has considerable bearing on its use in practice. It can not safely be used on moist materials such as vegetables because the solution of HCN in water is a dilute acid. Not only does this render these materials impalatable and possibly hazardous for human consumption, but its action in causing wilting, burning or discoloration may make them unmarketable. HCN is highly sorbed at atmospheric pressure and therefore does not penetrate well into some materials. The high boiling point contributes to this sorption factor, and the problem is increased at the temperatures desirable for the maintenance of general, overall quality in lettuce. Although HCN is not generally recommended for the fumigation of fresh fruits and vegetables, it was studied here because it was reported to be the acceptable treatment in Japan.

Materials and Methods: HCN gas was generated from potassium cyanide (KCN) in the test containers, using the pot method described by Metcalf and Flint, 1962. Concentrations of HCN in the test atmosphere were determined using the silver nitrate test (Quayle, 1941). A test container without lettuce was used to determine actual dosage applied since the determinations on the containers with lettuce reflected the concentration of free gas available for insect control, the difference between the two concentrations being largely a measure of the sorption of HCN by the lettuce. In the tests that involved only insects, small fumitoria were used, and a predetermined amount of HCN gas of known concentration was added by hypodermic syringe. Combinations of treatment temperatures of 5°C (41°F) and 20°C (68°F) with various dosages of HCN were evaluated. Durations of exposure used were ½, 1, 2 and 3 hours.

Results and Discussion:

1) *HCN effects on lettuce:* Injury to the lettuce occurred at fumigation concentrations as low as 1.8 gm/m^3 for ½ hour at both 20°C (68°F) and 5°C (41°F). Damage increased with concentration and time at both temperatures, although increasing concentration had a predominate effect over increasing time. For example, fumigation at 5°C with a dosage of 2 gm/m^3 for ½ hour had a more adverse effect than a dosage of 1 gm/m^3 for 1 hour, although both have a concentration x time product (Ct) of 1 $gm{\cdot}hr/m^3$. Pre-transit fumigation resulted in more injury to the tissue than post-transit fumigation. This is probably attributable to the longer period of time for symptom development when fumigation occurs before transit. Symptoms were somewhat more severe when fumigation was at 5°C as compared to 20°C, probably due to increased sorption with decreased temperature.

Symptoms of HCN injury to lettuce include a yellowing of cut surfaces and insect and mechanically damaged areas. This is generally evident at exposures as low as 1.8 gm/m^3 for ½ hour and become more evident as concentrations and times are increased and temperatures are lowered. At this dosage damage from the fumigant does not extend to the center of the head, but leaves internal to the surface may develop a yellowish appearance, especially on midribs and veins. In more severe cases, pale yellow diffused lesions are found on both surfaces of the leaf. Concentrations of HCN greater than 3 gm/m^3 for 2 hours result in a water-soaked, decayed appearance, along with a "cyanide odor" which persists for several days. It is interesting to note that at concentrations of HCN of above 5 g/m^3 for 2 hours the external yellowing of cut surfaces did not appear, but instead butts and damaged areas remained very white. This is believed to be a direct effect of high concentrations of cyanide on oxidase systems in the plant. To observe full extent of HCN damage, it is necessary to tear the heads apart, since the damage may be greater within the head (Figure 1).

2) *HCN effects on insect mortality:*

a. Aphids: The minimum effective concentration for 100% mortality was a Ct product value of 2 at both 20° and 5°C. This would represent an effective fumigant concentration of 1 gm/m^3 for 2 hours. It is felt that higher concentrations for a shorter period of time to achieve the same Ct value would be detrimental to the lettuce.

b. Corn earworm (5th instar): A minimum effective Ct product of 10.2 was required for 100% mortality. This is far above what is tolerated by the lettuce.

c. Cabbage looper: Were not controlled at Ct values up to 2.2. Due to a limited supply of insects it was not possible to evaluate their reaction at higher concentrations.

Summary and Conclusions: HCN does not appear to be a satisfactory fumigant for lettuce. Concentrations required for insect control resulted in injury to the lettuce. Under some treatment conditions the concentrations applied were reduced in 2 hours by as much as two-thirds due to sorption by the lettuce. This would mean that under such conditions to achieve an effective concentration actual dosage would have to be three times the effective concentration, which would result in severe damage to the lettuce. The high degree of sorption of HCN would probably necessitate a prolonged desorption period to rid the lettuce of residual HCN, a factor which would not permit immediate marketing after treatment.

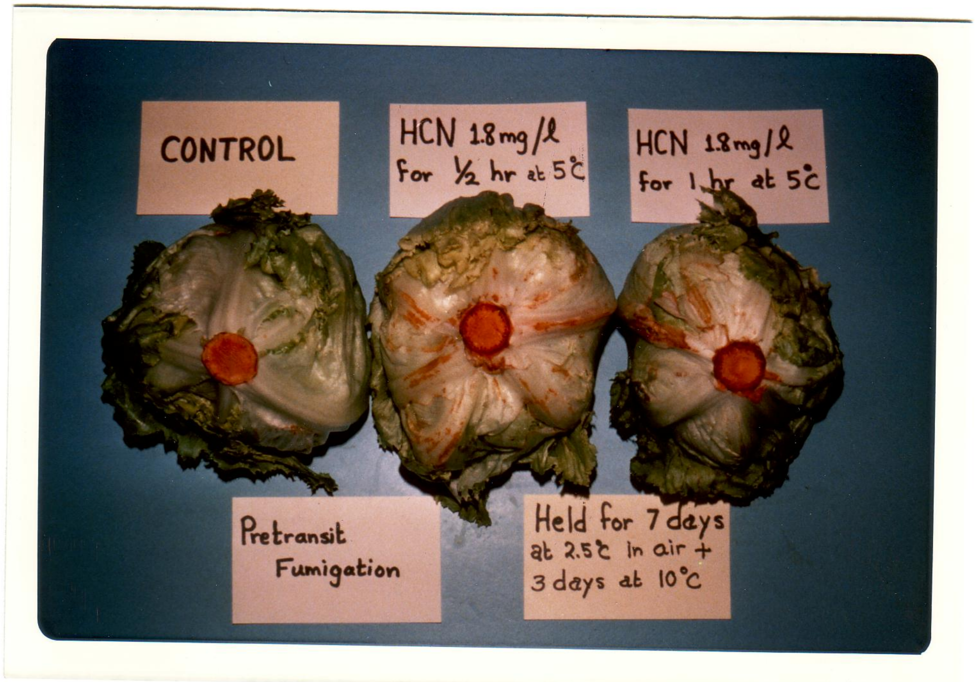
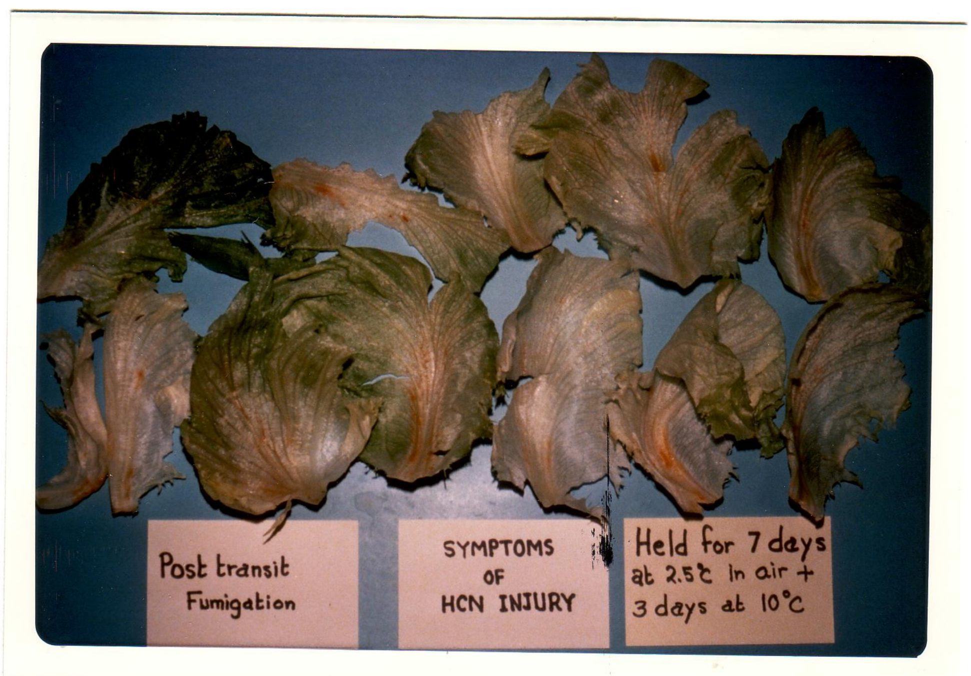


Figure 1.

a. Effect of HCN fumigation for ½ and 1 hr durations at 5°C and at the concentration required by Japanese regulations. Fumigation after simulated transit (7 days) showed slightly less injury.



b. Symptoms of HCN injury to interior leaves -- concentrations were greater than the required treatments (1.8 mg/l) and hence symptoms shown are more severe than would be expected from the commercial treatment.

VI. FUMIGATION STUDIES WITH METHYL BROMIDE

Introduction: Methyl bromide is not as toxic to most insect species as HCN; nevertheless, other properties make methyl bromide an effective and versatile fumigant. One of its most important attributes is that it is not highly sorbed by most commodities, and can therefore penetrate easily into them and would escape quickly on ventilation at the end of the treatment (Thompson, 1966). Methyl bromide's comparatively low boiling point (3.6°C) and low sorption by many products makes it more useful for low temperature treatments that are not practicable with many other fumigants. Treatments of a wide range of commodities may be conducted at temperatures down to 4°C or even lower in some cases. The possibility of fumigation temperatures in this range, which is suitable for lettuce storage, indicated a need to evaluate methyl bromide effects on harvested lettuce. Monro (1969) provides a schedule for methyl bromide fumigation of fresh vegetables. Although no data are presented for lettuce, a general recommendation for insect control in or on leafy vegetables calls for a 2-hour treatment of 4 lbs/1000 ft^3 at 4°-7°C (40°-45°F) or 2.5 lbs/ 1000 ft^3 at 16°-20°C (60°-69°F).

Materials and Methods: Because of the small volume of the fumatoriums, it was not possible to meter out the correct volume of methyl bromide in the gaseous state to achieve the concentrations desired. Therefore, the methyl bromide was handled as a liquid at 0°C and the prescribed amount of liquid methyl bromide was applied volumetrically into an evaporating pan inside the fumatorium. The fumatorium containing lettuce was equilibrated to the desired fumigation temperature, and then placed back into that temperature after the liquid methyl bromide had been added and the penetration resealed. Due to the low boiling point of methyl bromide (3.6°C), the liquid methyl bromide volatilized rapidly at the 20°C treatment temperature. However, a somewhat slower volatilization was assumed to take place at 5°C, although it was always complete, even in the ½ hour treatments. The slightly slower volatilization at 5°C would likely increase the sorption of methyl bromide, and could account for some tissue damage that would not have occurred if the methyl bromide could have been added accurately as a hot gas. This would be the preferred method in large-scale fumigation at 5°C. Actual concentrations of methyl bromide in the fumatorium were not determined but were assumed to be that which would result from total volatilization of the liquid methyl bromide added. Various combinations of concentration and durations were tested at both 20°C and 5°C; however, emphasis was placed on fumigation at 5°C, since this is a more desirable lettuce storage temperature.

Results and Discussion:

1. *Methyl bromide effects on lettuce:* Lettuce appears to be completely intolerant to any practicable concentration of methyl bromide. Dosages recommended for insects on green, leafy vegetables severely damaged the lettuce. A total of 15 time-concentration-temperature combinations were tested and only at a level of less than ¼ lb/1000 ft^3 for 1 hour at 5°C was there no noticeable effect on the lettuce. This is far less than the general recommendation for fumigation at 5°C and did not provide any insect control.

Symptoms of methyl bromide injury can be quite severe and are concentration related (Fig. 2). Initial symptoms appear as small, light yellow, sunken, oblong lesions. Internal leaves may be void of these lesions, but internal leaves take on a yellowish cast that is especially prominent on the midrib and veinal tissue. The penetration of methyl bromide is evident as damage can be found on the heart leaves. More severe injury is evidenced by an overall yellowing of tissue, especially the inner leaves. The lesions become more prominent and cover a larger portion of the leaf, especially on the midrib area. At this stage the lesions resemble the common lettuce postharvest disorder, "russet spotting," except the lesions are larger (up to 2 inches) and generally more diffused over the leaf than russet spotting. Severe damage is represented by an overall discoloration of leaves and identifiable lesions are hard to find because of the overall nature of the browning. Inner and heart leaves are completely destroyed, dark-brown in color with cell breakdown evident. A yellowish exudate in the form of small droplets is apparent on the butt surface and midrib of outer leaves. Severe symptoms appeared at dosages as low as 2 lbs/1000 ft^3 for ½ hour at 5°C.

2) *Methyl bromide effects on insects:* At 5°C fumigation, 100% mortality of both aphids and corn earworm was attained only at Ct product values that far exceeded dosages tolerated by the lettuce. Time of exposure was an important factor, as at 5°C a dosage of 1 lb/1000 ft^3 for 2 hours (Ct=2) resulted in 100% mortality of aphids while 2 lb/1000 ft^3 for 1 hour (Ct=2) gave essentially no control. Cabbage loopers were effectively controlled only at Ct products far beyond the allowable Ct product for lettuce.

Fumigants were generally more effective at 20°C fumigation temperature than 5°C fumigation; however, the required concentrations and times for insect control at the higher temperature still exceeded dosages that caused damage to the lettuce.

Summary and Conclusions: At this time it is concluded that methyl bromide is not a satisfactory fumigant for use on harvested lettuce. A very complete concentration x time series was conducted at 5°C and damage to the lettuce was observed at levels of fumigation much lower than levels which gave satisfactory insect control. The rate recommended for insect control at 20°C resulted in complete destruction of the lettuce.

Figure 2. Effects of methyl bromide at indicated concentrations and durations on quality of harvested lettuce.

VII. FUMIGATION STUDIES WITH CARBON MONOXIDE

Introduction: Carbon monoxide (CO) is extremely toxic to living systems in which hemoglobin respiratory pigments in the blood serve a major respiratory function as oxygen carriers. This function is inhibited by carbon monoxide to such an extent that exposure of humans to 1000 ppm (0.1%) can produce unconsciousness in 1 hr and death in 4 hours. Insect blood does not contain hemoglobin, therefore this mode of action was not anticipated; however, CO also acts on a site in cellular respiration which is common to both lower and higher animals. Lettuce tissue can tolerate CO concentration up to 20% (200,000 ppm) for periods of 7 days, providing carbon dioxide concentration is kept below 1%. This tolerance suggested the possibility that CO might be an effective fumigant.

Materials and Methods: Experiments were of two basic types: 1) short time exposures (1 or 2 hours at 20°C) to various levels of CO (0, 10, 30, 70 or 100%) and 2) continuous exposure for 1, 3, or 7 days at 2.5°C to various levels of CO (1, 5, 10, 20, 40 or 80%) in combination with 5 or 21% O_2 ± 1% CO_2. For the short exposure fumigation experiments the fumatoria were flushed with high flow rates of the desired CO concentration until equilibrium was established. They were then sealed and held at 20°C (68°F) for the desired length of time. The continuous exposure treatments were maintained by capillary controlled flow of the desired gas treatment. All continuous treatment experiments were at 2.5°C (36.5°F).

Results and Discussion: Carbon monoxide did not effectively control the insects in any of the short term fumigation type experiments. Likewise, the continuous exposure to CO for 1 or 3 days did not result in suitable control, regardless of CO level. A 7-day exposure resulted in no aphid control and only slight corn earworm control. Looper mortality was 100% in all levels of CO as well as in the control. Therefore, it must be concluded that other factors, such as the cold temperature, were the determining factors. The looper data cannot be taken as positive, since there was a possibility of a weakened looper population due to unknown bacterial disorders that appeared later in the colony.

Summary and Conclusions: Carbon monoxide in itself does not appear to be a suitable substitute for common fumigants. Results concerning insect mortality are not consistent and are not adequate for a quarantine treatment.

VIII. INFLUENCE OF CONTROLLED ATMOSPHERES AND TEMPERATURE

Introduction: Low storage temperature (0°-2.5°C) have been reported to kill certain stages of some insects (Glass *et al.*, 1961). It has also been our observation during several years of research that we seldom find live insects in lettuce after low temperature storage. As stated earlier, it was thought that temperature alone may be effective and eliminate the need for fumigation. Storage atmospheres of high carbon dioxide (CO_2) content and/or low oxygen (O_2) content have also been shown to kill some types of insects (Monro, 1969). Lettuce will not tolerate CO_2 concentrations above 1% at temperatures in the desirable storage range (Brecht *et al.*, 1973a & b).

However, lowered oxygen concentrations have not proved harmful to lettuce, especially when CO_2 is kept below 1% (Lipton, 1967, 1971). Realizing that high CO_2 levels would not be practicable under lettuce storage conditions, it was decided it would be worthwhile to look at it as short term, room temperature treatment, acting to asphyxiate the insects.

Materials and Methods: Carbon dioxide at levels of 10, 30, 50 or 70% at two levels of oxygen (5 or 21%) were used as fumigant-type treatment for 1- and 2-hour periods at 20°C (68°F). CA conditions of 5% O_2 or 5% O_2 plus 1% CO_2 were also tested in both static and flow conditions for 1-, 3- and 7-day durations. Along with normal air controls for all treatments at low temperatures, a temperature series of 0°C (32°F), 2.5°C (36.5°F), 5°C (41°F) was tested for effectiveness in insect control.

Results and Discussion: Under conditions tested no level of CO_2 provided any insect control. It is apparent that if such treatments were to be successful, considerably longer exposure periods to CO_2 might be necessary, a condition that lettuce would not tolerate.

Reduced oxygen treatments (5%) likewise did not give satisfactory control. There exists the possibility that even a lower oxygen treatment (2%) would be more effective. However, it is doubtful that satisfactory control would be obtained in a 7-day transit period. Lowered oxygen concentrations may prove to be more worthwhile under longer storage periods than 7 days.

Temperature alone was not an effective control agent. It appears that the effectiveness of low temperature depends to a great extent on the stage of development of the insect and on its general physical condition. On 3rd instar corn earworm larvae mortality was fair with exposure to 2.5°C (36.5°F) for 7 days. However, late 5th instar stages of the same insect remained alive after 7 days at 0°C (32°F). It is therefore concluded that exposure to low temperature alone is not a dependable method of insect control.

Summary and Conclusions: Although various degrees of mortality were found in both controlled atmosphere and low temperature treatments, they do not give dependable or predictable results.

IX. APPENDIX

REVIEW OF LITERATURE ON POSSIBLE METHODS OF INSECT IRRADICATION IN HARVESTED LETTUCE AND OTHER COMMODITIES

I. Controlled Atmosphere Cold Storage and Atmospheric Gases as Insecticidal Agents.

The problem of eliminating insects from a highly perishable commodity is not a new one. The shipment of eastern apples into California was once prohibited for fear of introducing the oriental fruit moth, the plum curculio and the apple-blueberry maggot. Import restrictions were dropped when it was shown by Glass *et al.* (1961) that cold storage (32°F) in air for 33 days was lethal to both apple-blueberry maggot and curculio larvae. The same mortality under controlled atmosphere conditions of 3% O_2 and 2-8% CO_2 required a slightly longer time period. Since the controlled atmos-here storage was at 38°F, it was concluded that mortality was dependent on temperature rather than on gas mixture.

Coddling moth larvae however were able to survive cold storage for more than 132 days. This is not surprising since this insect overwinters in the larval stage.

Moffit (1971) found that methyl bromide fumigation with 32 g/m^3 (2 lb/1000 ft^3) for 2 hours at 25°C followed by cold storage at0.56°C resulted in 100% mortality of coddling moth larvae. It is interesting to note that neither treatment alone was sufficient to kill the moth larvae. Higher dosages of methyl bromide were precluded since they result in unacceptable damage to some apple varieties.

A similar situation was encountered in the shipment of avocados and papayas from Hawaii. Slight temperature deviation from the recommended storage at 2.2°C for 10 days resulted in incomplete kill of Mediterranean and oriental fruit flies (Seo *et al.*, 1971). A combined methyl bromide fumigation and pre- or post-fumigation cold storage period were utilized to insure the required mortality, and equations were used to predict the separate effects of each subtreatment and of the combined treatments.

Although Moffit and Albano (1972) demonstrated that controlled atmosphere cold storage resulted in higher mortality of coddling moth larvae and pupae than equivalent cold storage in air, the use of various mixtures of atmospheric gases to kill insects on highly perishable commodities (i.e. fruits and vegetables) has not been used. The reason for this appears to be that the long treatment times and gas mixtures required to kill insects are also damaging to the commodity.

For example, Lindgren (1970) found that the time required for 95% mortality of granary weevil adults at 80°F was almost 24 hrs at 90% CO_2 and 3 days in 40% CO_2.

Alinazee (1973) used pure oxygen to kill confused flour beetle larvae, a process that required a 15-day exposure at 26.7°C to achieve 100% mortality. The same author (1972) tested the effects of 100% helium and 100% nitrogen on the confused flour beetle and the red flour beetle. In this case at 21°C the helium atmosphere produced 100% mortality in both species in 9 hours. The nitrogen was not as effective, requiring an 18-hour exposure to achieve the same result.

This preliminary search of the literature revealed no examples of the use of carbon monoxide to kill insects, although it is known to affect the respiratory enzymes. Preliminary testing shows that, like other atmospheric gases, exposure time and gas concentrations would cause damage to a commodity such as lettuce.

Although the use of atmospheric gases alone appears to be unsatisfactory for the elimination of lettuce insects, their use prior to a period of cold storage might be worth investigating. For example, pretreatment with enough CO_2 at a high temperature to induce narcosis followed immediately by 7 days of cold storage.

II. The Use of Fumigants to Eliminate Insects From Lettuce.

The materials that at this time appear to have some potential for use on lettuce are: Methyl bromide, hydrogen cyanide, ethylene dibromide and dichlorvos (DDVP).

Methyl bromide is the fumigant most commonly used on fruits and vegetables, with ethylene dibromide probably the second most common. Hydrogen cyanide has been used on citrus and ornamental trees, and in glasshouse fumigation of tomatoes, lettuce, and other miscellaneous-growing plants.

A wide range of vegetables has been evaluated for tolerance to methyl bromide and ethylene dibromide by Pratt *et al.* (1951), but on the basis of these results it seems impossible to derive generalities concerning the tolerance of a specific commodity to these fumigants. Methyl bromide damaged 5 commodities and ethylene dibromide damaged 6, but only 2 of these were damaged by both materials. It therefore appears that, even though MB and EDB are chemically similar, their physiological effects on plant tissues may be quite different. The effects of MB on mamalian tissues have been found to be attributable to the intact molecule rather than to bromine alone; therefore it follows that the effects of EDB may be quite different.

Another important point brought forward by the study of Pratt is that the history of the commodity prior to treatment can drastically affect tolerance. Bell peppers were found to be tolerant to both fumigants if treated before a simulated transit period, but damaged by both if treated after the transit period. Roth and Richardson (1968) reported similarly that Honey Dew melons could be successfully fumigated with MB at 48 mg/l for 2 hours at 10-15°C, but that melons that had experienced longer transit periods showed nontolerance at that dosage.

MB in preliminary testing has been found to damage lettuce at rather light dosages when fumigation is conducted at 5°C and after chilling, but time has not permitted testing very low dosages at higher temperatures prior to chilling. Under these conditions low dosages of MB might result in the desired mortality (when combined with a period of cold storage) without damaging the lettuce.

The same approach might be applied to the use of EDB; however, it has the disadvantages of high sorption and low volatility, and more lengthy desorption time due to its higher molecular weight. It has however been used in low concentrations as a dip treatment (Wolfenbarger, 1962; and Burditt *et al.*, 1963) but is doubtful for lettuce. Also due to its high sorption EDB can leave high residues of the unchanged chemical in some commodities, but this must be determined for each commodity on an individual basis.

In addition to tomatoes and other glasshouse crops hydrogen cyanide has been used extensively on grains (for which FDA tolerances have been established) and has been used successfully on avocados (Richardson and Balock, 1959). Although our tests have shown it to cause some damage to lettuce, very low dosages prior to chilling and combined with a 7-day cold storage period may produce the desired insecticidal effects without this damage. The problem of high sorption and water solubility always makes HCN fumigation of lettuce questionable.

Dichlorvos (DDVP) is an organo-phosphorous compound that combines high toxicity to insects with very rapid breakdown characteristics. Further, Pass and Thurston (1964) tested 42 plants at the dosage of 131 mg/m^3 at 20°C and noted no damage except a slight fading of chrysanthemum blooms. This material has been used on a wide variety of commodities including fresh meat and mushrooms. The greatest apparent drawback to the fumigation of lettuce with this material is its relatively low penetration which might preclude its effectiveness at the center of the head. However this material should be quite effective against aphids, loopers and other insects found in and around the outer leaves.

III. Residue Tolerances

The Environmental Protection Agency's Compendium of Registered Pesticides gives a list of chemicals registered for lettuce as of March 3, 1973. Calcium cyanide has been registered for greenhouse lettuce with a residue tolerance of 5 ppm. Ethylene dibromide is registered for soil treatment and the residue tolerance is 30 ppm as inorganic bromides. Dichlorvos is also registered on greenhouse lettuce with an allowable residue of 1 ppm. The dosage recommended is 1 gm actual/1000 ft^3 and time between treatment and harvest is 24 hours.

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