Section 10 - Non-chemical control methods

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Physical and mechanical methods of stored product insect control

by A.C. QUINONES

The problems associated with chemical control of insects and increased demand for hygienic food supplies have stimulated a search to alternate control of storage-insect pests. Among the approaches being explored are use of temperature, moisture and atmosphere control, airation, grain fuming facilities, cleaning operation and radiation.

1. Temperature Control - Temperature plays a significant role in such physiological functions as oviposition, fecundity, generation time, longevity which essentially determine the growth and abundance of insect populations in grain. Temperature ranges of 2238C are considered favorable to most insects, but optimal temperature preferences are quite variable which accounts for geographical distribution patterns often associated with particular species.

Heat was used to kill insects at least 75 years ago. Temperatures of 120 to 130F maintained for 1012 furs. are effective. Actually, these temperatures kill most insects quite quickly but where insulation, grain and materials are involved, the general temperature must be kept up for several hours to ensure complete penetration of heat. Heating vaults are used for control of insects in used bags and in bagged or packaged products. With vault temperatures of 180 to 200 F, 100 1b bags of feed or flour can be completely freed from insect life in 24 hours' exposure.

Low temperatures are probably the most important single factor in making long-term storage possible and economical. Freezing quickly kills many forms of organisms and low temperature is also important in maintaining seed viability.

Temperatures above 115F may damage seed viability unless the grain is cooled soon after drying.

Several methods of heating grain in bulk are now available. A recent method is the use of fluidised-bed heating (Thorpe, 1985) where air is used as the heat transfer medium because it transfers heat rapidly, can combine with good solid mixing ensuring that individual grains in the bulk will each receive appropriate treatment. Batches of each receive appropriate treatment. Batches of grains in fluidised beds could be disinfected by heating them to a temperature of 65C in 4 minutes or even in 30 sec. or less. It was also found out that heating did not adversely affect the moisture content, germination or baking quality of wheat. At this temperature of the grain all developmental stages of insect pests are killed.

Frying a small quantity of rice (22.7 kg) for about 7 minutes was found sufficient for control of common insects, but this method is ineffective against S. oryzae despite reduction in the rate of build-up of the weevils (Lim 1975). On the other hand microwave heating complete mortality of adult s oryzae was achieved in rice (13% moisture content) after 10 min. (Lim & Tea 1978)

2. Moisture Content Seeds are hydroscopic, thus grain either absorbs moisture from the environment (under high relative humidity conditions) or loses moisture (under low relative humidity conditions). This relationship is represented graphically by typical zymoldal curves, dependent on whether the grain is absorbing or desorbing moisture, until a situation where relative humidity of the surrounding air and moisture within the grain kernels reach a dynamic equilibrium. Thus, the maintenance of "safe storage moisture content" requires a corresponding low level of relative humidity, which can be achieved by:

1. Positioning of the storage in a region or location where relative humdity is naturally low.
2. Reducing relative humidity to an acceptable level by conditioning the storage environment with refrigeration and dehumidification.
3. Storing dry seeds in impervious sealed containers.

In regions where climate is more humid, grains are nearly always harvested with moisture favorable for insect development and unfavorable for safe storage. In this case, post harvest drying using heated air is a necessity.

The phenomenon of moisture migration is very pronounced in large grain bulks stored in warm subtropical climates where bulks of grain initially stored at elevated temperatures remain warm, while ambient temperature drops at night. A consequent increase in moisture content of the grain bulk periphery or condensation of moisture (sweating) on the bulk surface is generally imminent in vertical storage systems initiating microfloral activity and promoting excessive losses. Heavy insect infestation also causes moisture migration as a result of heat and moisture given off by the insects. Under this situation, the insects must be destroyed by fumigation. Methods of eliminating accumulations of moisture and reducing moisture migration are as follows: 1) drying the grain to a low, uniform moisture content, 2) keeping the grain uniformly cool by aeration or ventilation and 3) fuming the grain to cool it and disperse accumulation of moisture.

Most stored grain insects are unable to survive and reproduce in grain with moisture content below 9 percent. If by various means, it is possible to reduce and maintain the moisture below that favorable for reproduction and development, then we have, in effect, controlled the insects.

3. Grain Aeration - Another non-chernical method of insect control is grain aeration or ventilation. This is the process of cooling a grain bulk by passing air of suitable temperature and humidity through it. This process involves the formation of temperature and moisture fronts which move through the grain bed in the direction of the air flow. These fronts can interact which leads to an attenuation of grain temperature and moisture content profiles (Sutherland et al., 1971).

Grain temperatures of 15-18C prevent reproduction of Rhyzopertha dominca and Tribolium species and severely limit other Sitophilus spp. Dryness of grain also lowers reproduction of Sitophilus. Cooling to suppress moisture movement and removal of heat resulting from grain respiration, also decreased chemical protectant breakdwn rates, maintenance of grain freshness, expulsion of odors, and distribution or removal of fumigants.

Grain cooling can be achieved by aeration using ambient or artificially cooled air. The term aeration is usually taken to mean the forced movement of air of low airflow rates for the purpose of cooling rather drying, although some drying may occur. In tropical climates storage temperature are usually in the range of 25-30C, and although some cooling by aeration may be possible to prevent grain deterioration, it is often necessary to pay more attention to reducing grain moisture content as a means of suppressing biological, particularly microbiological, activity and use other methods to control insect infestation when this occurs.

An aeration system comprises a fan ductwork to distribute the air through the grain bulk, a controller to operate the fan only when aeration will be beneficial and for conventional systems, temperature monitoring equipment to observe and optimize the cooling performance. Fans and ducts are the two major pieces of equipment needed for the proper delivery of aerating air through grain mass in terms of the required rate and uniformity of the airflow.

Grain aeration is widely used in Australia, and the United States and is now practiced in Asean countries like Malaysia, Philippines and Indonesia. In Australia, about 30% of the permanent storage capacity have aeration facilities. Aeration has been applied to vertical silos (concrete and steel) and horizontal sheds of various shapes and sizes. Table 1 summarizes date on refrigerated aeration trials at Dalby Oueensland in 1967. This type of grain aeration was used in areas of Australia where the climate is too warm for the use of ambient air to be effective. Results of three trials with wheat carried out over two seasons showed that insect free wheat could be safely stored for 10 months and that insect population could be held at relatively low levels.

The airflow rates used in grain aeration systems must be adequate to cool all the grains before undesirable changes takes place. The airflow rate required depends on the purpose of aeration, the kind of grain, the size and type of stage structure and climatic conditions.

The recommended air flow rates in USA are measured in cubic feet per minute per bushel (c.f. m/bu), where 1 cfm/bu is equal to 0.0344 m/sec/m (Chung, et. al., 1985)

Sorenson and Crane (1960) recommended an airflow rate 12.9 x 10-3 m/sec/m for rouch rice with initial moisture content of 25% wet basis. With this airflow rate, the rice bed usually should be no deeper than 2.44 to 3.01 m (8-10 ft) for economical use of power.

In California where the climate in drier and cooler than in the Texas rice-growing area, Hederson (1958) recommended airflow rate of 5.7 x 10-3/sec/m and 9.2 x 10-3m/sec/m3, for rough rice with moisture content of 20-25%.

In Malaysia, studies carried out by Mohd/Jantan et. al., (1983), to determine optimum level of aeration for avoiding grain deterioration revealed that aerated storage at airflow rate of more than 0.1 m/t/m can reduce yellowing in paddy bulk stored up to 4 months in concrete tower silos to about 2% (weight rates of yellow kernels to that of total bulk). There was also a slight drying effect.

In the Philippines paddy held in sacks and aerated 8 hours a day with an airflow rate of 28 m/min/t showed no decline in grade up to 9 days, while paddy in bulk aerated with an airflow rate of 35 m/min/t lasted for 14 days without losing its initial grade quality. Wet moisture of 23-25% maintained milled rice quality within acceptable limits for about one week by aerating at an airflow rate of 6.0-35.0 m/min/t for 8 hours a day.

4. Turning of Grain - is the process of moving grain by transferring the contents of a silo to a nearby empty one. The result is a break-up of pockets of insect infestation, a slight loss of heat to the atmosphere, an averaging out of temperature and hence a reduction in maximum

temperature and the mixing of any moist grain with the rest of the bulk. However, moisture content of the bulk of the grain is not lowered appreciably by turning.

With limited handling facilities, grain turning is a tedious and time-consuming process. A complete turning of 750 t of grain in one silo may take 40 hours and often interferes with other drying and transfer operations of the plant (Loo, 1 985)

Caution must be exercised in turning grain which is already infested. It will diperse the existing infestation and subject more grain to attack. Surface-infesting insects should be destroyed and the damaged grain removed before the grains are turned.

5. Atmosphere Control - Insects are essentially obligate aerobes, hence, the atmospheric gas composition has a dramatic effect on insect populations in grain bulks. Under hermetic conditions, oxygen depletion will eventually wipe out insect populations. The application of lethal concentrations of CO2 is an established, commercial control tactic in many countries. The level of gastightness achieved by sealing is of paramount importance for successful application of the technique.

Cleaning Operations - Most modern storages have elevators with equipment designed to clean or condition grain before it is stored. Most of this equipment employ a shifting and/or an aspiration process. These two processes are capable of removing most of the free-living insects but have no appreciable effect on internal infestation. Proper disposal of the residues from cleaning restricts cross - infestation.

6. Radiation - The use of radiant energy for insect control offers many promises. Research in this subject is very active and it is anticipated that the practical use of radiant energy for insect control will increase. Several types of radiation are useful for stored-product insect control.

Many insects are attracted most strongly to "black light" or ultraviolet light. Advantage is taken of this attraction by using such light in insect traps to indicate population levels or degree of infestation.

Infrared radiation produces high temperatures in relatively short exposures. This radiation can be produced electrically (heat lamp), but gas-fired ceramic panel heaters produce it more economically on a large scale. Temperatures in the order of 160 F in irradiation periods of 10 to 20 seconds are said to be effective in drying grain and controlling insects.

Ionizing radiation (atomic energy) can be used to "cold sterilize" food products or grain. X-rays, accelerated electrons, and gamma rays are deadly to insects. These forms of radiation have been seriously considered for large-scale processing applications.

Table 1 Summary of results of three grain refrigerated aeration trials at Dalby, Queensland.

Trial No. Season	**1** **1967 - 68**	**2** **1967 - 68**	**3** **1968 - 69**
Average airflow rate			

(L/sect/t of silo capacity)	0.45	0.15	0.6
Quantity of wheat (t)	594	594	570
Storage periods (months)	10	10	10
Mean grain temperature (C)			
initial	26.4	33.0	28.3
final	10.4	12.1	15.5
Time to cool to 15C (months)	0.6	2.1	15.5
Mean grain moisture content			
(% wet basis)			
initial	10.8	10.7	11.1
final	12.5	10.9	10.9
Total operating time (hours)	5600	5600	1900
Energy used KWh/t of silo capacity)	34	11	18
Number of live insects			
inloading	0	0	0
outloading	0	35	3
Quantity of grain sieved (kg)			
inloading	76	72	82

outloading	214	94	91

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Controlled atmosphere storage technology (CAST)

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By R.L. Semple

INTRODUCTION

The use of residual contact insecticides for the protection of stored commodities and structural treatments, and the use of fumigation as an emergency disinfestation technique are components in an overall pest control strategy. However the most primary form of control, is adequate sanitation and hygiene within the warehouse and including its immediate surroundings, in storage bins, conveyance machinery, processing machinery (i.e., rice mills) and transportation.

Interest in alternatives to chemical control has been rekindled due to problems related to their use;

1. **Resistance to registered and available grain protectants resulting in control failure.**
2. **Lack of replacement insecticides that are relatively inexpensive and safe, which includes not only pest control operators but consumers and the enrironment.**
3. **Unacceptable pesticide residues especially imposed by some importing countries that are not recognizing codex MRL's.**
4. **High development costs and time required for chemical manufacturers to register grain protectants for commercial use.**

5. **The requirement for achieving nil tolerance for live insects in export grain commodities (including raw cereals and milled products).**
6. **Environmental and social imp/ications.**

ENVIRONMENTAL MODIFICATION TECHNIQUES

Stored grain, associated insects and microorganisms respire using atmospheric oxygen to convert carbohydrates in the grain into carbon dioxide and water. In poorly ventilated bulk storage systems, the heat generated by this reaction is not easily dessipated due to the insular properties of the grain. Consequently, temperature rises in localized regions or ``hot spots", respiration rate increases and the rate of deterioration is accelerated.

This phenomenon can be best illustrated by the simple oxidation equation of glucose (starch of grain changes to glucose before most organisms digest the food) in air.

Formula

If we review the ratios of atomic weights of the elements involved, we can compute the following relationship

Formula - continue

Where 180 parts of cereal plus 192 parts of oxygen, evolves 108 parts of water plus 264 parts of carbon dioxide. Any units of weight can be assigned to these values, so long as they remain

constant. (atomic weights of carbon = 12; hydrogen = 1; oxygen = 16).

The breakdown of 180 grams of cereal produces 2820 kJ of free heat (1 KCal = 4.1868 kJ; 1 BTU = 1.05506 kJ), which must be dissipated to the surroundings, the zone of generation being at the higher temperature. Deteriorating grain creates hot spots that may be detected by temperature sensing thermocouples or resistance thermometers (thermistors).

The respiration of dry, uninfested grain is low, but in the presence of insects, the rate of respiration as measured by the production of carbon dioxide, significantly increases and is greater at elevated temperatures and moisture contents. Most storage fungi are unable to proliferate at relative humidities below 70%, and in such cases when insects are not present, the respiration rate is similar to that of dormant seeds.

Most animals and plants require oxygen for their respiration and will die or cease to grow at low oxygen tensions. Some organisms (such as yeasts and bacteria), are capable of anaerobic respiration, breaking down carbohydrates to substances such as lactic acid, acetic acid or alcohol, but producing less heat than aerobic respiration.

Formula

In dry grain stored in air-tight conditions, an insect infestation will use up available oxygen and become asphyxiated (they are essentially obligate aerobes), if the oxygen concentration falls below 2% by volume of the intergranular air (Bailey, 1965). Fungi can still grow at oxygen

concentrations down to approximately 0.2% (they are micro-aerophilic), while yeasts (anaerobic), can proliferate very rapidly at an oxygen tension between 0.5 -1.0% under suitable temperatures, causing fermentation.

Safe storage is therefore achieved by keeping the grain under conditions in which spoilage organisms are not able to rapidly develop or become established. Variables that can be manipulated and controlled are temperature, relative humidity and therefore grain moisture content (EMC), and the gaseous composition of the storage atmoshphere.

Table 1. Carbon dioxide concentrations as an index of infestation.

CO_2 CONCENTRATION % per 24 hrs	**INDICATION OF INFESTATION**
0.25	Uninfested grain with less than 15% m.c.
0.30	Clean grain
0.35 - 0.50	A slight insect infestation or a rater high infection of microorganisms
0.50 - 0.90	Close observation required
1.0	Limit of dangerous storage conditions
1.0	Highly unsuitable storage conditions

DETERMINING GRAIN ACCEPTABILITY FOR STORAGE

Carbon dioxide evolution is directly related to dry matter loss (DML) of grain as a function of moisture. Teter (1979, 1981) stated equations for paddy as

$\log (CO_2) = 0\ 44\ Mw - 6.08$ for Mw 13.3- 15.6%
$\log (CO_2) = 0\ 21\ Mw - 3.04$ for Mw 10- 13.2%
$\log (CO_2) = 2.33 \sin | 7.5(M - 13)$ for Mw 15.7-25%

where Mw = % moisture wet basis.

The general equation is

$\log(CO_2) = AMW- B$ (Hall,1970) -------(4)

where CO_2 = mg CO_2 per 100 gm dry matter day m = % moisture wetbasis

A & B = grain constants as shown in Table 2 (Appendix I).

Carbon dioxide evolution is a direct ratio of DML, such that for each gram CO_2 evolved, 0.682

gram of dry matter is lost (180 $C_6H_{12}O_6$ to 264 CO_2; 180/264 = 0.682).

If follows that 1 kg of CO_2 generation gives;

- **0.682 kg of dry matter loss**
- **0.409 kg of H_2O**
- **10,680 kJ of heat generated (1000/264 x 2820 kJ)**

The acceptability of grain for storage has been stated that the deterioration at the conclusion of storage should not exceed 0.8% DML (Teter, 1981), or in the U.S., 0.5% DML.

This level can be computed from

% DML per day = $(CO_2).10^{-3}$ (0.682)

1. EFFECTS OF MODIFIED ATMOSPHERES ON STORAGE INSECTS

Monro (1967) has summarized some of the original observations of Dendy and Elkington (1920) concerning the effects of air-tight (hermetic) storage on grain insects.

1. Grain insects in hermetic inclosures succumb as soon as the oxygen has been used up with a corresponding increase in the amount of CO2 being produced.

2. The gases present under normal sealed conditions are oxygen, nitrogen and carbon dioxide.

3. The amount of CO2 given off by live cereal grains in hermetic enclosures is directly related to moisture content and temperature, and above a critical point of moisture, the production of CO2 increases very rapidly.

4. Above a certain critical level of moisture content, grain stored under hermetic conditions creates an environment lethal to insects (and other aerobic organisms) quite rapidly, but at lower moistures, the rate of "self sterilization" takes a comparatively longer time to achieve.

5. The amount of oxygen consumed by live cereal grains of low moisture is greater than the amount of CO2 given off (i.e., a respiration quotient less than unity).

6. At approximately 30C, 100 Sitophilus oryzae give off about 30 milligrams (i.e., nearly a fifth of their own body weight) of CO2 in 24 hours, while at lower temperatures (20-21 C), the amount of CO2 evolved is reduced to about 9.4 mg in 24 hours.

7. Sitophilus granarius is a less active weevil as compared to S. oryzae, and on a weight for weight basis, the amount of CO2 evolved is less. The respiratory quotient (RQ or ratio of CO2 given off to the amount of O2 consumed) for S. oryzae is 0.773, while for S. granarius, it is around 0.815.

8. It is generally accepted that the absence or lack of oxygen is sufficiently lethal to weevils,

without taking into account the presence of increased concentrations of CO2, although they may remain alive for extended periods under low oxygen tensions.

9. However, CO2 does exert a lethal effect on weevils irrespective of depleted oxygen tensions such that at approximately 30C, S. oryzae was killed in less than 12 days in an atmosphere containing 14.0822.56% CO2, although around 14% 02 still remained.

10. Pure (moist) CO2 is less fatal than CO2 with a small admixture of O2, where pure CO2 acts almost instantaneously as a narcotic under the influence that Sitophilus spp. remain in a state of "suspended animation" without losing their power of recovery.

Sealed grain storage therefore has the following advantages:

- **It sterilizes the grain, killing insects in all stages and other vermin that may be present.**
- **It prevents absolutely, the entry of insects and rodents and therefore obviates the problems associated with further applied control measures.**
- **It prevents grain of high moisture content from becoming mouldy and from heating, although it does not prevent anaerobic respiration and the associated rise in acidity.**

Table 2A. Constants A and B for equation (4).

Grain	**Moisture Range**	**A**	**B**
Maize, dent	10.0 -13.2	0.17	2.00

	13.3 - 17.0	0.27	3.33
Sorghum	10.0 - 13.2	0.125	1.65
	13.3 - 17.0	0.32	4.19
Rough rice	10.0 -13.2	0.21	3.04
	13.3 - 17.0	0.44	6.08
Brown rice	10.0 - 13.7	0.17	2.67
	13.8 - 17.0	0.44	6.41
Polished rice	10.0 - 14.1	0.16	2.83
	14.2 - 17.0	0.49	7.48
Wheat, soft	10.0 - 14.0	0.09	1.35
	14.1 - 17.0	0.36	5.14

- It prevents the absorption of moisture from the humid ambient atmosphere, so that if grain is initially stored in a dry condition, it remains dry.

The general conclusions are that grain stored in air-tight conditions can be maintained without appreciable deterioration in grain quality and germinative capacity, so long as it is initially dry. Grain above 17% m.c. deteriorates in time, while grain below 17% m.c., remains viable for 6 months or even a year, but shows low rates of germination and is therefore unsuitable for

seeding and malting. At temperatures of 25C, grain of 24% m.c. loses Its germinative capacity completely in 6 weeks, while grain stored at the same moisture content but at 15C, loses its germinative capacity in a longer period (19 weeks).

The diffusion theory in insect respiration:

Wigglesworth (1966) stated that;

"Although the tracheae are permeable to oxygen throughout their length, the abundant supply of tracheal capillaries in and around the most active tissues leaves no doubt that most of the oxygen enters the organs through these endings but it is only comparatively recent that the diffusion theory for insect respiration in the conveyance of oxygen from the spiracles to the tracheoles has been widely accepted."

Essentially, the elimination of carbon dioxide is performed in a similar manner. Generally, the amount of CO2 produced by the insect is usually less than the amount of oxygen absorbed. The rates of diffusion is proportional to the square roots of their densities and since they do not differ dramatically, the theory of diffusion therefore accounts for both oxygen uptake and carbon dioxide elimination.

CO2 diffuses more rapidly (presumably) through insect cuticle of the body (especially when this is thin), and through the larger tracheal trunks, than oxygen entering via these routes, and it is generally safe to say that in most terrestrial insects, oxygen is taken in by the tracheae.

Tracheal respiration is compounded by the requirement for conserving body moisture, since most of the water loss in insects occurs by evaporation through the spiracles.

The function of the spiracles has been termed the "diffusion control of insect respiration". When the spiracles are closed, any active organ or tissue can draw on oxygen from any part of the system, and oxygen levels can be re-established by the opening of a single pair of spiracles. Normally, spiracles are kept closed and are only opened long enough for the insect to be supplied with oxygen. If an insect struggles or is under stress, or the rate of metabolism is increased due to elevated temperatures, or at the height of digestion and egg production in females, they are opened more frequently and remain open longer with other spiracles being activated, which results in increased rates of water loss.

Spiracular movement is regulated by specific ganglia of the ventral nerve cord and the brain, and are stimulated by CO2 such that an exposure to 2% CO2 incluces the spiracles to remain permanently open, as well as by the accumulation of acid metabolites due to oxygen lack. In normal respiration, the reduced tensions of oxygen and accumulation of CO2 (except in thin-skinned insects where most of the CO2 diffuses through the general surface of the body, and consequently cannot serve as a respiratory stimulus) cooperate in the control of respiration.

Gaseous diffusion is adequate for small insects, but is insufficient for actively running or flying species with a high rate of metabolism and requirement for energy. A certain degree of mechanical ventilation is added to compensate for the higher requirement for oxygen.

The main function of collapsible tracheae and airsacs (tracheae with large dilatations which occupy a great part of the body cavity as in many Orthoptera and Hymenoptera) is the ventilation of the respiratory system. They are ventlated by the respiratory movements of the rigid body wall that encloses them, and to some extent by general body movements, such as during flight. Therefore, the greater part of the tracheal system is maintained full with a gas similar in gaseous composition to that surrounding the insect, but the supply of oxygen to the tissues is still effected by diffusion along the tracheal branches extending from these airsacks.

The extent to which the tracheal system is ventillated varies dramatically between different insect species, or in the same insect in different physiological states. Respiratory movements of insects are brought about by special musculature generally confined to the abdomen, but in some insects (aquatic), ventillation is maintained by aspirating movements of the metathorax.

So it can be seen that when oxygen requirements are great, ventilatory pumping movements are initiated and the rythm of opening and closing of spiracles is modified in such a way that a directed stream of air is forced through the system, a system which necessitates a complex nervous co-ordination between pumping movements and regulation of spiracular movement.

Either a lack of oxygen or an excess of carbon dioxide may stimulate the respiratory centres and cause hyperpnoea (= abnormally rapid or deep breathing).

The secondary respiratory centre located in one of the thoracic segments has an overriding action and control movements in the entire insect, can be stimulated by CO2 tensions of 0.2 -

3.6%, while the primary centres (usually found in the segmental ganglia and control the movements of their own segments, i.e., isolated segments of the abdomen can perform respiratory movements) can be activated at 12-15% CO2, while ventillation in other insects can occur at CO2 tensions of 10%, even when the insect is at rest. It is highly probable that a chemical stimuli is acting in all cases, and is probably an increased acidity in the nerve centres, due either to excess CO2, or an accumulation of acid metabolites due to oxygen lack.

Bailey (1965), and Bailey and Banks (1974), demonstrated that the oxygen tension should be maintained below 4.5% O2 to produce a lethal atmosphere for even the most susceptible stored product insect, however, a limit of approximately 2% O2 has been predicted to ensure mortality of stored products insects that have been so far tested (Oxley and Wichenden, 1963; Banks, 1979).

Girish (1978) has made several observations on the susceptibility of various life stages of Trogoderma granarium Everts under varying exposures of gastightness at optimum conditions for development (35C; 11.5% m.c.). These observations are summarized below.

1. Susceptibility to air-tight conditions varied from stage to stage and age of larvae.

2. No life stage was capable of surviving or completing development to the next stage under airtight conditions and 100% filling ratio of the container, except for pupae.

3. Reduction of free air space or headspace decreases available oxygen and is depleted more

quickly than if insects are maintained in an empty container.

4. Susceptibility to low oxygen tensions produced biologically by grain and insect respiration was egg < first instar larva < adult < full grown larvae and pupae.

5. Oxygen consumption was greater than CO2 production for all stages.

6. Carbon dioxide production and O2 consumption is suppressed by the depleted O2 and accumulated CO2 under airtight conditions.

7. Grain respiration is much less than different life stages under air-tight storage conditions.

8. Under air-tight conditions, the levels of CO2 and O2 to achieve complete mortality was:

Life Stage of T. granarium	**: Gas Concentrations (%)**	
	: CO_2	**: O_2**
Adults	12.56	3.39
Full-grown larvae	14.62	1.06
First stage larvae	12.34	5.35
Eggs	2.49	16.77

Observations (3) and (5) from this study require further clarification. Referring back to the oxidation equation of glucose (hexose polysaccharide) the volume of CO2 produced is equal to the volume of O2 consumed in the respiratory process, which represents a respiratory quotient (RQ) of 1 or unity.

This is rarely obtained in air tight conditions where the filling ratio is 100% (RQ = 0.7) mainly because CO2 is absorbed by the grain (in greater amounts than absorbed oxygen relative to the net volume of grain), and is therefore not measured in the interstitial airspace. Once the filling ratio in airtight containers is reduced (to < 5%), the RQ approaches unity (see Table 3). For this reason, even with high population densities of insects (assuming anaerobic respiration is not evident) in completely sealed containers, oxygen depletion to a lethal 2% is accompanied by a rise in CO2 to rarely more than 15% (RQ of approximately 0.75).

Table 2. Influence of moisture content and temperature on the respiration and mortality of S. oryzae in wheat (in relation to equilibrioum relative humidity) (Results from Lindgren, 1935)

Wheat moisture content (%)	**Respiration of uninfested wheat (mg CO_2/48 hours/100 g of wheat)**		**Respiration of S. oryzae (mg CO_2/48 hours/1 g of weevils)**	
	25C	**35C**	**25C**	**35C**

8.7	trace	trace	151.6	129.0
10.7	trace	0.44	300.0	215.4
14.0	1.34	2.90	412.8	522.6
15.2	2.00	4.48	403.8	578.8
17.4	26.4	42.4	409.2	464.0

Table 3. Apparent Respiratory Quotient (ratio of evolved CO2 to oxygen consumed) of equal numbers of S. oryzae adults in jars of equal size but containing varying proportions of wheat, i.e., the filling ratio.

Proportion of container occupied by wheat (%)	Apparent respiratory quotinet
100	0.69
75	0.78
50	0.86
25	0.94
5	1.01

Extracted from "The effect of restricted air supply on some insects which infest grain" by T. A. Oxley and G. Wickenden Ann. Appl. Biol. (1963): 51, 313-324.

The RQ of T. granarium approaches but is less than unity, as shown in (5). Girish (1978) and Bailey (1955) suggest it would be advantageous to completely fill air-tight silos because of more rapid O2 depletion (lack of air space means an interstitial air space of 40% of total volume with wheat), but Oxley and Wickenden (1963) found very little difference between filling ratio and rate of O2 depletion.

In air tight or hermetic storage systems, it is therefore oxygen depletion by the respiration of insects themselves that is the lethal factor rather than the accumulation of CO2.

With externally generated controlled atmospheres, the combinations of gaseous mixtures is easily manipulated and may produce synergistic effects. Atmospheres containing 15% O2/36% CO2/balance air have been found to be lethal to Tribolium castaneum (Herbs") and Plodia interpunctella (Hub.) larvae after an exposure of 10 days (Herein and Press, 1968). Calderon and Navarro (1979) exposed T. castaneum adults for 24-120 hrs at 57% RH and temperatures of 26C and 30C, atmospheres containing O2 concentrations of 2 - 5% with 5 - 35% CO2. A clear synergistic effect was found, since mortality at given O2 concentrations was higher when the level of CO2 was increased. Mortality was also subsequently higher at the higher temperature and if the exposure period was longer. With a 5% O2 concentration, no mortality was observed, but the addition of 35% CO2 caused 100% mortality at both temperatures. To obtain an LD95 in atmospheres containing 2% O2, the addition of 10% CO2 is required, but 5% O2 plus 15% CO2 produced only negligible mortality. When CO2 is present, O2 concentrations are depleted more quickly and adults die at higher concentrations. Both O2 depletion and presence of CO2 increases water loss which increases mortality (Navarro and Calderon, 1974).

Enhanced insect mortality with reduced O2/increased CO2 concentrations in the atmosphere emphasizes the advantage of using CO2 to obtain low O2 tensions in preference to purging with N2.

Calderon and Navarro (1980) further substantiated the synergistic effects of the addition of CO2 to low O2 atmospheres on adult mortality of T. castaneum and Rhyzopertha dominica (Fab.) which appeared more susceptible (100% mortality at 2% O2 with the addition of only 10% CO2).

Results on the effects of CO2 to reduced O2 atmospheres on 0-24 hr eggs showed that with T. castaneum, 100% mortality was achieved at 2 and 4% O2 without CO2 (96 hr exposure) while even 8% O2 inhibited almost 50% of the eggs to hatch (it should be noted that no adult mortality was achieved in O2 alone above 4%).

The addition of CO2 to given O2 atmospheres had little effect on egg mortality, but increasing CO2 concentrations (from 5-30%) did display an additive effect in the range of O2 concentrations tested (2-8%), but failed to exhibit any synergistic effect on egghatch. Presumably greater water loss in adults in high CO2 atmospheres as compared to eggs is the reason for differing response. Eggs are significantly less effected by low relative humidities than adults of Ephestia cautella and T. castaneum.

These studies have established the toxicity of various gaseous combinations beyond those established in earlier publications by Bailey. O2 concentrations of 3-4% or between 40-60% in

the presence of atmospheric O2 concentrations (21 %) at 23-32C with 14 day exposures depending on the species.

Various workers have found that mixtures of 90/10 (CO2/O2) were as lethal as 98/2% mixtures against T. confusum, and 80/20% mixtures were much more toxic than 100% CO2 against S. oryzae and S. granarius adults, both tests performed at 26.7C. (All Niazee, 1971; Lindgren and Vincent, 1970).

Jay (1983) found that 60% CO2 in air is more effective against the internal feeders or primary grain pests such as Sitophilus spp., R. dominica and T. granarium, while atmospheres containing 100% CO2 is more effective against the external feeders or secondary grain pests, such as T. castaneum, Oryzaephilus surinamensis, Trogoderma variable and T. glabrum, presumably because of adsorption and desorption of CO2 on grain. The practical inference is that different gaseous mixtures could be used depending on species composition present in storage.

Bell (1983) has studied the toxicity of O2 in high concentrations of CO2 on the eggs of Ephestia cautella and E. Kuhniella at 15 and 25C.

At 25C, gas mixtures containing more than 40% CO2 killed all eggs (aged 2, 20 and 44 furs) within 48 hours. Egg immediately after diposition are more susceptible to atmospheres containing 40% CO2 either in the presence or absence of O2, while the susceptibility to this level decreases as embryogenesis proceeds, in the presence or absence of O2. The presence of

O2 plays some part in the toxic action of CO2, since susceptibility is prolonged in the presence of 4-20% O2, (up to 44 hrs), while in the absence of O2, susceptibility to concentrations of 40% CO2 and above decreases in 20 hour old eggs.

At lower temperatures, longer exposures are required to obtain complete mortality with CO2 in the presence of O2 than in its absence.

The dessicating action of CO2 can be ignored for eggs since its action affects spiracular control but if development proceeds during exposure, mortality will be achieved when a more susceptible stage is reached. CO2 is actively toxic at higher concentrations (unlike N2, it does not rely on anoxia to be lethal) and may be more toxic in the presence of oxygen. The speed of action of CO2 in air is only slightly faster than CO2 alone, but is enhanced substantially at lower temperatures, where purging storages with CO2/O2 mixtures may achieve better results than purging with CO2 alone. Also, purging with CO2 remains effective for longer than N2, since the atmosphere is diluted progressively as air leaks back into the purged structure.

Jay (1983) claims storage insects are more susceptible under field applications than in laboratory evaluations, because of the gradual decline in concentrations during the ventillation phase. At 70% RH, 77% CO2, the exposures required for T. granarium and pupae of s. granarius is one month, but because of natural gas loss, an indefinite exposure for T. granarium maybe warranted. However, he emphasized the dearth of knowledge of CAST as related to grain moisture and relative humidity, especially conditions operative in the humid tropics.

Insects infesting stored products are capable of withstanding extremely low humidities, an important aspect of their structure and physilogy which allows rapid changes of O2 and CO2 while restricting water loss by efficient respiratory systems. Navarro (1978) suggests that with respect to controlled atmospheres, the structure and physiological characteristics of each species should be taken into consideration. For example, Ephestia cautella pupae in 3% O2 with low RH, mortality is due to dessication through effects on the spiracular regulatory system, but if the RH remains high, some survival is possible (Navarro, 1978).

At lower oxygen tensions (1 % O2 for E. cautella or 0.12% for T. castaneum adults) insect mortality occureed at high RH, but when the RH was low, the desiccation rate increased causing mortality in a shorter period, such that a relationship of higher RH, lower O2 to achieve similar levels of mortality exists.

Navarro and Calderon (1980) have emphasized the requirement of investigating temperature and relative humidity requirements for more efficient use of CAST. At a given atmospheric gas composition, the higher the temperature, the shorter the exposure time reguired to achieve an LD95, and is very pronounced in the range from 15-21C. Since different temperature gradients exist in grain bulks, the lowest recorded temperature should govern the length of exposure period required. Also since the response of storage insects differs to CAST, the most tolerant species present will influence the system used.

Controlled atmosphere treatments appear more effective when the moisture content of the grain is low. Due to moisture migration, moisture contents in localized parts of the bulk maybe

considerably higher than the remaining bulk, constituting the need for applied gas concentrations to achieve effective control of insects residing in higher moisture grain.

Damp pockets of grain can cause insect survival with CAST, and although Navarro and Calderon (1980) claim drying of the grain bulk for obtaining efficient insect control with modified atmospheres is impractical, the requirement for grain drying in the humid tropics is a prerequisite for good storage.

The effectiveness of sealing techniques to prevent excessive use of gas mixtures has been established, but to render storages completely gaslight is an expensive if not impractical undertaking.

Table 4. Survival rate (%) of pyralid eggs exposed to gas mixtures for 24 hours at 25C. (After Bell, 1983).

If aiming for a lethal modified atmosphere of less than 2% O2 under hermetic storage, and if the walls of the container allow diffusion of oxygen From the atmosphere, Insects will also be killed dependent on the rate of diffusion (which is also dependent on the difference between internal and external concentrations) and the initial population density (Oxley and Wickenden, 1963).

There is also the possibility of surviving insects aggregating around leaks at higher O2 tensions (Navarro, et a/., 1979). In experiments where O2 levels were measured at 17% around leaks,

O2 concentrations approximately 90 cm from the leak dropped to a lethal 1.6% (Navarro, et. al., 1976).

Distributions of R. dominica, S. oryzae and 0. surinamensis have been monitored under a range of leakage rates and gas gradients in a simulated laboratory silo (Navarro, 1977). Results using atmospheric gases suggest that none of the insects examined are able to migrate to high oxygen concentrations (ca. 21 %) in a nitrogen or carbon dioxide enriched atmosphere. Insect movement is apparently inhibited in very low oxygen concentrations (c 1 %) in nitrogen, and by high CO2 enriched atmosphere (>65%) in air with O. surinamensis (Navarro, et. al., 1981).

Relative short purge periods with N2 to achieve 1 % O2 (approximately 12 hrs in a 2000 tonne bin of wheat at a purge rate of 3 m3.min-1 (Banks and Annis, 1977) dispersion from low to high O2 tensions existing around cracks in the bin structure is remote, but insects already present in this region may survive the treatment (Navarro, et al., 1981).

The response of O. surinamensis to CO2 atmospheres, dispersal is restricted to concentrations up to and including 66% CO2. Other studies with Tribolium spp. have shown attraction to CO2 concentrations ranging from 0.25- 15%, while repellency occurs between 60 - 90% CO2.

In practice, the purging phase to achieve 20% CO2 is short enough (< 24 furs) to severely restrict insect dispersion to regions of low CO2 concentration.

These observations suggest there is an advantage in using CAST for insect control, since the

infestation will not redistribute itself to regions of high O2 or low CO2 concentrations in the period of time required to obtain the controlled atmosphere.

A potential problem of CAST is the development of resistance, since stored grain insects do possess the genetic potential to develop CO2 resistance (Bond and Buckland, 1979), although no field strains have exhibited this ability. Insects are essentially obligate aerobes, which may indicate a potential advantage of using N2 to create lethal O2 tensions, in preference to the toxic action of CO2.

2. EFFECTS OF MODIFIED ATMOSPHERES ON MICROORGANISMS

Christensen (1978) listed the advantages of CAST as inhibition of aerobic fungi, prevention of mycotoxin production and conservation of desirable quality factors of the grain, while some disadvantages are the elimination of microorganisms that compete under aerobic conditions, development of populations of certain aerobic microorganisms before the atmosphere has been sufficiently modified, expensive and complex technical facilities and capabilities, and generation of some adverse quality factors.

Busta, et al., 1980 consider the following as major considerations influencing the effectiveness of CAST such as the types and numbers of microorganisms present including both storage and field fungi, yeasts, including Candida and Hansenula spp, and bacteria including the genera Lactobacillus, Clostridium, Bacillus and general groups such as the coliforms.

Other important considerations are grain type, and primary physical parameters, both intrinsic and extrinsic factors, influencing microbial activity such as grain moisture or water activity, storage temperature, atmosphere surrounding the grain during storage and storage duration.

Temperature and moisture requirements of fungi have been sufficiently reviewed elsewhere (Christensen and Kaufmann, 1974; Christensen an Kaufmann, 1977, Northolt, 1979).

In commodity modified or biologically produced atmospoheres, respiration and basic metabolism reduce available O2 and increase CO2, where a rapid increase in the production of CO2 above a grain moisture content of 15% mc was accompanied by a relative increase in the number of moulds on the grain (Milner, et al., 1947). If fungicidal or fungistatic substances are applied, respiration remains low even at 20% mc.

Similar reductions can be achieved with CAST by the precise and constant maintenance of CO2, N2 or O2 at specific pressures or under a partial vacuum, but is expensive and technically demanding, while the atmosphere developed under air-tight storage is self developed and dynamic, depending only on the activities of biological organisms and the grain itself (Busta, et at., 1980). Aflatoxin inhibition has been demonstrated in atmospheres containing 90% CO2/10% O2 at 28C (Shih and Marth, 1973).

Table 5. Relationship of moisture content to respiratory rate and mould growth (Regent wheat after respiratory trials at 30C). From Milner et al., 1947.

Initial Moisture Content (%)	Respiratory rate, (mg CO_2/100g grain)	Mould colonies (per mf of grain)
12.3	0.07	0.5
13.6	0.11	0.1
13.8	0.23	0.1
1 4.5	0.57	0.4
15.4	2.53	4.8
16.3	23.35	396
16.8	20.3	209
18.5	111.0	2,275
20.8	604.9	11,300
25.2	1,724.8	37,500
30.5	1,282.0	63,500
38.6	4,666.5	67,000

Table 6. CAST inhibition of growth or toxin production on corn and wheat *

Grain Type	% Composition of Atmosphere $O_2/CO_2/N_2$	

Corn	0.5/13.5/84.8[1]	(at 27C)
	0.3/0/99.7	(at 27C)
Wheat	0/0/100[3]	(22 - 32C)
	4.5/0/95.5[3]	(22 - 32C)

1. **Wilson and Jay, 1975.**
2. **Wilson, Huang and Jay, 1975.**
3. **Shejbal, 1979**
4. **Extracted from Busta et al., 1980.**

Various interactions between temperature, relative humidity, and gaseous composition have been evaluated and it appears that a 3-way interaction exists. The required CAST system and gas composition will be very dependent on temperature and relative humidity (or water activity, Aw) operating in any particular storage situation.

Lillehoj et al, (1972) have estimated mean germination of Penecillium martensli in various gas mixtures after 16 hours. It appears normally distributed about 30C where mean percent germination was approximately 37% in air, 30% in 20% CO2, 20% O2 and 60% N2., approximately 10% in 40% CO2, 20% O2, and 40% N2., and approximately 2% in an atmosphere containing 60% CO2, 20% O2 and 20% N2

3. EXTERNALLY MODIFIED CONTROLLED ATMOSPHERE STORAGE

The techniques involved in modified or controlled atmosphere storage of grain involves the alteration of the normal atmospheric gases present (21% O2, 0.03% CO2, 1% argon and other gases, balance nitrogen) so as to obtain an artificial atmosphere that is insecticidal and prevents mould growth and quality deterioration of the commodity.

Three types of controlled atmospheres have been under investigation by the Commonwealth Scientific and Industrial Research Organization (CSIRO), Canberra, Australia which are:

1. **Low oxygen atmospheres generated by purging with nitrogen, and maintained by addition of further nitrogen to compensate for air ingress (less than 2% O2).**
2. **Low oxygen produced and maintained by the combustion of hydrocarbon fuels (13% CO2, 1 % O2, balance N2)**
3. **High carbon dioxide atmospheres (35-80%) either allowed to decay through its active range or if this is too rapid, maintained with further CO2.**

The methods used are based on the ancient process of hermetic storage where the respiratory activities of insects and moulds alter the atmospheric composition in a gas-tight structure by gradually using up the available oxygen and depleting it to around 2% with a subsequent rise in CO2 to around 13%, (anaerobic fermentation in damp grain under hermetic conditions may increase CO2 as high as 60%). It should be emphasized that the technology involved in CAST differ substantially from that of modified atmospheres generated by biological processes within a sealed structure, such as hermetic storage (see separate section on airtight storage), whereby the appropriate atmospher is supplied externally and introduced into the structure,

and additional gas added if necessary to maintain the lethal atmosphere.

The first successfully recorded large scale treatments in Australia occurred during the World War I, but the process was not used widely again until 1979 (Banks, et al., 1980).

Externally generated atmospheres for disinfesting stored bagged grain in Australia from 1917-1919 was probably the first extensive use of such a system in the world (Winterbottom, 1922). Disruption of world trade had resulted in large stocks being stored in Australia where approximately 60,000 tonnes was treated with low oxygen atmospheres genearated from burning coke in a modified producer gas generator.

3.1 Modified atmospheres using liquid nitrogen: (From Banks and Annis, 1977)

Controlled atmosphere storage with nitrogen can be viewed in two phases, the purge phase, where the desired atmosphere is obtained in the bin or structure, and the maintenance phase, where this atmosphere is maintained for the desired exposure.

Liquid nitrogen supplied under pressure by road tanker is passed through a heat exchanging facility where it is vaporized and bought to ambient temperature (within 2C). It is then passed through a flowmeter (rotameter) and then into the gas introduction system or manifold. Gas flows of 3 m. min1 has been suitable for purging bins from 300-7000 tonne capacity. Increasing the gas flow rate maybe less efficient in reducing oxygen tensions during the purge phase in silos other than those fitted with aeration ducting, since pockets of air may remain with high

O2 levels before being removed by diffusion and convection. thus wasting gas. A vent of at least 50 cm must be left open in the roof during the purge in order to prevent dangerous pressure build up. A pressure release safety valve must also be installed to prevent any posit,ve pressures from endangering the structure.

Gas input must continue uninterrupted until the headspace has been reduced to 1 % O2. Purging in the grain mass occures as a defined front through the grain and direct displacement of the interstitial air. In the headspace, where free gas mixing occurs, decay is exponential.

The quantity of nitrogen required during the purge phase is dependent on the filling ratio and the porosity due to different commodities (i.e., barley requires higher gas input then wheat).

Once the headspace has been reduced to 1 % O2, the purge phase is discontinued, the top vents and introduction ports closed, and the maintenance input of gas commenced by addition of nitrogen in the headspace.

Maintenance gas is also supplied by liquid nitrogen in an insulated vessel on site and charged into the structure through an atmospheric heat exchanger. A simple continuous gas input system has been found superior to using a demand system which maintains a small positive pressure of 120 Pa in the storage, since the former used less nitorgen to maintain the same O2 tension.

Once the exposure period has been met, the atmosphere of a partly sealed structure will

slowly return to normal atmospheric composition containing 21% O2, since air excluded by the maintenance phase leaks in.

This is dependent in levels of gastightness and weather conditions and may be accelerated by forced draught ventillation using fitted aeration equipment or simply providing screened openings (60 mesh) to restrict insect movement at the base and in the headspace. It should be emphasized that oxygen tensions should always be evaluated before entering storges that have been disinfested in this manner.

Choice of exposure period:

If disinfestation of grain is only required, prolonged CAST appears unnecessary. However, a perfectly sealed storage should be insect proof and therefore prevent reinfestation, but is dependent on the gastightness requiements with relation to the leak size (either one large leak or comprised of many smaller leaks) which may permit insect entry, and also their ability to locate suitable entry points.

Nitrogen storage reduces the formation of aflatoxins from the Aspergillus flavus oryzae group of storage moulds, and reduces total mould count. This may allow the safe storage of grains beyond the 12% mc limit that is imposed in Australia (where mould infection is not a serious problem compared to the humid tropics), and also reduces the hazards of heating in oil seeds as well as eliminating the risk of fire and restricting mould growth, but the little effect on the rise of FFA's. Shejbal (1979) has made the observation that the viability of malting barley could

be preserved for long storage periods under nitrogen.

Table 7. Exposure periods and concentration requirements for modified atmosphere disinfestation of grain (12% me).

Requirements	**Atmosphere Source**	
	Carbon Dioxide	**Nitrogen**
Initial target concentration in storage	70% CO2 in air (80%)	1 % O2 in N2
Final target concentration in storage	35% CO2 in air (40%)	1 % O2 in N2
Period of exposure within these limits.	25C: 10 days	24 wks at 15C
	18 - 25C: 14 days	6 wks at 20C
		3 wks at 26C
		2 wks at 30C
		1 wk at 35C

SOURCE:
Banks and Annis (1977)
Banks et al., (1980)

*** The exposure periods for nitrogen CAST are tentative, and based on S oryzae, the most tolerant grain pest to low oxygen atmospheres. If Tribolium spp or Oryzaephilus spp are present, shorter exposure periods maybe used, but if there is mixed populations, or any doubt to the indentity of the grain pests present, the recommendations for S. oryzae should be adhered to.**

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3.2 The application of modified atmospheres based on the external addition of carbon dioxide

The processes of either lack of oxygen under nitrogen CAST or the toxic action of high concentrations of carbon dioxide in air can kill insect pests and rodents in storages and control the growth of moulds in wheat with moisture contents between 1216% mc (Banks, 1979). The toxic action of CO2 rich atmospheres is independent of CO2 concentration above 60% in air, while it declines between 60-35%, and some species are capable of surviving or are tolerant to CO2 levels immediately below 35%. Reproduction rates are however much reduced even at concentrations approaching 10% CO2 in air.

The application technique and introduction of CO2 into the structure is similar as for liquid nitrogen but after the initial purge, it has been demonstrated that there is a need for gas recirculation in order to avoid regions of inadequate CO2 concentration in the upper parts of the storage (Wilson, et al., 1980) (See Fig. 1.).

Recirculation is required in tall structures because CO2 is 1.5 times as dense as air and there is a natural downwards movement of CO2 which reduces the concentration in the headspace. Blowers rated at 3 m3.min-1 are suitable for this purpose. Recirculation should continue for 10 days (ensures headspace concentration does not fall below 40%) or preferably 14 days (CO2 maintained above 35%).

One advantage with CO2 atmospheres as compared to low oxygen in nitrogen atmospheres, is that CO2 may be applied in a "One-shot" operation in welded steel bins after gastightness specifications have been adequately fulfilled. No further gas needs to be maintained while the target regime for insecticidal activity can still be achieved (Banks, 1979; Wilson, et al, 1980).

For modified control atmospheres to have any insecticidal effect, it is necessary that certain levels of gastightness must be achieved. The specification corresponds to a hole area of about 1 cm in the structure which maybe composed of a single leak or a number of small leaks giving

an equivalent combined effect. Both wind and thermal expansion can cause extensive gas leakage during both controlled atmosphere storage and during fumigation. Wind is more important in leaky situations, while problems with thermal expansion of the headspace (Temperature effects within the bulk can be considered negligible) can occur in both poorly and well sealed silos (see Section on gastightness requirements). Retarding solar radiation by insulation or painting the external surfaces with a high reflectance paint (a matt white acrylic paint) reduces the amount of gas loss, and therefore the amount of maintenance gas that needs to be added to compensate for this loss. Moisture migration and condensation problems on the grain surface due to convection currents within the bulk are also reduced.

Controlled atmosphere storage in Australia has favoured CO2 in preference to nitrogen. This is attributed to nitrogen's low boiling point, where the onsite equipment and power supply was unable to cope with the rapid vapourization process and purge times were consequently lengthy. Even silos that attained the levels of gastightness reguired, need a continuous bleed of maintenance gas. It was difficult to fine-tune the system so that the correct bleed rate was reached without releasing the safety valves under excessive pressure and consequently losing nitrogen to the atmosphere. Alternatively, a situation could be created where the bleed rate was insufficient to compensate for the natural decay rate, thus allowing oxygen levels to rise to a point where insect survival was imminent.

Fig. 1. Scheme for nitrogen purging and maintenance of the nitrogen in a sealed grain silo. When CO2 is used only the purge apparatus is needed, but the atmosphere must be recirculated.

From 'Alteration of storage atmospheres for the control of pests' by H.J. Banks in Grain Storage Research and its Application in Australia, CSIRO Division of Entomology ADAB Research for Development Seminar, February, 1979.

NOTE: DIMENSION IN MM

Fig. 2. CO2 introduction and recirculation in vertical welded steel bins of 2000 tonne capacity pressure relief valve: 200 mm drain recirculation and vent pipe fits over 75 mm galvanized pipe within the pressure relief valve. Water added to cover 200 mm pipe by 75 mm. Oil added to prevent evapouration. Internal bin pressure - max 750 Pa (75 mm watergauge). Gas introduction: tanker head pressure 2000 kPa through vapourizer at gas inlet temperature of 25-35C. Application at 3.5 tonnes CO2.hr-1 purged in pairs using 80% CO2 20% air. Gas introduced in bin base until CO2 level reaches 80% in the 210 mm recirculation pipe just above pressure relief valve. Normal dose. 1 tonne CO2 1000 tonnesl of wheat. Bin sealing completed by filling pressure relief valve, and closing slide valve on gas inlet. Recirculation fan and piping connected to gas inlet, 0.4 Kw, 75 mm above suction fan achieving 0.1 atmosphere changes per day (10% of internal volume). The purge/recirculation piping must be placed as near to bin apex as possible. Grain at least 1 m below this point to obtain even distribution in cone. Elbow at roof/walls join 0.5 m flexible PVC (200 mm drain) to allow some movement.

SOURCE: Wilson, A.D., Buchanan, S.A., and Sharpe, A. G. (1983), "Use and recirculation details of CO2 atmospheres" in the Int. Symp. on pract. Aspects of CA and Fum. in Grain Storages, April, 1983, Perth, Australia.

Practical aspects to CO_2 application to vertical bins:

The welded steel Ascom Type 1900 tonne capacity bins are typical of those used by Bulk Handling Authorities in Australia. These are 18.2 m high (14.8 m height to eaves, 3.4 m height of cone), 13.9 m in diameter, with a total internal volume of 2418 m. Critical areas for sealing are:

- **Wall to floor joint - storages are flat bottomed on a concrete base with a central gravity feed opening in foof apex and central outlet to an underground conveyor system at the base. This area is subject to leakage due to differential expansion and contraction of concrete and steel.**
- **Doors: door openings are lined with a 40 mm width by 8 mm thickness of silicone rubber plus the application of a sealing membrane to the external perimeter (i.e., Envelon or mastic).**
- **Walls: porous sections (slag holes, bolts, etc) sealed with 2-3 coats of a sealing membrane.**
- **Roof hatch: Sealed, hinged, disc batch operated under pressure maintaining contact of rubberto-rubber seals has been shown effective. The peripheral areas require an additional mastic type seal.**

Details of purging/recirculation are found in Fig. 2. Problems of moisture migration has been discovered in some bins that had an initial grain moisture content greater than 12%, resulting in some mould growth and 'bin scalding'. To overcome this, overfilling the bins must be avoided (1 m below roof apex). Grain turning should be performed before the CO_2 purge to

redistribute any hotspots.

4. PRACTICAL ASPECTS OF CO2 APPLICATION IN HORIZONTAL STORAGES

Most conventional horizontal storages or warehouses have not been designed for the application of the CAST technique nor the efficient use of conventional grain fumigants. Some engineering modifications, as described by Ellis (1983), need to be incorporated in bulk handling facilities to accommodate this form of pest control. These modifications include;

1. Change Ventilator Design to Allow for Sealing. Existing storages normally ventilated through a number of convection type roof ventilators require the design to be modified to allow for the addition of a sealing support plate, sprayed with a sealing material. These may be removed at a later date if required.

2. Change Entry into Storage from C1 Conveyor. The overhead belt conveyor from the elevator generally through the wall into a penthouse from which the grain flow is either split or distributed on to overhead conveyors. Therefore the conveyor is elevated above the roof line and spouting (closed and sealed by a manually operated slide plate) passes through the roof to the distributing belts. Access by personnel is through a lockable, sealable man-hatch.

3. Eliminate Bird Netting by Using Sealing Plates. Ventilation has sometimes been achieved under the eaves, with nylon netting to prevent the entry of birds. This is replaced with inclined galvanised sheeting which is sealed when required.

4. Modified Sealing of Main Doors. The main doors of the storage are used for access of front end loaders during outloading. A sealing membrane is required on the hinge line. The doors are also fitted with a 'last man out hatch' fisted with rubber seal flanges so that sealing can be fully carried out from the inside, after which the operator crawls through the manhole hatch and bolts it into position.

5. Install D.l.P. Lighting. Sufficient lighting with natural light normally enters through the eaves, doors, ventilators and transluscent sheeting. In sealed storages it is necessary to install artificial D.l.P. (Dust Ignition Proof) lighting along the conveyor gallery (Light Specification:- D.l.P. HAZLUX No. 3.400 WHigh Pressure Sodium DS25B).

6. Add Girts to Allow Installation of Fans. Air convection within is a sealed storage minimised, and the use of reversible fans in each of the gable ends of the storage disperses respirable dust held in suspension.

7. Add Purlins for Strength on Roof Sheeting.

8. Eliminate any Transluscent Sheeting. The heat input is found to be high with transluscent sheeting in existing storages. When the storage is sealed, these are sprayed over with a clear sealing material, or in new storages, simply eliminated.

9. Colourbond Roofing and Access. White 'colour bond' roofing material can be selected, to reduce solar radiation. Air movement due to the daily temperature changes is therefore

considerably less.

It must be emphasized that storages which are capable of controlled atmosphere disinfestation (either by modifying existing horizontal storages, or incorporating the required design criteria in new facilities) are less than desirable unless all other functions of a modern storage can be performed without breaking the seal. O'Neil (1983) has described the low cost (less than 50% of the cost per tonne of more conventional vertical storages) "total concept" 60,000 tonne capacity horizontal storage situated at Moura, Queensland, Australia. The storage has been built at a cost of $ Aus. 3 million i.e. $ Aus. 50. Tonne^{-1} storage capacity.

The provision of integrating the functions of fumigation unloading/outloading and aeration as a simultaneous operation is the first of its kind in horizontal storages.

The containment of modified atmospheres, such as nitrogen or carbon dioxide, appears easier than with fumigants such as phosphine, where some problems in theplacement and retreival of fumigant strips, pellets or other phosphine generating formulations is anticipated.

Although not fully commissioned, it is envisaged that by the end of 1983 most of the following difficulties will be overcome:

1. **In loading: from transfer shute to ridge conveyor.**
2. **Sampling/inspection/detection of infestations.**
3. **Monitoring of temperature, moisture levels and density and distribution.**

4. **Remote control of reclaim conveyors.**
5. **Design of gaslight valves for underground reclaim conveyor.**
6. **Positive fresh air pressure in tunnel to allow maintenance operations.**
7. **Fresh air venting of outloading elevator shaft.**
8. **Purging gas from outloading grain stream and prevention of excessive gas escaping from the storage.**
9. **Monitoring equipment for 8.**
10. **Aeration systems.**

CO_2 Application in Transit

The control of insects in containerized transport using methods involving CO2 disinfestation have been demonstrated by Banks and Sharpe (1979). Nineteen tonnes of bagged wheat loaded in a Graaf container (total internal volume of 21.2 m) which had a leak rate of 6 x 10-3m3.sec-'of air (at 250 Pa excess pressure) was dosed with 30 kg of dry ice directly onto the wheat stack, and about 31 kg of additional dry ice was kept in an insulating box which provided a release rate of 3 kg of gaseous CO2 per day.

The concentration of CO2 after 240.7 hrs was approximately 49% measured manually by a Riken 18 interferometer and remained between 42-52% at all sampling points over a period of 10 days. The load initially had a light infestation of R. dominica and C. pusillus, and hidden infestations of C. pusilius, E. cautella, J. castanceum, but mainly R. dominica. Test cages containing multiple resistant S. oryzae were also added to this natural infestation, none of

which survived the treatment after 252 hours (10.5 days) exposure. It was viewed that the insulating box of dry ice may not be required, if headspace temperature fluctuations were minimized by stowing the container below deck.

5. AIR-TIGHT OR HERMETIC SYSTEMS

The principles that apply to the control of insects in dry grain also hold for high moisture grain (i.e. grain of a moisture content over about 15%), but here it is the micro-organisms, mostly fungi, that create the oxygen-free conditions. Most moulds need oxygen for their growth, and die, or at least become inactive, in its absence. Once oxygen-free conditions have been established, however, certain other micro-organisms, known as anaerobes, can grow. These are mainly yeasts and bacteria. The latter need a very high humidity for their growth, and flurish best at grain moisture contents over 22%. Their respiration, as described earlier, involves incomplete breakdown of the carbohydrates of the grain, and results in the production of alcohol and other volatile substances. These impart a sour-sweet or "beery" taint to the grain, which is not completely removed by subsequent airing or drying. The grain is therefore normally unacceptable for human consumption, although suitable for animal feed. At high moisture contents (over 22%) or with prolonged storage, the gluten of the grain is affected, so it cannot be used for breadmaking. The germination capacity is also reduced, rendering the grain unsuitable for seed, or for malting.

Successful hermetic storage requires the creation and maintenance of low-oxygen tensions in which most moulds are unable to grow even in equilibrium relative humidities above 70% ERH

(approximately 14% me). This would enable growth in open storage systems. Many storage fungi are microaerophilic but their subsequent growth is much impaired in low oxygen tensions (which is the limiting factor), while the increase in carbon dioxide concentrations may also have a slight additive effect.

If oxygen entry is restricted to a minimum, and the internal oxygen concentration remains somewhere between 0.5 -1.0% O2 (most readily obtainable O2 levels in commercial airtight stores rather than 2.0% O2) then the grain remains bright and free from visible moulding.

The field fungi are eliminated at moistures above 20%, correlated with the depletion of O2 and accumulation of CO2, while the numbers of bacteria and yeasts usually increase, especially the yeasts of the genera Hansenula and Candida at O2/CO2 levels of 1 - 2% and 15 - 40%. At higher O2 levels found in partially empty silos, mesophilic fungi such as species of Penicillium and Aspergillus, and mesophilic fungi with higher temperature optima and true thermophils such as Mucor may be found associated with developing hotspots. Other thermophilic organisms such as actinomycetes can also be present causing bridging and spoilage of the grain.

Several of these organisms are capable of causing infections, inducing allergic responses or respiratory irritation from people handling mouldy grain, and mycotoxicoses in both man and domestic animals consuming infected or mouldy grain.

Because of the more stringent requirements for gastightness with high moisture grain, many of

the structures suitable for airtight storage of dry grain are therefore unsuitable. Specially constructed metal silos are sometimes galvanized or treated with epoxy-resins, or more commonly coated in a dark blue or green vitreous enamel finish to protect the steel from corrosion manifested by silage acids (lactic, acetic, propionic). To cope with pressure changes, they are fitted with pressure release valves (a few centimeters of water) in the top, while the American vitreousenamelled or "glass-lined" silos are provide with a breater bag in the beadspace to compensate for temperature induced internal pressure changes with respect to atmospheric pressure, which prevents free air interchange below the pressure setting of the safety valve.

Smaller flexible bag silos made of butyl rubber or PVC supported in metal cages of approximately 20-40 tonnes, or smaller heavy gauge polythene sacks (50 kg) are also widely used. These are easily handled and transportable, but are also easily damaged by rodents, birds, and machinery.

The Waller bin developed from European grain storage practices was simply modified by replacing the hessian or paper liner with plastic or rubber bags. The sealing becomes increasingly difficult as the diameter increases. A desirable safety feature is the inclusion of a safety door which facilitates cleaning and ground level ventillation, to reduce CO2 levels to normal atmospheric conditions. The liner cannot be hung on the mesh cylinder, so the liner is inflated by using a fan (rated at 140 m.min^{-1} at 100 mm W.G. which is equivalent to 5000 c.f. m. at 4 in. W.G.) during loading. When filling operations have ceased, the silo is simply allowed

to collapse onto the grain surface. This also occurs during emptying, thus reducing the headspace volume.

With undamaged liners, complete disinfestation can be rapidly achieved with an initial high population density. insect penetration of the butyl rubber sheeting has also been reported, but O'Dowd (1971) found Callosobruchus maculatus capable of penetrating polythene but not butyl rubber. Butyl rubber has limited ability to withstand U.V. radiation and high ozone concentrations, while the natural black colour and pigmented polythene is responsible for extreme temperature fluctuation in an unshaded silo resulting in moisture migration to the outer grain layers, particularly on walls facing east/west. Shading by providing a roof is necessary, since painting the walls white did not reduce the internal temperatures to acceptable levels.

Observations were carried out over a 10-month period in a flexible silo consisting of a butylrubber/EPDM welded liner inside a circular supporting weldmesh wall designed to provide an airtight seal with a capacity of 900 tonnes of wheat by Navarro (1976). Development of insect populations which become evident two months after the beginning of storage was arrested due to the airtightness, and gas concentrations unfavorable to further insect development were maintained during three stoage seasons. The insect population remained low until the end of the storage period and in the order of decreasing abundance, were Cryptolestes sp., Oryzaephilus surinamensis L., Tribolium castaneum (Herbs"), Rhyzopertha dominica F., and Sitophilus oryzae L. Grain was preserved at a satisfactory guality throughout the storage seasons. Insect accumulation occurred at the silo apex and was

accentuated by migration from within the bulk. This was attributed to interrelated effects of oxygen tension within this region which permitted survival through O2 diffusion; moisture accumulation in this region attracting grain insects and providing a most favourable environment; and temperature gradients where temperatures within the centre of the bulk varied between 33-40C, a condition which is less favourable for those insects listed and therefore promoted their outwards and upwards movement. It was concluded from these tests, that moisture accumulation at the silo apex and damage caused to the sheeting will ultimately result in some deterioration.

Both of these systems, ranging in sophistication and capacity, are suitable however to either dry grain or high moisture air-tight storage, depending on the final grain requirement (animal feed or human consumption).

Various systems for dry drain grain airtight storage hve been documented. These include traditional methods such as dried fruit cases of cucurbits, commonly known as gourds, used in the tropics for storage of small quantities of grain often intended for seed purposes, but are not sufficiently gaslight unless a surface treatment is applied and attention is given to adequately seal the stopper.

Metal bins display great potential for use as airtight containers, provided that shading or the application of external reflectant treatments offset or minimize temperature induced moisture migration by diurnal fluctuations.

The semi-underground bins developed by J. E. Waller in Cyprus and Kenya have been used for longterm storage and some were initially sealed for over 3 years with excellent results. Moisture migration was also a problem, but moulding was reduced by either placing trays on top of the grain surface below the mancover, or lining the metal covers with polystyrene sheets to prevent translocated water vapour from coming into contact with the cold metal.

Maize has been stored for three years at 12% mc and wheat for 18-24 months at 13% mc with low levels of deterioration mainly related to rise in FFA's (wheat 20-30 to about 40, maize 15-20 to about 50 based on mg KOH per Kg of grain) and loss in seed viability. After 3 years, moisture was within a 60-75% ERH range.

The airtight underground storages (Aus) in Argentina have been used for 2-3 years with loss amounting to 0.5%. Many of the 2.5 million capacity Aus storages were not built gaslight (because of the change to permanent vaulted roofs made of hollow blocks covered with reinforced concrete, rater than flexible roofs that allowed some movement) but gave better storage conditions than above ground silos through superior thermal insulation. Repeated fumigation was however still necessary to prevent insect infestation.

Grain loaded at 12% mc and at 20C, resulted in altered atmospheres of 3-4% O2 and 10% CO2. If loaded with grain at higher temperatures, moisture migration became a problem, and construction of Aus systems have been limited to wheat producing zones where grain is harvested with a low moisture content.

The Grain Elevators Board of N.S.W., Australia, has developed a system of bunker-type storage which was originally intended as temporary storage facilities to offset lack of storage capacity during bumper harvests. It has evolved from an original C.S.I.R.O. concent of underground storage which has been practiced since time immemorial,but has since been greatly modified. Further trials by CSIRO involved the provision of an above-ground bunker storage covered by earth or sand. This involves building earthen banks and lining the base with PVC sheeting to prevent moisture migration and seepage. During the 1979/80 harvest, about 1/4 million tonnes of wheat was stored in Victoria, Australia in this manner. Bunker walls measure 13 meters at the base, rise 2.5 meters and are 6 meters wide at the top. Each bunker is about 30 meters wide and built to a length which determines its capacity. When full another earth wall was built to enclose the bunker and the surface of the wheat was covered with another polythene sheet and about 1/2 meter of earth.

Excellent results have been obtained with phosphine fumigation equivalent to a dosage rate of approximately 0.2 tablets/tonne (ca one pellet). This is due to the high level of sealing obtained combined with prolonged exposure periods.

The earth covering presented appreciable operational problems, and with the availability of high grade PVC sheeting, the earth covering has since been omitted.

One major disadvantage with this system of storage is the disruption to inloading and outloading operations caused by rain. Concrete banks (or the movable 'A' frame type of temporary storage of wooden supports covered with G.I. sheet as in Western Australia) have

now replaced earthen banks which are water-proofed by a bitumen surface. A special machine has been developed which is capable of inloading at 250 tonnes. fur-' and outloading at 200 tonnes.hr-1 or better.

Problems that are associated with bunker storage, although essentially overcome, are,

1. **Admixture of some earth to the grain.**
2. **Admixture of stones and other foreign matter.**
3. **Damage to PVC sheeting from various causes, including hail.**
4. **Adhesive failure allowing PVC sheets to come apart.**
5. **Inability to load in high winds.**
6. **Vandalism of storages not protected by fences.**
7. **Rain during inloading and outloading.**

Problems (1) and (2) are associated with earthen banks, while the provision of concrete banks and butimenising the storage surface completely eliminates these. Problems of hail damage still remain, but (4) has been obviated by sewing rather than using a sealant, but a suitable sealant over the stitching must be provided to ensure gas tightness. Item (5) has been minimized by the "Lobstar" machine, which can be operated in moderate winds, depending on wind direction, Item (6) is removed by supplying "man-proof" fencing.

PVC bunker-type storage of 50,000 tonne capacity costs approximately $6.00 per tonne to utilize, if used annually, but if used as an emergency storage 3 years out of 10, the costs

increase to $13.20 per tonne. The equivalent costs of steel bins of 10,000 tonne capacity is $11.65 and $38.37 respectively (Druce, 1982).

Yates and Sticka (1983) claim that bunker storage has now reached a stage where

"It is quite a satisfactory form of permanent storage where only minimal capital requirements are warranted, where capital is simply not available for the construction of storages, and where a storage facility must be provided at very short notice.

The PVC-covered bulk grain storage system is shown to be capable of directly responding to the two major factors:

- **The need to minimise handling and storage costs, and**
- **The need to control insect infestation in grain without the use of chemical protectants and, possibly, without the use of fumigants.**

These storages are now routinely being constructed to 50,000 tonne capacity. A major advantage of the PVC bunkertype storage is that it can be fumigated very satisfactorily by the use of phosphine. This is a very cheap form of fumigation and experience to date indicates that it is completely effective at very low rates of application as the storages are sufficiently gas-tight."

6. SAFETY ASPECTS OF CAST

Although nitrogen is non-toxic, atmospheres containing less than 14% O2 or atmospheres containing more than 5% CO2 maybe dangerous for human life. The rapidity of onset of symptoms may vary with individuals and even at 6.5% O2, may take a few minutes. Death will ensue if an unconscious subject remains in such an atmosphere, but if removed quickly and given air, recovery is complete. There are no reliable warning symptoms concerning nitrogen asphyxia. Carbon dioxide enriched atmospheres strongly stimulate breathing, and becomes uncomfortable in atmospheres containing 3% CO2. Death occurs in 20-30 minutes on exposure to CO2, but if an affected subject is removed to fresh air even after a brief exposure to very high CO2 concentrations (e.g.70%) no irreversible physiological damage results.

No space under controlled atmosphere should be entered without protective respiration devices, and then only in emergencios. Fully self contained or compressed air breating apparatus should be permanently on hand. Gas masks provide no protection against low O2 or high CO2 atmospheres. A storage that has been under CAST should be tested for oxygen and where relevant, CO2 levels should also be checked. If CO2 levels are less than 0.5% and oxygen levels are greater than 18%, the area can be declared safe for entry.

7. ADVANTAGES OF CONTROLLED ATMOSPHERE STORAGE

CAST (or modified atmosphere or inert atmosphere storage) has some distinct advantages over practices of insect control using residual grain protectants.

- **It leaves no chemical residues on the grain.**

- **It can act as a guality preservation system.**
- **It can provide long-term protection as well as disinfestation.**
- **Since it makes use of chemicals that naturally occur in the atmosphere (although at varying concentrations), they do not possess high intrinsic toxicity and are therefore safer to use than many fumigants and grain protectants.**
- **A sealed storage which is a prerequisite for success of the system prevents pilfereage.**

Some disadvantages are that the process of disinfestation is slow (taking up to 2 weeks even under tropical conditions), sampling during CAST is difficult and the development of a suitable remote sampling method to detect infestations if it occurs is required. The commodity is immediately liable to reinfestation after outloading from CAST, so a system of transportation of the insecticide free grain from storage to its ultimate destination which does not allow the grain to become sensibly infested en route also needs to be developed.

Rather than aiming for the present 'one-shot' method of CO2 introduction (- 70% CO2 decaying to less than 35% in 10 days at temperatures greater than 25C) it may be more efficient to introduce the intermediate CO2 system (achieving > 40% CO2 in approximately 30 days or more).

CAST systems are now at a stage in Australia where they can be regarded as one of the strategies commercially available for control of insect infestations. Their adoption is related to cost benefits derived from the system, and because of increasing costs and lowered efficacy of existing systems, CAST is already becoming competitive in many situations where there is a

requirement for treatments that do not leave any chemical residues.

It also appears highly unlikely that a strain of stored products insects will evolve which is capable of completing its life cycle in micro aerophilic or oxygen free atmospheres, since they are essentially obligate aerobes. Since the possibility however remote, does exist, it is advisable to utilize controlled atmospheres as efficiently as possible to avoid undue selection of more tolerant strains.

8. APPLICATION OF THE SYSTEM IN DEVELOPING COUNTRIES

Annis (1982) has described comparative trials with bagged stacks of milled rice. With the Rice growers Cooperative Mills in Griffith, New South Wales, Australia, and Badan Urusan Logistik or BULOG, the Indonesian National Logistic Agency.

For large structures that are sufficiently sealed, a single introduction of CO2 will ensure lethal concentrations be maintained long enough to disinfest the commodity. However, with small enclosures, the same level of sealing is not practically attainable and gas loss through leakage must be replaced.

Normal disinfestation techniques with bagged commodities is to fumigate with methyl bromide or phosphine under plastic sheets secured to the floor by placing chains or sandfilled snakes (roughly 2 kg.m-2) on the sheet margins. Once the correct exposure has been achieved, the fumigation sheet is normally removed, which allows the commodity to be liable to

reinfestation by resident insects within the warehouse or godown, and the requirement for repeated fumigation on a regular basis. This becomes an expensive and inefficient exercise, and in the case of methyl bromide, the possibility of objectionable levels of bromide residues being reached. It was viewed that CO2 may be an attractive and viable alternative to fumigation, especially in Jakarta in tropical conditions with a naturally high insect infestation pressure. The treatment involved three main phases.

1) Covering and sealing the stack and testing the seal.

In Jakarta, four stacks each of 180 tonnes of hagged milled rice was manually stacked on timber dunnage, and enclosed by Wavelock 41 nylon reinforced PVC sheeting built on a 0.76 mm PVC ground sheet. The outer edge of each enclosure was bonded with an urethane sealant to the ground sheet. Pressure testing was carried out after searching for obvious leaks, by connecting a small vacuum cleaner to the gas inlet port and creating a negative differential pressure of eve 1500 Pa (6 in. water gauge) with respect to atmospheric pressure. Pressure change was recorded (-ve 500 to eve 250 Pa) over time.

2) Adding the CO2

Carbon dioxide was added as snow from inverted 30 kg cylinders of food grade gas in a specially constructed frame and piped through a 17 mm copper pipe between the timber layers of dunnage out of contact with the bags. The snow sublimes and CO2 was added until the gas leaving the exit vent (200 mm x 200 mm) in the top of the stack reached 60% CO2 after

which the enclosure was completely sealed. Gas concentration were measured by a Riken 18 interferometer daily for first 10 days, then subsequently by Evrite gas absorption apparatus twice a week.

3) Prolonged insect proof storage

This trial demonstrated the potential of CO2 using one 30 kg cylinder CO2 per 10 tonnes of rice, or between 2.2 - 2.9 kg.tonne-1 if using bulk gas, for up to 5 months protection in tropical conditions. The enclosures remaining in situ offered considerable protection against reinfestation from within the warehouse. At this stage the milled rice had not deteriorated detectably, and insect control and quality maintenance were satisfactory as compared to normal operational experiences in Indonesia. A stack size of 200 tonnes can be adequately sealed to permit single CO2 introduction, while the sealing achieved with 100 tonne stacks in Greffith was only just effective (see Table 8).

A second trial was began in January, 1982, by BULOG at their National Food Technology and Research Centre at Tambun, West Java (Sukardi and Martono, 1983). It was designed to compare an initial disinfestation using phosphine where the fumigation sheet was retained to provide an insect barrier, and disinfestation using carbon dioxide.

At this trial, carbon dioxide was evaluated over a period of 16 months using 8 x 200 tonne stacks of locally produced milled rice and 8 x 200 tonne stacks of imported milled rice, where no protective insecticidal films were applied. The same system of stacking was used for the

phosphine treatment, and 4 stacks of both CO2 and phosphine were opened for inspection every 4 months.

In trial 2, an application of 1.7 - 2.9 kg CO2 per tonne of rice was used to achieve a 62 - 81.8% CO2 concentration leaving the exist vent in the top of the stacks. The CO2 concentration naturally decayed to 17.4 - 22.9% after 4 months and to 1.2 6.2% after 8 months storage.

Table 8. Comparison of trials of bagged milled rice under CO2 in Australia and Jakarta.

	Griffith, N.S.W.	**Tambun, W. Java**
Size of bagged stacks	100 tonnes	4 x 200 tonnes
Pressure decay (500- 250 Pa)	5 mins.	10 - 17 mins.
Purge	10 cylinders	13 - 17 cylinders
True CO2 application	2.9 kg. tonne^{-1}	2.2 - 2.6 kg. tonne^{-1}
	Caged cultures of all	Initial natural infestation of 15 live insects.kg^{-1}, composed of R. dominica, T. castaneum, and smaller numbers of S. oryzae, C.

Bioassay insects	developental moryzae stages of S. througtrout the stack.	ferrugineus, C pusillus, O. surina mensis, C. pilosellus, larvae of Ephestia sp. and Liposcelis entomo philus abundant on the floor dunnage and lower stack.
Results	No survival.	One stack opened on, 28, 58, 94 and 133 days after treatment. No live insect found except L. entomophilus at 58 and 1 live S. oryzae at 133 days, but may have originated externally and not survived the treatment.

Observations at 4.8 and 12 months revealed no live insects at the surface and on the floor, while the rice was in good condition with no appreciable signs of mould. When the four phosphine treated stacks were broken down and inspected after 4 months, mould damage was apparent, and moisture condensation evident on the internal surfaces of the enclosures. To stabilize these conditions in phosphine treated stacks (equalize temperature and moisture

contents) it has been necessary to adapt a system of Low Volume Exhaust Ventillation.

Several conclusions can be drawn from this interesting study.

- **The use of CO2 at 2.1 - 2.4 kg.tonne-1 is effective in disinfesting bagged stacks of milled rice under plastic sheets for several months under tropical conditions without adverse effects on quality, so long as the initial moisture content is less than 14% mc.**
- **By initially fumigating with phosphine and maintaining the bagged stacks in sealed conditions, the period of storage must not exceed more than 4 months. Beyond this point, forced ventillation is required with fresh air from outside the fumigation sheets to remove excessive heat and moisture from localized regions within the stacks.**
- **Pest control in both carbon dioxide and phosphine treated stacks can be achieved without applying residual contact insecticides to the bag surfaces, therefore lessening the degree of reliance in this form of protection, and retarding the onset of resistance.**

Annis and J. van Someren Greve (1983) described trials in Lae, Papua New Guinea, which established that carbon dioxide can be successfully used for preserving the quality of green coffee beans in small stacks of 10 tonnes under plastic sheets. An average CO2 concentration of > 95% CO2 was obtained by adding 4.5 kg CO2.tonne-1. Insect control under these conditions is again comprised of .

- **elimination phase: disinfestation. It is importent to obtain a perfect fumigation to prevent respiration in the sealed environment.**

- **prevention of reinfestation phase, by leaving the sheet intact. If using PVC sheeting, chemical bonding to the ground sheet will give an adequate seal, while polythene sheeting needs to be heat sealed.**

Normally, deterioration of coffee beans stored in open stacks is rapid (3 weeks), while in the sealed enclosures treated with CO2, quality can be maintained for 6 months or more.

Calverley (1983) stated that grain quality preservation is not simply a function of storage but affected dramatically by environmental conditions operating in the region where grain is stored such that the moisture content can be cited as the "single greatest factor of grain loss throughout the world." This cannot be overemphasized where national grain agencies in the humid tropics receive a large volume of grain on procurement that has such diverse moisture content and grain quality as compared to Australian conditions where large volumes on receival are homogenous with respect to moisture (< 12% for wheat).

Apart from extending simple airtight storage, CAST is unlikely to have any application to farm storage systems because the benefits to be gained are insufficient to justify the very considerable effort needed to establish a system requiring such a level of technology."

More relevant and low cost control measures in this form of grain storage are necessary, while at the national level, more capital intensive but cost effective control measures could be implemented.

Government storage functions cover

- **storage of operational stocks to meet seasonal demand and effect price stabilization.**
- **maintain carryover or buffer stocks between seasons.**
- **establish and maintain strategic or long-term reserves as an insurance against crop failures and other unforeseen calamities.**

Storage periods are often extended beyond 12 months, with management relying on insecticides, fumigants and often to a lesser degree, good storage hygiene to effect control. Control failures are common and losses in these types of storage are oftentimes extremely high.

Through research programmes initiated by the Australian Centre for International Agricultural Research (ACIAR) in Southeast Asia, the modifications of the "Australian experience" in controlled atmospheres necessary for adaptation in this region has be or investigated. With an appropriate gas supply by generation of controlled atmospheres on site, the technology of CAST can be adapted under high moisture content regimes.

Whether we are investigating modified atmospheres using CO2 or N2 (in which there is an abundant supply in the atmosphere) or looking towards more efficacious use of phosphine or potentiating fumigation (either CH3Br of PH3 in CO2 enriched atmospheres), the need for a high standard of gastightness in whatever structure or enclosure is being used through efficient sealing methods becomes apparent.

The problems of grain quality preservation exist in those countries constrained by the environment, i.e., in the humid tropics, and not in large exporting countries. It becomes imperative that systems involving controlled atmosphere storage, and other related pest control techniques, should focus directly at problems of high moisture grain storage in developing nations.

In the humid tropics, the use of natural airtight or hermetic storage should be restricted to grain of moisture content not exceeding 13-14% m.c. Most of the grain is destined for human utilization, which therefore makes the taints that develop at high moistures unacceptable. Seed germination is also affected at warm temperatures in relatively short storage durations under sealed conditions. The deterioration of damp grain is extremely rapid in warm environments (< 24 hours during the wet season harvest).

The degree of gastightness required to prevent mould growth is much more critical than that required to control insects in dry grain. Sufficient oxygen maybe present to allow the growht and development of the harmful organisms such as the toxin producing Aspergillus flavus oryzae group of storage fungi. Even if these are controlled by subsequent air-tight conditions, aflatoxins that they produce would remain, and invasion by moulds after outloading from hermetic storage would be rapid.

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INSECTS AND MITES:

- **Difficult recognition of developing infestations, especially in large bins.**
- **Losses of the stored grains, due to the biological activity of all stages of insects, before the recognition of infestations, cannot be avoided.**
- **Formation of off-odours and residues due to insects.**
- **Temperature and moisture increase due to the formation of hot spots.**
- **Treatments using toxic chemicals cause harmful residues in the products.**
- **Fumigations are difficult to carry out in large storage facilities.**
- **Fumigations require specialized personnel. In the event of leakage, fumigants are dangerous for the outside environment.**
- **It may be necessary to transfer the whole content of the bins to allow efficient pest control measures.**
- **The effect of the treatments is limited in time and reinfestations are possible.**
- **Chemical insecticide-resistant strains may build up.**
- **In the future further pest control chemicals will be prohibited by Health Authorities.**
- **Explosion and fire hazards exist for many chemical fumigants.**

RODENTS AND BIRDS:

- **Losses of the stored grains before recognition of the attack.**
- **Difficult chemical and mechanical control. Necessity of transfers and cleaning of damaged products.**

MOULDS AND YEASTS:

- **At critical moisture content, frequent shifting of grain is necessary in order to avoid moulding.**
- **Drying may be necessary, shortened storability in time.**
- **Formation of off-odours. Death of caryopses.**
- **Contamination by mycotoxins.**
- **Hot spots, increase in moisture and temperature of the bulk.**

OXYDATION REACTIONS:

- **Take place without control, especially at critical mositure contents of the products. Formation of off-odours.**
- **Degeneration of fats.**
- **Heating, autocombustion may occur.**

VIABILITY OF CARYOPSES:

- **Free respiration causes a progressive redution of the germinative energy and capacity.**
- **Frequent transfers, aeration or refrigeration are required.**

TECHNOLOGICAL PROPERTIES:

- **Progressive deterioration especially at critical temperatures and moisture contents.**

- **Necessity of ventilation.**

NITROGEN ENRICHED ATMOSPHERES

INSECTS AND MITES:

- **Full kill of insects, at all stages of their development, by relatively short treatments.**
- **Any loss is excluded, since the biological activity of insects at all stages of their development is inhibited.**
- **No formation of off-odours.**
- **No formation of Hot spots or variation in the temperature and moisture content of the bulk.**
- **No residue in the stored products.**
- **Easy disinfestation due to total penetration of the inert gas throughout the bulk.**
- **Simplicity and automatic requlation of most of the operations.**
- **No danger from accidetal leakage.**
- **Shifting of the stored cereals is not required. By maintaining an inert interstitial atmosphere, reinfestations are excluded.**
- **Insect resistance to anoxia is excluded.**
- **Health hazards due to the inert gas treatment are excluded.**
- **Inert gas interstitial atmospohere excludes explosions an fire outbursts.**

RODENTS AND BIRDS:

- **Infestations excluded.**
- **No loss of the stored grains.**
- **No control measure needed.**

MOULDS AND YEASTS:

- **Their proliferation is significantly retarded, and completely inhibited in convenient conditions.**
- **Grains can be preserved for significantly longer preiods at critical and high moisture contents.**
- **Formation of off-odours excluded.**
- **Viabllity of caryopses preserved.**
- **Mycotoxin contamination during storage excluded.**
- **Increase in moisture and temperature of the bulk excluded.**

OXIDATION REACTIONS:

- **Their evolution may be checked and stopped at the desired stage.**
- **Formation of off-odours excluded.**
- **Protection of fats.**
- **Explosions and left-ignition are excluded.**

VIABILITY OF CARYOPSES:

- **The germinative energy and capacity are maintained for long periods of time.**
- **Possibility to store seeds and cereals for malt production in large storage structures.**

TECHNOLOGICAL PROPERTIES:

- **Satisfactory preservation for long periods without any special measure.**
- **No ventilation necessary.**

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Section 11 - Biological control methods

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Biological control of stored grain insect pests

INTRODUCTION:

Biological control maybe defined as,

"a method of reducing or eliminating damage inflicted by a pest by means of a biological agent, traditionally a parasite or a predator, or by the introduction of a disease where the causal organism is specific in action."

Biological control may also be defined in a much broader sense to include,

"the manipulation of other biotic or demographic facets on the system (s) of a specific pest or pest species complex (since rarely do stored grain insects exist as a single species in an infestation) such that the reproductive processes (governing the growth of populations and their consequent abundance) and physiological processes (governing behavioural and developmental aspects) are impaired."

In combination with other applied control measures, the overall level of effectiveness (i.e. by reducing the dosages or frequency of application of the more traditional insecticides and grain fumigants) will therefore be enhanced.

Of recent development is the use of sterilization (induced chemically, or by exposure to a low-level irradiation source) which is effective for pest species where the female only mates once during its life-time (Sterile-Male Release Technique, or SMRT) or in noncontiguous areas such as islands geographically isolated from migrating reproductives. Another development is the use fo sex attractants or lures (pheromones) which can attract searching male insects to an insecticidal source, or as a disruptive or confusing agent which completely swamp the naturally produced pheromone of the female, consequently inhibiting mating. Both male and female pheromones play an integral role in the mating behaviour of stored products Phycitid moths, while Coleopteran pests such as Trogoderma inclusum and Attagenus megatoma (among others) have been shown to emit short-range pheromones that initiate the mating process. The major developmental problem for their adaptation as commercially effective control agents, is that these highly specific, volatile substances exist as complexes and not single entities, which makes their chemical elucidation and manufacture expensive.

Another "specialized" form of biological control is the use of Insect Growth Regulators (IGR's) which have been demonstrated to induce morphological aberrations; induce ovicidal effects, sterilization, and parental mortality (under some circumstances); impede metamorphosis (preventing adult emergence) and; prolongation of the immature larval stage, resulting in intermediate forms that are not reproductively viable.

Genetic manipulation (mainly by irradiation techniques) aims to displace field populations with new "artificially synthesized" populations which are more easily controlled by conventional means, or lack the characteristics which cause them to be initially regarded as

economic pests.

The sequence in which genes are normally present on chromosomes can be rearranged by a series of irradiations where a new strain is developed which has full fecundity, but when mated with the existing field strain, infertile hybrids result, and the irradiated strain becomes dominant. If this strain which has been developed is highly susceptible to current control measures (such as insecticides), then the benefits become obvious. Other possible advantages are the introduction of a lethal gene i.e., susceptability to cold where a pest may be multi-voltine (several generations per year) but one generation must survive in defined winter periods, or the creation of a genotype which has the ability to be a vector for a disease pathogen removed. This last approach is seen to be advantageous for the control of tsetse flies, mosquitoes, etc., which are of extreme medical and veterinary importance and have a limited number of chromosomes, thus making manipulation less difficult. This approach has some definite attractions for stored products insect control but has not been researched in depth, possible due to the difficulty of encorporating the new genotype into the existing field population without rejection or elimination. I would also consider the use of plant extracts which have been shown to possess insecticidal properties as a "transient" form of biological control. Man has utilized plants or plant parts for a multitude of different uses since the early millenia and have become the basis of many synthetic organic processes, due to higher costs of production using the natural derivatives. The commonest plant extracts which are still widely used are natural pyrethrum extract (a mixture of pyrethrin esters, Pyrethrim I and ll and Cinerin I and ll occurring in various proportions), from Chrysanthemum cinerariaefolium. The extract has replaced the earlier use of dried flower heads (usually available as concentrated

extracts of 25%; acute oral LD50 (rat) 1500 mg.kg-1) and derris dust, containing rotenone. This is extracted from the roots of the leguminous plants Derris elliptica and Lonchocarpus spp, first isolated in 1895 (acute oral LD50 (rat) is also 1500 mg.kg-1 and therefore has low mammalian toxicity, although irritating to the eyes and mucous membranes; of short persistence, and usually applied for the control of garden pests, ticks, lice and fleas). Many other plant extracts have subsequently been cited for their potential as insect control agents (such as neem powder from Azadirachta indica).

The following sections will cover in depth the more traditional biological control agents, such as hostplant resistance of stored cereal grain to post-harvest insect attack; parasites and predators; and entomopathogens (Bacteria, viruses, fungi, protozoa and nematodes).

Biological suppression of stored product insect pests

Adelaide C. Quiniones

Introduction

Biological suppression of stored-product insect pests is becoming recognized as an acceptable strategy. copple and Mertins (1977), described "biological insect pest suppression" as man's ues of living organisms or their products to reduce populations of pest insects. Topics here will

concentrate on methods of manipulation (used singly or in combination) of parasitoids, predators, pathogens and other living organisms, their environment, or their natural products for the purpose of protecting stored commodities.

In recent years, studies of biotic agents and their products e.g., pheromones, have led to consideration of their potential for the suppression of stored-product insects. This marks a considerable change in thinking. Previously, all organisms were considered unacceptable contaminants of stored products; but now the presence of biological control agents is considered less harmful than residues of chemical pesticides. In addition, insect pests of stored produts are developing resistance, the cost of pesticides and their application is rising rapildly, and there is growing awareness of woild food shortages. All these factors have made it necessary to find new strategies for managing insect pests of stored - products.

Pheromones

Pheromones seem to have the promise for early practical use in manipulating stored product insects, and they have many advantages. They are active in minute quantities and over considerable distances, they are safe, and they are often effective most immediately. They also are highly specific. Pheromones can be used. (a) for surveillance and detection of an infestation and (b) for control of infection, sometime by communication disruption or mass trapping with lures and devices that will attract and kill the insects.

Surveillance and detection of infestation. In one study conducted in Milwaukee, Wisconsin,

the pheromones of Trogoderma and the black carpet beetle, Attagenus megatoma (F), were used either singly or in combination to bait traps, and the traps were then placed in three warehouse and grain elevator locations. The traps treated with pheromones caught significantly more target insects than untreated traps; a previously undetected population of Trogoderma variable B. was discovered; and the seasonal emergence of A. megatoma was observed and charted oven a two-year periold. The primary component of the Trogoderma pheromone, 14 - methyl 8 - hexadecenal is now used routinely by the Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture, in the major U.S. port facilities where large numbers of mostly T. variabile are caught.

In other trapping studies conducted in California military warehouses, the location and efficiency of similar traps received particular attention. Traps near the exterior walls accounted for the most of the captured Trogoderma, and the pheromone - baited traps caught significantly more Trogoderma than unbaited traps. Dichlorvos was the killing agent in these traps. (Burkholder, W. E.).

Wheat germ oil is another material that is an attractant for Trogoderma larvae, and several attractive components of the oil have been identified. As a result, a combination of wheat germ oil, and Trogoderma sex hormone is now being used in the APHIS Trogoderma - trapping program for Khapra beetle detection.

Male lesser grain borers, Rhyzopertha dominica (F.), produce an aggregation pheromone that attracts both sexes. The pheromne is responsible for the characterestic "whitish" color of grain

infested with these borers. When the synthesized pheromone (Williams et al, 1981) was tested in a warehouse in Texas, baited traps were effective in monitoring populations for as long as five weeks. Thus, perforated grain - probe traps baited with this pheromone might provide a means of suppressing populations of the grain borers that develop deep within grain bins.

The pheromones of other grain-infesting beetles and weevils are being isolated, identified, and developed and will probably be available within a few years. Those for Tribolium spp., Sitophilis spp., Stegobium paniceum (L) (drugstore beetle) and Lasioderma serricorne (F) (cigarette beetle) could be especially useful. In addition pheromones are available for monitoring some of the moth species that are of concern in warehouses. For example, several species of stored-product Pyralidae respond to the synthesized sex pheromone (Z, E) - 9, 12-tetradecadien -1 - 01 acetate (Brady et al 1971; Kuwahara et al 1971).

Control of infestation. The use of pheromones for control depends on the efficacy of traps in capturing the attracted populations of placing them to an area where other suppression methods can affect them. Also, mating disruption with pheromones may produce some suppression, although this technique most often has greatest impact when it is used in combination with other techniques.

Manipulation and Suppression of Stored - Product Insects with Pheromones and Entomopathogens

One innonative strategy involves the use of pheromones as lures in devices that contain insect

pathogens, a technique that is particularly successful against insect parts in stored products. Their habitats provide ideal conditions for inducing disease epizootics, especially when the pathogen have desiccation - resistant spores. Even small habitats with localized populations can contain highly concentrated populations of insects that have excellent potential for the creation of epizootics. Of course, a pathogen would not kill the insects immediately. Rather, an infected, spore-ladened insect returns to its natural habitat and infects others of its kind. It is an especially promising method for long-term control of insect pests of stored products when the insects can be exposed to the pathogen by using an effective pheromone baited device.

The combinations of pheromone with pathogens or biotic agents for suppression of dermestid beetles (Coleoptera: Dermestidae) was first proposed by Burkholder. The effect was to suppress population of Trogoderma glabrum Herbst. In this trial, the sex pheromone of the beetles (14 - methyl - 8 hexadecenal) was combined with a protozoon pathogen, Mattesoa trogodermae Canning (Neogregarininda: Ophoryocystidae). Because failure to bring the insect into contact with the pathogen would doom the strategy, the following conditions were established. The adult males used as the test population were situated downwing from the sites where the pheromone plus spores was available; adult males that come to lure were redistributed among emerging females and mated; and dead adults were available as food for offspring.

In this model system, subsequent generations of T. glabrum were substantially suppressed by a single introduction of M. trogodermal spores into high density (32 adults/m) population of adult males. The treated populations increased only fourfold in the first post treatment

generation and fell belwo pre-treatment levels in the second generation. Meanwhile, the control had a 24 - fold increase in the first generation and 100 - fold increase in the second generation. (The low-density treated populations (2 adults/m) and also the low density controls increased 12-fold during the first generation). Results demonstrated that with the dense population, a 48-hours exposure of the pheromone plus spores was sufficient to achieve distribution of effective doses within a radius of 1.25 m around the sites of the treatment and the attracted males attempted copulation with the pheromone source, which increased spore transfer. Spore transfer to the subsequent generations, however, was mainly the result of larvae that ingested either dead, contaminated adults, or larval food the adults had contaminated by contact. Although the maximal distance of the spore transfer was limited by the size of the experimental arenas (2 x 2 m), it is likely that in a natural environment, the pathogen would be dispersed substantially further, since flight by contaminated adults is possible.

Burges and Hurst (1977) noted sudden and spectacular mortality of stage moths (Plodia interpunetella) in maize storage facilities in Kenya when the insects were exposed to bacillus thuringiensis Berliner, although mortality of similar moths exposed in the same way in laboratory jars was only progressive. As a result, they suggested that cadavers of larvae are the most potent source of infective materials because they contain many spores and crystals of B. thuringiensis, adults, frass or eggs and are capable of rapidly killing larvae that feed on them. In fact, when Burges and Hurst spread B. thuringiensis spores and crystals in the surface to the grain in the jars, rather than applying the material to one point source on the surface, significantly more spores and crystals were required to start an epizootic. Therefore, the

presence of infected adult moths in newly harvested and untreated grain could produce epizootic of this pathogen, but epizootics may also arise because infected larvae immigrate into the storage area from adjacent stored grain, residues of food from local farms, transport vehicles, or terminal stores or bags contaminated with freass and insect bodies.

Since the severe mortality of moth larvae caused by B thuringiensis in the Kenya study (Burges & Hurst 1977) was enhanced by high concentrations of spores and crystals in the larval cadavers, it might be possible to mimic nature by providing a high concentration of B. thuringiensis spores and crystals in a sumulated larval cadaver (SLC). Since some laboratory studies indicate the existence of promising attractants or feeding stimulants for insects larvae, these compounds could be combined with the pheromones that attract the adults. Such baits might be formulated with pathogen or small paper clips (to simulate a larval cadaver) or other devices and placed in grain. In this way an epizootic might follow the introduction of a few SLCs, and the initial rapid kill of feeding larvae would provide actual cadavers thereby accelerating the suppression of the insect population.

Also, McGaughey (1976) reported that B. thuringiensis prevented infestations of Indian meal moths, P. interpunctella, and almond moths, Ephestia cautella (Walker), in stored corn and wheat when cat 120 mg of a formulation of B. thuringiensis was applied to 1 kg. of grain. Treatment of the surface layer (to a depth of 100 mm) was more effective than treatments at depths of 33 or 67 mm and as effective as treatment of the entire grain mass. The formulation was less effective in controlling the Angoumois grain moth, Sitotroga cerealella (Olivier), since doses that gave complete control of the Indian meal moth and the almond moth reduced

emergence of adult Angoumois grain moths by only about one-third. In addition, Kinsinger and McGaughey (1976) reported that viability of applied B. thuringiensis and granulosis virus (GV) was only slightly reduced one year after treatment of wheat in a farm grain bin, and believed that with proper timing of application, either pathogen could protect the grain from Indian meal moth for one year. They also suggested that residual activity of the materials would extend protection even longer.

An SLC formulated with an attractant bait would lure the young feeding stages that are particularly susceptable to the pathogen. As a result, the larave would theoretically die before inflicting extensive damage on the stored grain. Ideally, such an SLC should be attractive both physically and chemically; it should contain a feeding stimulant to insure ingestion, and it should contain enough pathogen to be lethal. It could consist of pieces of corrugated paper, paper straws, or a a natural material (such as wheat straw) that are coated with the pathogen and the attractant. The ideal system should be a laminated structure made of safe and biodegrable materials. Adjuvants or stickers of the type curently used in the pesticide industry might be useful in binding the pathogen to the attractant. Distribution of the SLCs in the wall or cracks of empty bins, under conveyors, or other areas where residual populations exist would also enhance population suppression.

Another method of effectively distributing a pathogen among stored-product insects would be to provide a pheromone - baited or light-baited device with an open reservoir containing a pathogen such as B. thuringiensis. The insects attracted by the pheromone or light would become dusted with the pathogen and would distribute it within the insect population and

habitat.

Beneficial insects

The use of beneficial insects to control pest insects in stored products has not received extensive study because there are problems with the introduction of insects into a food product and because control is prompt. Studies have demonstrated effective suppression, however. Perhaps the most promising use of beneficial insects would be with commodities such as seed corn, peanuts, and other unprocessed materials that may already contain predators and parasitoids.

Predators. The predacious bug Xylocoris flavipes (Reuter) and several other anthocorid bugs of the sub-family Lyctocorinae that frequently occure as predators in storage ecosystems appear to be promising agents for suppression of both Coleoptera and Lepidoptera in stored products, since they prey on most stages of many of these species. Also, the predator has a high capacity to increase its numbers so as to reduce the population when prey is scarce. The difficulty is that X. flavipes. although it is effective against many unprotected insects, is incapable of penetrating hard materials like seeds, and therefore it is ineffective against weevils that infest grain and pulse. Nevetheless, its role in an integrated suppression program for certain commodities warrants further study.

Parasitoids. Two common parasitoids that in stored products are Bracon hebetor Say (Braconidae) and Venturia canescens (Gravenhorst, Ichneumonidae).

Takahashi (1973) studied the suppression and regulation of E. cautella populations by the action of V. canescens. He noted that the adults attack host larvae on the food surface and do not penetrate the food surface and do not penetrate the food surface appreciably. Bracon hebetor can attack host larvae in deep layer of food, but the population disappears. Takahaski therefore suggested that procedures should be developed to maintain the population level of the parasitoid. The parasitoids could be attracted by either a food (Nectar) source or by their pheromones.

Laeluis pedatus (Say) (Bethylidae) is another parasitoid associated with dermestid larvae. Burkholder, and Carlson (1978) showed that Anthrenus flaviceps Le Conte (furniture Carpet beetle) possesses a supra - anal organ that serves as a defense mechanism against this parasitoid, but that several other species of Anthrenus do not and thus can serve as hosts. Another bethylid, Cephalonmia tarsalis (Ashmead), a parasitoid of Oryzaephilus surinamensis L. (sawtoothed grain beetle), was studied as a possible means of supressing the host insect.

Finally, there are several promising pteromid (Hymenoptera; Pteromolidae) parasitoids of grain and pulse weevils. Anisopteromalus colandrae (Howard) has long been known to be of considerable economic importance in the control of grain weevils and a number of other stored-product pests. Lariophagus distinguendus (Forst) and Choetospila elegans Westwood are other cosmopolitan parasitoids of grain weevils.

Insect Growth Regulatiors

Chemicals that have juvenile hormone activity (IGRs) have been studied in a closed environment and have been found relatively successful against several stored-product moths and beetles (Leshiavo 1976; McGregor and Kramer 1975; Strong and Diekman 1973). For example, Methoprene and hydropene applied at a rate of 20 mg/kg to diets prevented emergence of pupae in Tribolium castaneum (Herbs") and substantially reduced emergence of pupae in Tribolium confusum Jacquelin du Val (Leschiavo 1976). Methoprene at 1 mg/kg or higher prevented emergence of adult (Oryzaephilus mercator (Fauvel) and O. surinamensis in treated rolled oats or cornmeal; and hydropene at 10 and 20 mg/kg in wheat almost completely inhibited production of adult progeny by Sitophilis granarius (L.). Incresasing concentrations of both compounds reduced the populations of adult progeny of Sitophilus oryzae (L.), but not enough to provide useful control.

Likewise, Walker and Bowers (1970) demonstrated that eggs of Lasioderma serricorne (F.), a cosmopolitan pest of stored products, were sensitive to IGRs. Thus, pey suggested that methoprene maybe useful in controlling L. serricorne on package commodities.

IGRs could be added to attractant impregnated baits instead of directly to food. The insects might then cease to develop or behavior may be disrupted and reproduction impaired.

Conclusions

Biological suppression procedures can be an important part of pest management programs for stored-product insects. Whit th increased availability of pheromones, pathogens, IGRs,

parasitoids and other suppression methods, systems of biological suppression that make use to innovative manipulation are increasingly promising. These approaches should be developed further and evaluated for efficacy, cost, and safety.

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Table 1. Predators and parasites of stored product insect pests in tropical and subtropical storage

INSECT ORDER/FAMILY/SPECIES	**TYPE OF OCCURRENCE**
Hemiptera	
Reduviidae	
Amphibolus wenator (Klug)	Spr*
Peregrinator biannulipes (Montrouzier)	Spr**
Reduvius spp	Gpr*

Anthocoridae	
Lyctocoris spp	Spr*
Xylocris flavipes (Reuter)	Spr****
Other Xylocoris spp	Spr*
Diptera	
Scenopinidae	
Scenopinus fenetralis (L.)	Spr*
Hymenoptera	
Ichneumonidae	
Ventura canescens (Gravenhost)	Spr*
Braconidae	
Bracon hebetor Say	Spa***
Phanerotoma spp	Spa*
Chalcididae	
Antrocephalus spp	Spa*
Euchalcidia spp	Spa*
Pteromalidae	

Anisopteromalus clandrae (Howard)	Spa****
Cerocephala spp	Spa*
Choetospila elegans West wood	Spa***
Dinarmus laticeps (Ashmead)	Spa*
Habrocytus cereallelae (Ashmead)	Spa**
Lariophagus distinguendus (Forster)	Spa*
Encyrtidae	
Zeteticontus spp	Spa*
Bethylidae	
Cephalonomia tarsalis Ashmead	SPa**
Cephalonomia waterstoni Gahan	SPa **
Holepyris hawaiiensis (Ashmead)	SPa *
Plastanoxus munroi Richards	SPa *
Plastanoxus westwood (Kieffer)	SPa **
Rhabdepyris zeae Turner and waterston	SPa **
Coleoptera	
Carabidae	

Various spp	GPr **
Histeridae	
Carcinops pumilio (Erichson)	SPr *
Carcinops troglodytes (Paykull)	SPr *
Teretriosoma spp	SPr *
T nigrescens	SPa *
Teretrius spp	SPr *
Staphyulinidae	
Various spp	GPr **
Dermestidae	
Dermestes ater De Greer	SPr *
Trogossitidae	
Tenebroides mauritanicus (L.)	SPr *** -
Cleridae	
Necrobia rupifes (De Geer)	SPr ***-
Thaneroclerus buqueti (Lefevre)	SPr ** -
Various other spp.	SPr *
Passandridae	

Laemotmetus rhizophagoides (Walker)	SPr *
Coccinellidae	
Various spp	GPr *
Colydiidae	
Various spp	GPr *
Tenebrionidae	
Lyphia spp	SPr *

GPr = general predator; not particularly adapted to storage environment, usually highly polyphagus, often found only in small numbers and usually not significant in the natural control of pests.
SPr = storage predator; adapted to storage environment although some species are also found in other habitats, usually oligophagus and sometimes found in large numbers.
SPa = storage parasite; adopted to the storage environment, although some species are also found in other habitats, usually oligophagus.

(sometimes with a very harrow host-range) and sometimes found in large numbers.

*** - occasional or rare species where they occur, maybe abundant**
**** - infrequent**
*****- common**

***** - very common**

- These species are not obligate predators; they are also secavengers or secondary pests on certain commodities, in some situations, therefore, they are economically undesirable rather than beneficial.

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Insect growth regulators

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By R.L. Semple

Principles

Physiological changes are associated with metamorphosis. This is the change from the larval or immature stage to the adult or imago and maybe totally different in appearance and habits, which are regulated by ecdysones that initiate moulting, and juvenile hormones that regulate growth and development of immature stages and sometimes oogenesis in the adult. The pioneering work done by the Italian entomologist Berless, the famous work of Sir Vincent B.

Wigglessworth with the bloodsucking reduviid bug, Calliphora spp., and Fukuda working with the larvae of the commercial silkworm Bombyx mori, helped to elucidate the mechanisms of insect hormones on growth and development.

Professor Carroll M. Williams (1956) was probably the first to recognize the potential of applying insect hormones in pest control, and in 1967 hailed them as "third-generation pesticides, the first generation is exemplified by arsenate of lead; the second by DDT. Now insect hormones promise to provide insecticides that are not only more specific but also proof against the evolution of resistance." These compounds have been commonly termed juvenile hormone (J.H.) mimics or analogues and may act to inhibit, retard or even accelerate developmental process. WiIliams and Amos (1974) referred to them as "insect developmental regulators", while now they are more readily termed "insect growth regulators (IGRs)."

Because of the discontinuous growth pattern imposed on insects by the rigidity of their exoskeleton, moulting, or the process of secreting a new larger cuticle and shedding their old one, must occur to accommodate the increase in size. This process is common to all arthropods. It generally takes place a number of times during the larval and nymphal stages, and in holometabolous insects, once more when the adult emerges from the pupa. The number of instars (stages between successive moults) is usually greatest in the more primitive insects, such that members of the order Thysanura continuously moult throughout their lives, while in the more advanced endopteryzotes (such as members of the higher Diptera, i.e., muscid flies) may have typically five instars; three larval, one pupal and finally the adult.

The insect cuticle

The cuticle covers the entire external surface of insects and continues into the fore and hind guts, covering the ducts of the dermal glands and the entire tracheal system. It possesses a multilayered structure which provides a one-way barrier for water loss and protection against many external conditions.

The epidermis (or hypodermic) secretes the cuticle during ecdysis, and is supported on a thin sheet of connective tissue called the basement membrane. The cuticle itself is sub-divided into a thin, outer non-chitinous epicuticle and an inner, thicker chitinous procuticle.

The outer epicuticle is 2 to 3 microns thick, comprising four separate layers; an outer cement layer followed by a wax layer impermeable to water but allowing rapid entry of lipophilic insecticides, a layer of polyhenols and protein under which is finally the cuticulin layer containing polymerized lipoprotein.

The procuticle contains protein (arthropodin) and chitin (a polysaccharide in which the unit sugar is Nacetylglucosamine, arranged in an outer exocuticle and an inner layer of similar nature and thickness (approximately 10 microns) but less densely tanned called the endocuticle.

Polymerization of the chitin and protein into a stable copolymer or glycoprotein, by linking a large number of molecules to form long chains, produces an immersely strong, resilient but

chemically inert compound, giving the insect cuticle its characteristec strength.

Sometimes layers of a unique rubber-like protein called resilin alternate with layers of chitin. It is particularly abundant in areas of elastic cuticle such as the flexible intersegmental membranes, and in the thorax forming hinges and elastic ligaments for the articulation of wings and aiding energy conversion during flight. It is a long, freely rotating polypeptide chain without free amino-acid end groups and relatively low tyrosine residues held together by infrequent stable bonds caused by a small number of cross-linkages. This gives the structure plasticity and elasticity of movement.

Integument changes at moulting

The beginning of the moult is the detachment of the epidermis from the cuticle, a process called apolysis. The rearranged epidermis secretes a thin lipoprotein layer which is the precursor of the final cuticulin layer of the new epicuticle. At this time, a moulting fluid is pumped into the exuvial space containing digestive enzymes which breakdown protein to amino acids, and chitin to individual sugar molecules of the old endocuticle. The epicuticle previously formed is permeable to molecules in solution, since the products from the old cuticle are recycled back into the epidermis for the formation of new cuticle or passes into the haemolymph and stored in the fat body.

The epidermis secretes the new cuticle by small cytoplasmic papillae which eventually form the pore canals through which the protective outer layers are secreted. The new pharate instar

is protected to a certain extent by the cuticle of the previous instar, but is still aware of external conditions since in many cases, the sensory setae of the old cuticle maintain contact through the moulting fluid and new cuticle to the original sensory cells.

After the moulting fluid has dissolved the inner layers of the previous cuticle, it is resorbed by the epidermis and replaced by air. Wax layers are secreted over the new cuticle rendering it impermeable, and through contraction of abdominal muscles and increasing blood pressure in the thorax, the remaining old cuticle splits at the weakest point, usually the mid-dorsal line. This process is known as ecdysis; the Insect emerges leaving the cast-off skin or exuviae behind; while swallowing air and stretching its much folded and creased new cuticle until it assumes its final shape and size. The inner layers are secreted, while additional layers of endocuticle are added throughout the duration of the instar.

Fig. 1. Structure of cuticular proteins (diagrammatic): A, Resilin; B, Sclerotin. From Insects of Australia, CSIR.

Hardening and darkening

The cuticle of a newly emerged insect is often soft and colourless, and is converted into the horny exoskeleton by the reaction of the polypeptide chains with quinones under the action of specific enzymes.

This process, referred to as sclorotization, where the tanning quinones react with the free amino groups of the protein of the epicuticle and outer procuticular layers, causing extensive crossbonding and dehydration.

The quinones are derived from the amino acid tyrosine which accumulate in the haemolymph before ecdysis, and migrate into the cuticle during the hardening process. The intermediary in this reaction is the diphenol N-acetyldopamine, which is derived from tyrosine. In the presence of the oxidative enzyme phenolase, the quinone is 'ultimately formed which tend to condense with one another and fill the spaces between protein chains or form the polymerized indole pigment melanin. The cross-linked, pigmented protein of the exocuticle or sclerotin, produced by the action Of quinones, is responsible for the common brown or black colouration of the insect integument. Many other integumentary colours are formed from interference by the interaction of light through thin films separated by a material of different refractive index or fine striations on the cuticular surface, and by the accumulation of other pigments in the blood or epidermal cells below a fairly transparent cuticle.

Hormonal balance

Cuticle synthesis and moulting are controlled by three groups of growth regulators - the brain hormones, the ecdysones and the juvenile hormones. Under normal concentrations, these growth regulatiors also control other developmental processes in insects including sexual maturation, colour differentiation and reproduction.

Fig. 2. Reaction pathways in the formation of sclerotin. From Insects of Australia, CSIRO, various authors.

Briefly, the mechanism and interaction of these hormones in metamorphosis are as follows:

The brain hormone, the prothoracicotrophic hormone (PTTH), is secreted by neurosecretory cells of the brain and released by the corpora cardiaca (CC). It activates the prothoracic glands (PTG) which stimulates the insect to moult. The kind of replacement cuticle secreted by the epidermal cells at each moult or ecdysis, is regulated by a third group of hormones, the juvenile hormones (JH) which are secreted by the corpora allata (CA), a paired organ situated in the head. In the presence of JH larvae moult into larvae. However, when the JH titre in the haemolymph is reduced or absent, larvae or nymphs of hemimetabolous insects such as grasshoppers, metamorphose into adults, while larvae of holometabolous insects such as butterflies moult into pupae and then into adults.

Moulting is controlled by regulating the release of the brain hormone; maturation and metamorphosis are regulated by controlling the release of juvenile hormone (Fig. 1).

Insects use alpha ecdysone, beta ecdysone and perhaps others as moulting hormones. Work by Krishnakumaran and Schniederman (1968) have proved that ecdysones appear to be moulting hormones for all arthropods in general.

Moriyama et al. (1971) also suggests that beta ecdysone is the active material and that alpha

ecdysone is rapidly synthesized into this form by tissues such as the fat body.

It must be emphasized that the misnamed steroid ecdysone initiates apolysis in the moult cycle (and puparium formation in flies culminating in tanning of the old larval cuticle) and not ecdysis. The moult cycle consists of the following chain of events; apolysis - moulting fluid production initiation of cuticle formation - moulting fluid activation - exocuticle secretion - ecdysis tanning - endocuticle deposition (Lock, 1964).

Fig. 3. Changes in the endocrine activity of the corpora allata of the cecropia silkmoth during the third, fourth, and fifth larval stages and during metamorphosis. Activity was assayed by transplanting corpora allata from cecropia at each stage into test polyphemus pupae. The break in the time scale represents storage of the donor pupae at 6C for 10-20 weeks to break diapause; the cross-hatched zones represent larval molting. From Williams (1961).

The final stages of moulting are regulated by ecdysial brain hormones released from the C.C., and identified in the pupal ecdysis of some insects as the eclosion hormone (Truman and Riddiford, 1970). A second hormone released from me Perivisceral organs (P.V.O.) called bursicon, regulates the postecdysial hardening and darkening of the new cuticle (See Fig. 4). The deposition of endocuticle during the course of the instar appears to be controlled by another brain factor (Locke. 1970).

The other fact to consider, as pointed out by Schneiderman (1971), juvenile hormones (JH) and moulting hormones (MH) do not act antagonistically, but rather act in harmony with one another in their effects on morphogenesis (N.B.: not a synergistic effect).

In the endopterygotes, when the larvae hatch from the egg, they already contain the blueprint for the ultimate adult form by a small group of cells or imaginal discs. These do not begin to develop until the pupla stage where the larvae structures are broken down and replaced by new adult structures developing from the imaginal discs (in more advanced forms).

Willis (1974) emphasizes that the inhibitive action of cuticle synthesis in imaginal discs is not related to antagonism between ecdysone or juvenile hormone, but that J.H. favours the formation of pupal cuticle and inhibits the formation of adult cuticle.

Chemistry of juvenile hormones

Attempts to obtain active juvenile hormone by extraction of Corpora allata failed, until an unpurified extract was obtained from the abdomens of the adult males of Hyalaphora cecropia (= Platysamia cecropia), a Saturnid silkmoth (Williams, 1956). Purified extracts were not isolated until 1967 by Roller et a/. and by Meyer et al. in 1968. which differed only slightly from the farnesol derivative obtained by Bowers et al. (1965) (Fig. 5A).

The first compound identified as possessing juvenile hormone activity was isolated from faeces of the yellow mealworm, Tenebrio molitor by Schmialek in 1961. This was the sesquiter

terpenoid alcohol farnesol, but was not as active as some of the synthetic derivatives (the terpenes farnesyl methyl ether and farnesyl diethylamine) which were similar in potency to the isolates from Cecropia.

Other mimetic substances displaying juvenile hormone activity were straight chain aliphatic ethers such as dodecyl methyl ether and dodecyl ethyl ether, which possessed similar activity as the farnesyl ethers when assayed in T. molitor and the waxmoth, Galleria mellonella (Fig. 5B). Slama and Williams in 1966 discovered that the paper towelling used in rearing jars possessed morphogenetic effects on the linden bug, Pyrrhocorus apterus by inducing supernumerary moulting. This "paper factor" from wood pulps of fir, hemlock, yew, larch, spruce and pine was found to be specific to members of the Pyrrhocoridae (fire bugs and stainers), and the active component was later isolated and identified from balsam fir wood as juvabione, a sesquiterterpenoid unsaturated methyl ester which was also active against other insects such as Tenebrio (Fig. 5C).

Many compounds that possess activity to naturally occurring insect hormones have subsequently been developed, a vast majority of which has an acyclic terpenoid skeleton to which various functional groups are attached. Naturally occurring juvenile hormones isolated from the tobacco hornworm, Manduca sexta (L.) have been terpenoid ethers, epoxidised aromatic ethers, bans, bans dienoate ethers and unepoxidised dienoates.

The most widely studied juvenile hormone analogues have been methoprene and hydroprene synthesized by Zoecon, Palo Alto, California. Various terpenoid compounds have recently been

evaluated for their potential in reducing the productivity of Rhyzopertha dominica (Fab.), Sitophilus granarius (L) and S. oryzae (L) at concentrations of 5 and 20 ppm (Amos, et a/., 1982). An all bans alkyl-terpenoid ketone was the most active against these species, while the arylterpenoid alkylethers were not promising although previously demonstrated as being highly effective against numerous dipteran species. The most effective arylterpenoid epoxides were not considered as potential candidates due to their relative inactivity against Tribolium castaneum and T. confusum in previous studies.

Certain commercial insecticide synergists such as piperonyl butoxide (PB) have been shown to be effective in blocking metamorphosis of Tenebrio and the milkweed bug, Oncopeltus fasciatus. Bowers synthesized the methylendioxyphenyl group chatacteristic of PB with terpenoid moieties similar to cecropia JH which greatly enhanced its activity. In a more recent study, Pratt et a/., (1981) found that either natural pyrethrins or permethrin were more highly synergized and active against T. castaneum when mixed with Hoffman La Roche RO 20-3600 (6, 7-epoxy-3, 7-dimethyl-1-(3-, 4(methylenedioxy)-(phenoxy)-2-nonene) than PB at 1:10 and 1:7 ratios. The usefulness of this juvenile hormone analogue as a pyrethroid synergist is due to its ability to inhibit the microsomal mixed function oxidases that would otherwise degrade permethrin via oxidative pathways as demonstrated in cockroaches, houseflies and cabbage looper larvae.

Fig. 4. The insect endocrine system (above) and the effects of its hormones on growth and development (below): br, brain; cc, corpus cardiacum; ca, corpus allatum; ptg, prothoracic gland; pvo, perivisceral organ; PTTH, prothoracicotropic hormone; JH, juvenile hormone.

Source: "Biochemistry of Insects" Edited by Morris Rockstein - Biochemistry of insect hormones and insect growth regulators Lynn M. Rddiford and James W. Truman, p309.

Figure 5. Some early compounds possessing juvenile hormone activity. JH-I and JH-II posses equal activity against Lepidoptera, while JHII was less than one-fifth as active against Tenebrio. All insects tested were sensitive to cecropia JH-I.

Compound

Toxic effects of IGR's

Professor Scneiderman from the school of Biological Sciences, University of California (1971) as well as Riddiford (1972) and Wigglesworth (1970) have shown that the timing of application of IGR's during insect development is critical in its subsequent effects. He states,

"If insects are experimentally exposed to large amounts of ecdysones or exposed to ecdysones during the life cycle when they are not normally active, they may be toxic and cause various developmental abnormalities, including a juvenilizing effect. Normally, low concentrations of ecdysone stimulate DNA replication and cell division, whereas larger amounts stimulate the immediate synthesis of new cuticle. From work done with Tenebrio pupae, injection of a few micrograms of beta ecdysone caused it to moult into a second pupa instead of an adult. The

reason being for a nymph or pupa to develop into an adult it must "clean its genes" before it can make adult cuticle, and this programming can only occure at times of DNA replication. The high level of ecdysone subjected the Tenebrio pupae to make old cuticle before DNA replication could take place."

Many effects of IGR's have been documented from experimental research on a wide range of economically important insect pests. Impairing embryogenesis when IGR's are applied early in egg development has been studied by Riddiford (1972) and MacFarlane and Jameson (1974) with the tortricid moths "codlin moth" Cydia pomonella L. and the "oriental fruit moth" C. molesta Busck.

The application to larvae results in the disruption of pupal development and adult emergence and the formation of various intermediate forms between larva and pupa, pupa and adult or larva and adult, where reproduction is inhibited and normal ecdysis is not achieved. Application of IGR's to larvae when some cells are insensitive while others are not, leads to the production of these mosaics, and has been demonstrated in stored products insects by Strong and Diekman (1973), Loschiavo (1975), Metwally and Sehnal (1973), Amos et al., (1974) among others.

If treated larvae do complete development they may give rise to morphologically deformed adults (Amos and Williams, 1974). They recognized a range of adult deformities with Tribolium castaneum Herbst including aberrations of the tars), legs reduced to unchitinized stump-like appendages, lack of differentiation and poorly chitinized antennae clubs, to crumpled and

greatly diverging wings and elytra and developmental intermediates or "adultoids" (pupal-adult mosaics). Some IGR's increased developmental mortality while others inhibited complete development of adults depending on applied concentration.

Leftwich (1976) has referred to the delay in the appearance of adult characters due to excessive JH (induced exogenously, naturally or by the presence of parasites) by disrupting the hormonal balance between CA and PTG as "metathetely" whereby the production of insect intermediates may be formed. Conversely, "prothetely" is the terminology used to refer to precocious formation of adult characters through insufficient JH.

Not only have morphologically deformed adults been produced when exposed to various concentrations of JH analogues, but also adults whose reproductive physiology has been impaired in some way. In stored products insects, this sterilizing effect has been observed in the "khapra beetle" Trogoderma granarium Everts (Metwally et a/., 1972) as well as Tribolium castaneum (Herbs") when adults were previously reared in flour incorproating either the IGR methoprene or hydroprene (Amos et a/., 1977). The productivity of adults was impaired depending on the concentration of IGR in its diet and its sex, but was independent of whether the D not the individual was morphologically deformed (Table 1).

Reproductive behaviour in adults may also be impaired as found in the "lndian mealmoth", Plodia interpunctella (Hubner) when larvae were treated at sub-lethal doses of IGRs (Oberlander et al., 1975). Prolonged treatments during late larval life did not prevent pupation and eclosion but greatly inhibited the adult moths from mating. Obviously, the advantage is

that the concentration of IGR required is considerably less to inhibit mating or reducing reproductive potential than that required to prevent metamorphosis or deleterious morphological effects.

Mode of application of IGRs

For the practical application of IGRs in pest management systems, three possible modes of exposure by surface contact, vapour action and ingestion of treated media have been investigated (Oberlander et al., 1979).

Marzke et al., (1977) studied exposing sexually mature females of the "cigarette beetle" Lasioderma cerricorne (F.) to filter paper impregnated with methoprene. The productivity of treated females with non-treated males was markedly inhibited and dependent on concentration.

Metwally et a/., (1972) exposed T. granarium Everts to the vapours of various IGRs which inhibited productivity of treated adults and prevented larvae and pupae development.

Amos and Williams (1977) have also demonstrated insect growth regulators that possess enhanced activity due to toxic avpour action. They studied "the effects of two IGRs on the productivity of Rhyzopertha dominica, Sitophilus oryzae and S. granarius by exposing insects to

wheat treated with methoprene (isopropyl 11-methoxy-3, 7, 11-trimethyldodeca-2, 4-dienoate) or hydroprene (ethyl 3, 7, 11-trimethyidodeca-2, 4dienoate) at concentrations of 1, 5, 10 and 20 ppm. Parental adult mortality was generally higher on wheat treated with methoprene than with hydroprene, and this effect was usually enhanced under unventilated conditions (Table 2). The productiovity of the three species was markedly reduced, in some instances suppressed, under unventilated conditions, whereas only R. duminica productivity was depressed under ventilated conditions (Table 3). When progeny were produced, their productivity was, in general, lower than normal . "

Hydroprene was generally less active than methoprene. Strong and Diekman (1973) observed no adult mortality or ovacidal effects when these species were exposed to merhoprene or hydroprene treated wheat, while McGregor and Kramer (1975) also found these IGRs non-toxic to adults. This study establishes significant parental mortality under non-vetillated conditions due to the existence of a towic vapour effect. Sitophilus spp appeared to be relatively insensitive to these analogues (Steal, 1975), but from these findings, control or at least suppression of productivity would be expected in bulk storages which would more approximate to the unventillated conditions used in this experiment.

Most of the previous literature cited on the effects of IGRs on stored products insects has been administered through their diet. This provides only a feeding insect with continuous exposure, whereas the surface contact or vapour action maybe equally effective with larvae, pupae and adults (which may not feed).

Bhatnagar-Thomas (1976), studied both injection and vapour action of the Juvenile hormone analogue (JHa) altozar for controlling T. granarium Everts. JHa absorbed on inert materials such as chalk and cellulose retained most of its activity even after 6 months storage. Not only did hydroprene impregnated tablets and filter paper strips suppress development, but terminated larval diapause earlier and the lifespan of the normal larvae was not prolonged (by the production of supernumerary larvae). The production of larval/pupal intermediates from normal larvae took 22 days at 0.5 ppm JHa, while diapausing larvae required 32 days at the same concentration. Metamorphosis of 4-hour old female pupae was completely inhibited at 1.0 - 1.5 ppm JHa, while concentrations of around 0.5 ppm produced a mixed population of sterile adults and adultoids

Table 1. Viability and productivity of adults of T. castaneum previously reared in flour containing either methoprene or hydroprene

IGR and concentration (ppm)	**Adult type (N or D)* crosed with control adult**	**Male Number of crosses set up**	**Number of crosses producing progeny**	**Mean number of progeny********	**Female Number of crosses set up**	**Number of crosses producing progeny**	**Mean number of progeny********

Methoprene							
0.001	D	14	11	142.7ab	26	15***	131.6a
	N	30	27	129 8ac	30	30	138.9a
0.01	D	18	14	173.9b	22	15***	138.9a
	N	28	27	132.6a	30	25	121.3a
0.1	D	28	3* * *	69.3c	30	5* * *	99.6a
	N	30	16* * *	80.8C	30	27	119.7a
Hydroprene							
0.001	D	4**	4	112.0	3**	0	-
	N	30	24	121.2a	29	27	138.1a
0.01	D	29	19***	145.8a	28	8***	126.4ab
	N	30	27	146.5a	30	26	143.3a
0.1	D	30	20***	130.1a	30	16***	98.4b
	N	30	28	161.0a	29	27	135.0a
Control	30	29					

Fig. 6. Diagrammatic scheme of insect growth regulator actions during the insect life cycle.

Adapted From:

"Advances in insect growth regulator research with stored grain insects" Oberlander, et al., (1979). Proceedings of Symp. on prevention and control of insects in stored food products.

Table 2. Percentage mortality of parental adults of three stored grain insects exposed to IGR-treated wheat for 7 days under ventilated and unventilated conditions

IGR	Concn	Ventilation	Percentage mortality of adults of:		
			S. oryzae	S. granarius	R. dominica
Methoprene	0	V	0	2	0
		UV	11	1	1
	1	V	12	73	4
		UV	81	11	96
	5	V	5	84	5
		UV	86	39	98
	10	V	8	94	6
		UV	96	30	100
	20	V	3	86	18
		UV	100	100	100
Hydroprene	0	V	13	1	9

		UV	1	0	1
	1	V	3	1	16
		UV	4	1	6
	5	V	7	3	11
		UV	6	1	13
	10	V	23	2	39
		UV	12	1	17
	20	V	5	4	45
		UV	97	74	25

V, ventilated, UV, unventilated

Extracted from "Insect Growth Regulators: Some effects of methoprene and hydroprene on productivity of several stored grain insects." T. G. Amos and P. Williams, Aust. J. Zool., 25,1977.

Table 3. The difference between the productivity of adults of three stored grain insects when on wheat treated with methoprene or hydroprene and when on untreated wheat under ventilated and unventilated conditions

IGR	Concn	Ventilation	Percentage mortality of adults of:

			S. oryzae	**S. granarius**	**R. dominica**^{A}
Methoprene	1	V	+ 10	18	- 99
		UV	-1 00A	89*	-1 00
	5	V	+ 9	12	- 99
		UV	- 98A	72*	-100
	10	V	+ 65 *	73	- 99
		UV	-1 00A	91 *	-1 00
	20	V	- 12	- 14	- 99
		UV	- 100A	- 100A	- 100
Hydroprene	1	V	+ 54	+9	- 28
		UV	-2	- 22	- 86
	5	V	+20	-5	- 98
		UV	-29	-87*	- 99
	10	V	-37	-25	- 99
		UV	-51	-98A	- 100
	20	V	-19	+8	-100
		UV	-100A	-100A	-100

V, ventilated. UV, unventilated. Significant at P = 0.05, Duncan's Multiple Range Test.

[A] No statistical test for significance carried out.

Extracted from the same source as Table 2.

DISADVANTAGES OF APPLIED IGRs

Prolonging larval life

Many reports have been made on the effects of e%posing larvae of stored products insects on IGR treated diets. Firstenberg and Silhacek (1976) found that the larval feeding period of P. interpunctella was prolonged approximately double the feeding period of control larvae, and dependent on applied concentration, but did not after the rate of feeding. Metwally and Sehnal (1973) found that T. granarium larvae treated within the first nine days of the last instar which lasted a total of thirteen days, underwent up to six extra larval moults, increasing the longevity of the larval trophic phase by more than four months compared to the controls.

Most superlarvae either died or pupated usually into morphologically normal adults while a small proportion formed adultoids. Pupla adult intermediates were also produced when the IGR compounds were applied within the first third of the pupal instar.

When larvae were subjected to vapours of the JH analogues for 6 weeks, the last instar larvae performed 1-4 extra moults finally developing into either larvalpupal intermediates or normal pupae which produced both morphologically normal adults and adultoids. When pupae alone were exposed, normal adults were produced although fecundity was lower than adults from untreated pupae (the hatchability of these eggs was reduced by 75% while most larvae died within the first two or three instars).

Ishaaya and Yablonski (1976) found also that treating T. castaneum larvae with hydroprene (ZR-512) or triprene (ZR-619) in their diet at 10-1000 ppm prolonged larvae feeding by 10x and doubling larval weight as compared to untreated control larvae. The period between fourth instar larvae and pupation was considered critical for JH effect. Loschiavo (1975) also found that the developmental period of larvae was delayed by 5 days in the "confused flour beetle" Tribolium confusum Jacquelin du Val and pu to 6.5 days for the "rust red flour beetle", T. castaneum (Herbs") when reared on a diet containing hydroprene and methoprene at 10 ppm as compared to larvae reared on untreated media. Alternatively, triprene or kinoprene (ZR-777) had no effect on larval survival or development in either species. Williams and Amos (1974) exposed eggs (0 - 2 days old) of T. castaneum to a range of JH analogues. With hydroprene at 5 ppm, percentage survival was similar to controls, but at 20 ppm and methoprene at both 5 and 20 ppm, development of immature stages was prolonged with individuals either dying in the larval stage or as adultoids. Adults were not produced.

They commented that, "Prolonging this developmental trophic stage is likely to result in more food being consumed or contaminated, or both. For control of existing insect infestations it

may be more desirable to use a JH compound which does not appreciably increase the larval life span, which preferably should be reduced. In this situation, the compound should indeally either inhibit the development of the egg or young larva, or affect the prepupal and pupal stages so that metamorphosis is either prevented or the adults produced are sterile."

Time of application

Most reports on the effectiveness of IGRs on stored grain insects have emphasized that those possessing juvenile hormone activity must be available to target insects early in the last larval instar and must be maintained to be effective (Brieger, 1973).

IGR's exert their influence during limited periods of the insects life cycle, resulting in a variety of morphological and physiological deformities. For example bugs, locusts and other hemimetabolous insects are usually only sensitive to juvenile hormone analogues or mimics shortly after the last larval ecdysis during the first thired of the last larval instar, or after adult emergence, where JH then has an ovicidal effect. Larvae of most holometabolous insects such as Lepidoptera and Coleoptera are sensitive only at the end of the last larval instar, while the pupae are sensitive for several hours or at most a few days after the lastlarval ecdysis.

Therefore to be effective in the field, juvenile hormone analogues must be applied at critical times to be effective and persist long enough to expose all members of the pupulation during periods of sensitivity to juvenile hormone. They must be stable and effective for several weeks under field conditions if these control agents are to be of any practical use (Schneiderman,

1971).

Cross-resistance to IGRs

Certain strains of insects that exhibit resistance to insecticides can also show some level of cross resistance to certain IGRs. There is a number of reported incidences where pests of medical importance and field pests have disphlyed crossresistance, including houseflies (serf and and Georghion, 1974) mosquitoes (Brown and Brown, 1974) and aphids (Hrdy, 1974).

Dyte (1972) reported that malathion resistant strain of T. castaneum also showed resistance to a juvenile hormone analogue. However, in many other instances, no cross-resistance to IGRs have been reported in stored-products insects. Silhacek, et al., (1976) did not establish cross-resistance in three malation-resistant strains of Plodia interpunctella while Amos and Williams (1974) found that methoprene and hydroprene reduced or suppressed the productivity of both susceptible and multi-resistant strains of Rhyzopertha dominica Fabricus. The resistant strain had a resistance foactor of x 37.7 to malation, which suggests no crossresistance to the two IGRs. Amos et al., (1977) compared the effects of both methoprene and hydroprene on the developmental survival of three malathion resistant strains of T. castaneum and one of T. confusum with a malationsusceptible strain of each species. In either case no crossresistance to the IGRs was detected. With T. castaneum the two IGRs were in fact more effective in reducing developmental survival of the malathion-resistant strains than the malathion susceptible strain. Development was completely inhibited at 20 and 5 ppm, while at 1 ppm, developmental survival was low with most adults being deformed in the susceptible strain,

and only adultoids being formed in two of the resistant strains.

Similar effects with hydroprene were observed with T. confusum where at 20 ppm no individuals completed development and at 5 ppm, adult emergence was low with most being deformed. Adultoids were visible at both these concentrations (see Fig. 3).

Beetles of different families posses different responses and sensitivity to JH analogues, and consequently results of assays on one species cannot be extended to all Coleoptera (Metwally and Sehnal, 1973).

Schneiderman (1971) also states that, "At certain stages in their development, insects normally inactivate, sequester or excrete juvenile hormone and many juvenile hormone analogues. Thus nature has endowed insects with a built-in mechanism to resist the artificial application of JHa's at specific stages. Mechanisms which inactivate JH or its analogues normally function only at specific times in development, but the existence of such mechanisms guarantees that natural selection could produce populations of insects which would be resistant to exogenous juvenile hormone analogues. Differences in sensitivity among different families stems from differences in rates of penetration, breakdown, excretion, storage or differences in behaviour feeding habits, etc. of insects tested. The fact that insects of one genetic make-up respond to JHa, whereas closely related insects of a different genetic make-up fail to respond, indicates that resistance to JHa could be selected in nature."

Candidate IGRs should therefore be evaluated for the potential of cross-resistance with the

appropriate target species before a full evaluation of these compounds can be ascertained.

Other effects of IGRs on insects

The application of IGRs during the development of a polymorphic insect can upset caste and phase determination. Certain social insets such as ants and termites are of considerable economic importance and difficult to control with conventional insecticides. If an IGR with morphogenetic activity was introduced into the nest which maintains a reproductive queen and also feeds and grooms their young, it may seriously effect caste systems and the developing progeny. French (1974) observed a caste shift from workers into soldiers in the Australian termite Nasutitermes exitiosus (Hill) when reared on a diet containing 0.1 % hydroprene.

Different phenotypes of locusts resulting in distinct phases (solitary and gregarious) are dependent on pupulation density. Variations between phases including colouration, rate of development and behavioural characteristics are controlled hormonally, with the CA and PTG being implicated in these coulur changes. Locust nymphs exist in a green or yellow form in the solitary phase, and yellow with extensive black patterning int the gregarious form. Gregarious behaviour, where nymphs or hoppers from large bands, is passed onto the winged adults who form devastating migratory swarms. This enables reproductives to escape from overcrowded areas and find suitable breeding grounds with sufficient soil moisture for egg-pod survival and emergence of another generation of hoppers. About 10 species of Acrididae rank as locusts because of this phase change, the desert locust Schistrocerca gregaria being a good example.

Kentromorphic phases triggered by overcrowding is not exclusive to locusts and is known in Phasmida and larvae of some Lepidoptera. Sehnal et al., (1976) have reviewed the effects of applied juvenoids to eggs, larvae and pupae of noctuid moths, which contains many destructive "armyworm" species.

Juvenile hormones are also unquestionably involved in the regulation of diapause, so the possibility exists for the application of JH analogues which can either prevent or induce diapause. Diapause is a condition of physiological arrest which permits survival during conditions of extreme heat, cold or drought. It can be inherited and therefore obligatory, or facultative and initiated by various environmental stimuli including photoperiod, temperature, humidity or by various combinations. Therefore, the termination of diapause by application of JHa can have disastrous consequences on insects which are dependent on this phenomina for survival.

Fig. 7. Developmental survival, together with information on adult deformity and adultoids, of malathion-resistant and -susceptible strains of T. castaneum and T. confusum when reared in diets containing either methoprene or hydroprene.

Extracted from "Susceptibility of malathion resistant strains of Tribolium castaneum and T. confusum to the insect growth regulators methoprene and hydroprene," Amos, et a/., (1977).

Termination of diapause by IGRs has been demonstrated in various field crop pests such as "the pink ballworm", Pectinophora gossypietla (Sauna), the alfalfa weevil Hypera postica

(Gyllenhal) and the infamous "Colorado beetle", Leptinotarsa decemlineata (Say).

Bhatnagar-Thomas (1976) has shown that hydroprene at 0.1.-1.5 ppm in stored food would protect it from T. granarium Everts infestation and when applied as tablets or strips, terminates larval diapause comparatively earlier than mixed with the diet. Hydroprene is 7 times more active than ethyl farnesoate which at 20 ppm, arrested metamorphosis of normal as well as diapausing larvae (BhatnagarThomas, 1972).

PHEROMONES

Definition: Pheromones (once classified as ectohormones) are chemicals secreted from dermal glansa of insects into the external environment, that elicits a specific reaction in the receiving individuals of the same species. Such substances maybe conveniently grouped into categories relating to their specificity of action.

Sex pheromones, sex attractants or lures:

Ofter the behavioural sequences in response to sex pheromones is extremely complex. The male reaction to the volatile odour emitted by the female is considerably more complex than a simple attraction to odour source. Usually the sex pheromone is released by the female insect to guide the male, as in the majority of the Lepidoptera and can be effective over quite astonishing distances.

Behavioural control programmes using sex pheromones are difficult in that the insect immediately involved is the wrong sex. Certain male insects do release sex pheromones but these are generally shortrange sex stimulants for mating.

The sex pheromones elucidated from the silkmoth Bombyx mori and "gypsy moth", Porthetria dispar have been identified as long chained unsaturated alliphatic alcohols, which are related to acids and alcohols of the cuticular wax. Others are more complex compounds such as terpenoids, many of which are responsible for the fragrance of plant oils and resins.

Alarm or warning substances

An example of this is pinene, and alarm substance which arouses aggressive behaviour in soldiers of Australian termites of the genus Nasutitermes.

Marker substances

Honeybee workers deposit an abundant source of nectar, a substance called Geraniol, which acts as a marker for following workers. Ants also leave odour trails which other members of the colony will follow.

Importance in promoting cooperative behaviour in social insects

Larvae of social Hymenoptera such as members of the family Vespidae, yield a secretion which is actively sought by the workers (sterile females) and probably acts as a unifying force in the

colony. The wasps solicit the secretion so avidly that the growth of the larvae maybe stunted. In the honeybee, Apis mellifera, all larvae from fertilized eggs are capable of developing into queens, if fed throughout their lives on a special secretion or "royal jelly" by the worker nurses. Unlike other colonial hymenoptera, the larvae of honeybees do not return any secretion in return of nourishment supplied by the nurse bees. However, a potent pheromone or queen substance can suppress ovarian development in workers that imbibe it, since most colonies have one solitary female reproductive. This action in colony adhesion is supplemented by food exchange between adult bees, by need to conserve warmth by clustering, and by visual factors and responses to vibration and odours.

The presence of an active king and queen in a termite community also inhibits development of other reproductives except in remote parts of the colony. Caste determination (it is now believed that each newly hatched nymph has the potential to develop into any caste) is under hormonal control and is passed from individual to individual by licking and food exchange and is able to induce specific actions, such as stimulating the development of reproductive organs or inhibiting the development of particular structural features. Nutritional control, the amount and type of food as well as tactile and olfactory stimuli also play an integral part in caste determination in Isoptera.

Aggregation

An aggregation pheromone has also been noted which keeps members of a specific population grouped together. Such substances have been recently recognized in members of Blattodea or

cockroaches accounting for their typical aggregation in harbourages.

Figure 8. Major pheromone components of some species of Coleoptera, Lepidoptera and Orthoptera infesting stored products.

Sourece: Levinson and Levinson (1978); and Burkholder (1978) from Proceedings of the Second International Working Conference on Stored Products Entomology, September 10-16, Ibadan, Nigeria.

FAMILY SPECIES AND SEX THAT PRO DUCKS PHEROMONES	**CHEMICAL DESCRIPTION AND PHEROMONES**
Dermestidae:	C = O OH
Attagenus megatoma (F.)	(E, Z)-3, 5-tetradecadienoic acid
Attagenus elongatulus (Casey)	C = O OH
	(Z, Z)-3, 5-tetradecadienoic acid
Anthrenus flavipes (Leconte)	C = O OH
	(Z)-3-decenoic acid
Trogoderma inclusum (Leconte)	C = O H CH_3
Trogoderma variabile (Ballion)	(Z)-14-methyl-8-hexadecenal
Trogoderma glabrum (Herbs")	C = O H CH_3

	(E)-1 4-methyl-8-hexadecenal
Trogoderma granarium (Everts)	C = O H CH_3
	(Z)-1 4-methyl-8-hexadecenal (92 %)
	CH_3 C = 0 H
	(E)-14-methul-8-hexadeceanal (8%\
Anobiidae	2, 3-dihydro-2, 3, 5-trimethyl-6
Stegobium paniceum (L)	(1-methyl-2-oxobuty 1)-4H-pyran-4-one
Bostrychidae	1 -methyl buty 1 (E)-2-methyl-2-pentenoate
Rhyzopertha dominica (F)	1-methylbutyl (E)-2, 4-dimethyl-2-pentenoate
Bruchidae	C = O OCH_3
Acanthoscelides obtectus (Say)	
	methyl (-)-(E)-2, 4, 5-teteadecatrienoate
Tenebrionidae	1-pentadecene
Tribolium confusum (J. du Val)	
	1-hexadecane
	1 -heptadecene
Gelechiidae	

Sitotroga cerealella (O.)	CH_3 OC = 0
	(2, E)-7, 11-hexadecadien-1-yl-acetate
Phycitidae	
Ephestia elutella	
Plodia interpunctella	CH_3 OC = 0
Ephestia cautella	
Ephestia kuehniella	(Z, E)-9, 1 2-tetradecadien-l-yl-acetate
Ephestia figulilella	
Blattidae	CH_3 CH_3 O
Blattella germanica	3, 11 -dimethyl-2-nonacosanone
	HO O CH_3 CH_3
	29-hydroxy-3, 1 1-dimethyl-2-nonacosanone

Stimulation of the mating process

Both male and female pheromones of phycitid moths seem to be important and beetles such as the khapra beetle, Trogoderma granarium Everts and Attagenus megatoma (A. pellio, the fur beetle) have been shown to emit pheromones in the mating process (See Fig. 4).

Other developmental effects

An interesting substance from the fifth instar larvae of E. Kuehniella accumulates when population densities increase, increasing the developmental period, reducing fecundity of the female and promoting migration.

Pheromones themselves may therefore be used in specific behaviour control programmes, but have been more importantly used in estimating population densities, to predict the timing of control measures. In the more restricted environment like a warehouse, they can be used as a disruptive or confusing agent which completely swamp naturally produced pheromones of the female and therefore inhibit mating. Successful control in this way has been achieved with the mediterranean fruitfly Ceritatus capatita, the oriental fruit moth Cydia molesta and the cabbage moth, Plutella tella xylosterva

THE POTENTIAL OF IGRs AS PEST CONTROL AGENTS

Although still very much in its infancy as a control measure in post-harvest storage of cereals, the potential of IGRs has been documented in laboratory trials and has undoubtedly a role to play in future strategies involving an integrated approach for control of insect pests.

McGregor and Kramer (1975) have shown that whole cereals treated with IGRs are not effective against the primary or internal grain feeders such as Sitophilus spp. For JH analogues to be effective against weevils, they will have to penetrate into the endosperm. Rowlands

(1976) reported that similar compounds were preferentially distributed in the aleurone and germ layers and therefore insufficient IGRs were found in the endosperm where the immature weevils are developing.

Because IGRs tend to be specific for certain related groups of insects, combinations with biological control agents becomes a feasible control approach.

Bracon hebator a parasitic wasp of many stored products moths is not as greatly affected as its hosts by certain IGRs. Treatment with 50 ppm of MV-678 allowed 100% eclosion of the parasite but reduced adult emergence of Ephestia cautella (Walker) the "tropical warehouse moth", by 85%. Also, the prolongation of larval life would be seen as an advantage by maintaining housts in a suitable state for the parasites, and therefore maintaining their population numbers. Preventing the eradication of beneficial parasites and predators as well as maintaining low numbers of hosts by the application of specific IGRs may become a viable alternative to the overall detrimental effects of broall-spectrum, nonspecific insecticides.

JH compounds may prove more useful as grain protectants (i.e., preventative) rather than infestation control measures (i.e., curative). When applied to insect free grain or grain containing relatively low numbers, they could prevent buildup of infestations. Under these conditions, prolonging development of small numbers of immature larvae would prove insignificant, as little contamination or loss would occur. In combination with more deleterious control measures at lower rates than are now normally used, would also be effective where JH analogues are applying stress to existing populations.

Application of IGRs that possess juvenile hormone activity to larvae prevents subsequent development to the adult stage, thus reducing the reproductive potential of the population. it becomes clear that the usefulness of IGRs in pest control is under those conditions where the adult is the harmful stage of the insect pest.

Synthetic IGRs are degradable in sunlight, under high temperatures and humidity, or in water with high microbial populations. Methoprene (Altosid, ZR-515) has been successfully used against flood water mosquitoes. Anopheles spp. in a micro-encapsulate slow release formulation, which extends its persistence in water.

The integration of IGRs and pheromones can also become a viable component of an integrated pest management concept. Pheromones have shown to disrupt mating of Plodia interpunctella (Hub.) at low population densities (Sower et al., 1975) while low adult numbers could be achieved by the application of IGRs which inhibit metamorphosis and adult eclosion, or at lower concentrations, possess a sterilizing effect.

Levinson and Levinson (1978) have described the practical application of the use of pheromones in an integrated pest control programme. It comprises of a continuous monitoring and supervision system of a pest population by pheromone traps in combination with appropriate insecticide control measures implemented with respect to population densities found by trapping. Three treatments phases are involved as described below.

The first phase aims to detect an infestation and to estimate its magnitude. For this purpose a

few permanent traps are strategically placed in the storage environment. In the second phase, suppression of the discovered population below the economic threshold level is required. This step demands an adequate increase in the number of suspended pheromone and food attractant traps. Control of localized infestations may also be supplemented by insecticide application or fumigation (e.g. with phosphine). In the third phase, complete fumigation of the storage environment is carried out if and when a dense insect population has become evident. If rapid insect eradication in a commodity is necessary, one has to proceed directly from the detection of an infestation to complete fumigation. Integrated manipulation of storage insects by pheromones combined with insecticides is considerably cheaper than insecticidal control alone. It also meets the demand for methods of prevention rather than of control.

Burkolder (1978) has similarly advocated the potential of pest management systems that integrate the use of chemical pesticides, biological chemicals such as pheromones and juvenile hormone analogues or mimics, biological control agents such as parasites, predators and microbial pathogens, physical and nonchemical methods such as appropriate design of facilities, aeration, controlled atmosheres, thermal disinfestation, as well as cultural (sanitation) and legislative control measures.

Several approaches with pheromones could be utilized. As mentioned previously, indirect control by using pheromone traps to monitor presence, population density and location of pest species, in order to evaluate the timeliness of an applied pesticide application could form one strategic approach.

Another approach would be direct control by using pheromones to discrupt communication between sexes, by mass trapping, or by luring insects to nonfood materials treated with pesticides. However, the effectiveness of pheromones in the context of communication disruption is reduced under high population densities, while mass trapping would be substantially more effective if both sexes were removed. This may be possible for example with the population agregation pheromone, (1-methylbutyl (E)-2methyl-2-pentencote) produced by the male of the lesser grain borer, Rhyzopertha dominica (F) either in suspended traps or traps placed directly on the grain surface. These traps can then be impregnated with insecticides, therefore reducing the concentration and problems with residues since the insects are lured to the toxicant which can subsequently be removed.

It is also possible to use pheromones to lure insects to devices containing entomopathogens. The approach will not produce immediate mortality as with the insecticide baited traps, but the infected insects would return and transmit the disease spores to other members of the population. To attract insects to a reservoir containing a pathogen such as Bacillus thoringiensis, either pheromones or light could be used.

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Host plant and varietal resistance to post harvest insect attack

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by R.L. Semple

INTRODUCTION

It is now well established that various staple cereals, such as rice, maize, wheat, sorghum, oats and barley, and legumes such as cowpeas, vary quite significantly in their inherent resistance or susceptibility to both field infestations, and to postharvest insect attack in storage by the more recognized grain storage insects.

The full yield potential of the growing crop is seldom realized due to the interaction of many factors (climatic, ecological and edaphic) of which pre-harvest insect infestation and consequent damage is one of the most important.

Studies in Nigeria by Raheja (1976), as reported by Dobie (1981), have revealed that field pest infestation in cowpeas can reduce the yield of that crop by 92-97% of the potential yield in the absence of insects, principally by the bruchid, Callosobruchus maculatus. Pimentel (1978) estimates crop losses due to pests to be around 35% worldwide, while post-harvest losses may range from (a maximum value of) 9% in the United States, to 20% or more in some developing countries, especially in the tropics. Total losses could therefore approach almost half of the potential yield of that particular crop, if certain restrictions on the development of pest populations is not implemented.

Plant breeders at the International Rice research Institute (IRRI) believe that rice intensification programmes can be achieved by:

1. **producing high yielding varieties (HYV's) with inherent disease and pest resistance characteristics with maximum protein content and enhanced taste which will make these**

varieties more acceptable to consumers than the traditional varieties;
2. multiple cropping characteristics of introduced dwarf HYV's that allow two or more crops per growing season due to the shorter period required to maturation (15 weeks instead of 25 weeks);
3. minimizing field crop losses by improved agronomic and cultural practices, water management, and improved fertilizer and pesticide usage in terms of frequency and timeliness of application.

The so-called "Green Revolution" has in fact intensified these losses by;

1. increased susceptibility of HYV's whereby they have become biological weaklings unable to compete in a natural environment, or reach their full yield potential, because of their heavy dependence on fertilizers and pesticides for disease, insect and weed control;
2. market specifications demanding product uniformity have all too frequently narrowed the gene pool from which we select our varieties, thus reducing the number of genotypes that naturally harboured alleles resistant to insects and other pests. Genetic uniformity tends to provide an ideal ecological environment for pathogens to evolve and severely attack these genotypes, and;
3. continuous cropping techniques ("monoculture", or the establishment of "homoclines") provides a ready source for infestation by a range of pests such as insects, mites and nematodes, and infection by bacterial and fungal pathogens from one crop to the next.

One can conclude from these observations, that there has not been a reduced reliance on the

use of applied pesticides with the introduction of improved varieties, but in fact a higher requirement for increased pesticide and fertilizer usage to adequately protect and sustain these crops (rice and cotton are too good examples).

To meet the growing demand for rice in Southeast Asia, increasing areas are being brought under irrigation (since the HYV's do not perform well under waterlogged conditions) and existing irrigation areas are being more intensively managed through the continued widespread introduction of HYV's in a multiple cropping system. This changing production pattern has created post-production problems that were not so obvious with the traditional, "non-improved" varieties, and traditional cultural practices. With the advent of multiple cropping, the offseason crop is therefore harvested in the wet season, where the combination of high moisture and the inability to adequately dry the crop by traditional sundrying methods have spawned the problem of rapid biological deterioration, manifested by discoloration and fermentation, before and during storage. In addition, the shortseason, photo-insensitive HYV's typically grown in a multiple copping system are easy shattering, and tend to have a wider range in kernel maturity than the traditional varieties at harvest, resulting in a higher percentage of high moisture, immature kernels or chalky kernels in storage. Therefore the introduced HYV's as a solution to the problem of food shortage for an evergrowing population has accentuated post production losses. These are likely to become increasingly important as production limits are reached due to limited availability of arable land, and the increase in production costs because of the increasing costs of required inputs, such as pesticides and fertilizers.

Apart from the increased strain on post production systems to adequately dry and store the increased production of paddy, it has also been repeatedly demonstrated that the HYV's developed by plant breeders are more susceptable to insect attack than the traditional, non-improved varieties, and that the increased yields per hectare being experienced in the field, can be lost due to their greater, inherent vulnerability to stored products insect pests in storage.

In small-scale storage systems where adequate pest control techniques are not employed, due to volume stored or rapid turnover of stocks, the advantages of varieties less susceptible to damage and loss inflicted by storage insects becomes very obvious. Haines (1982) reporting on unpublished work of Supriati in Indonesia, showed that among eight rice varieties grown in that country, the weight loss of paddy caused by an introduced infestation of Rhyzopertha dominica was more than seven times (7x) the loss on the most susceptible variety in the first two (2) months of storage, and had expanded to more than ten times (10x) in the third and fourth month of storage, when compared to the most resistant variety. Even in stores where pest control techniques are normally used, the utilization of the most resistant variety would lead to a reduction in the costs of whatever pest control strategy was employed. Duff (1979) found that in the Philippines, farm stored grain was generally in good condition because they gave a high priority to cleaning and drying that portion of the crop which was destined for home consumption. The storage problem is most definitely compounded with the marketable surplus. This is specifically with national government agencies maintaining buffer stocks in lieu of their price support/stabilization programmes, where stocks oftentimes enter long-term storage, and in spite of minimum quality standards, are buyers of the last resort.

PRINCIPLES AND TERMS USED IN INSECT RESISTANCE TO PLANTS:

The rate of increase of a pest population is influenced by a multitude of many interacting factors, one of which is the quality characteristics of the food medium on which the pest/pests are feeding. Many varieties of the same grain species appear to be less suitable than others for insect development, and are often described as being "resistant" (or in fact, less susceptible) to insect attack. Different definitions of resistance have been put forward. These include,

''relative amount of heritable qualities possessed by the plant which influence ultimate degree of damage done by the insect" (Painter, 1951)

"The collective heritable characteristics by which a plant species may reduce the probability of successful utilization of that plant as a host by an insect species" (Beck, 1965)

"Resistance is all those heritable traits of a plant, that lessen insect damage, while other plants of the same species and in the same environment receive greater damage. Resistance is therefore a relative phenomenom" (Owens, 1975)

It must be remembered that stored products insect pests are capable of inflicting serious damage to stored commodities, due to their very rapid capacity to increase in numbers, and to migrate and infest separate lots thus spreading and expanding the infestation. The use of resistant varieties, particularly in farmers and village cooperative stores, will more than likely extend the period that produce can be stored safely without the use of pesticides. When

susceptible HYV's have been planted, rural storages maybe forced to utilize expensive pesticides that are often unavailable, or if so, in packaging and formulations that are not suitable for small-scale use.

Tolerance as a particular mechanism for resistance in actively growing crops in the field is related to endurance to insect attack and repair capabilities once pests are established. This component of resistance is therefore not applicable to grain in storage, since although seed respiration continues, individual kernels do not possess the capacity to "tolerate" damage by the above mechanism. Preferential feeding activities on the germ or starchy endosperm, irreversibly effect seed viability, dry matter loss and increases in moisture content that cannot be compensated for by the grain. Resistance to post-harvest insect attack is therefore attributed to the interrelated component factors of antibiosis and nonpreference.

An insect population will rapidly increase in numbers when 1) a high rate of egg laying is achieved, 2) growth and development is rapid, and 3) when the mortality rates are so low, that few insects die before reaching sexual maturity, and begin producing progeny.

The rate of population increase can therefore be adversely effected when utilizing a resistant variety that it;

1) causes a reduction in the rate of egglaying by;

- **varieties with mechanical or physical barriers that prevent access thus reducing the**

number of eggs laid and therefore the insects productivity,

- **varieties that repel (anti feedants) or that are unattractive to them,**
- **varieties that are unsuitable for ovipostition either because they are too hard for species that adhere their eggs to smooth substrates,**

AN D/OR

2) extends developmental periods by:

- **hard-textured varieties that are difficult to injest or digest,**
- **varieties that are partially toxic or antibiotic,**
- **varieties that are nutritionally inadequate to support optimal development rates of the pest, AN D/OR**

3) causes high mortality of the immature and developing stages of insects, therefore reducing the number of sexually reproductive adults emerging by;

- **varieties that cannot be penetrated by the larvae after hatching from the eggs, and therefore restrict their feeding capability,**
- **varieties that are nutritionally inadequate or toxic to the feeding insects.**

In this context, antibiosis refers to plant characteristics that adversely effect insect mortality, size, and life history. The abnormal effects that may occur when a particular insect population

feeds on an antibiotic plant (or seed) are;

1. **death of early stages of larvae (or nymphs) resulting in little or no insect infestation;**
2. **death of pupae and late larval or nymphal stages reducing the size and potential of the next reproductive population;**
3. **decreased fecundity reducing subsequent insect populations;**
4. **reduction in the size of insects that emerge;**
5. **reduction in weight of those insects surviving and emerging where both 4) and 5) have cumulative effects on insect feeding ability and increase the effects of environmental variation on the insect;**
6. **decreased accumulation of insect food reserves affecting survival potential during periods of hibernation and aestivation (diapause);**
7. **abnormal behavioural and physiological affects reducing the ability of the insect to continue feeding; and**
8. **abnormal survival/longevity where immature survival may be unduly extended (a juvenile hormone effect). This exposes nymphs and larvae to natural enemies leading to fewer generations in a given time period; and, reduced adult life which limits time for mating and oviposition, therefore severely reducing fecundity.**

Certain phenomena which are not heritable traits of the host plant to induce resistance (either single, or more preferably, multiple resistance) have been described by Horber (1983) as "pseudo resistance factors". Some have been utilized to great advantage in economic entomology, and play a substantive role in stored grain protection. These include;

1. **Evasion by potential hosts when insect numbers are low, such as when some cultivars mature early and evade field infestation (lack of "phenological coincidence").**
2. **some individual kernels in a generally susceptible host variety may escape infestation as a purely random event. Insects in stored grain are generally not randomly distributed and ate located (and survive) in large numbers where conditions are most amenable and accessible. Grain outside of this area may therefore not become sensibly infested.**
3. **Temporary resistance of a susceptible host maybe induced by treatment with oils, repellents or inhibiting materials. The possibility of substituting insecticides with repellents or feeding inhibitors, such as extracts of neem and black pepper, and the use of various vegetable oils has been covered elsewhere, while trypsin inhibitors have been investigated in wheat for their effect on Sitophitus oryzae by Su, et.al., (1974).**

SOME CAUSES OF RESISTANCE:

PHYSICAL:

Much of the present knowledge on the resistance of crops to storage pests has been primarily concerned with establishing those factors that confer resistance. It is considered essential knowledge for plant breeders wishing to embark on breeding programmes to incorporate inherent varietal resistance to storage pests as an integral and desirable selection criteria for the suitability of varieties that are likely to be released for future productivity programmes.

1) Physical barriers:

The extreme value of a complete, well-fitting set of sheating leaves in maize to reduce pre-shelling infestation by Sitophilus spp. has been well recognized for many years. Tight husks are an important resistance mechanism for maize, wherever climatic conditions encourage field infestations.

In unhusked rice or paddy, the tightness of the glumes that cover each grain is of primary importance in reducing damage done by Rhizopertha dominica, Sitophilus oryzae, and Sitotroga cerealella, the success of these species, particularly Sitophilus spp., being dependent on the presence of hull defects. Rosotto (1966) screened 1700 varieties of rice collected worldwide for resistance to S. zeamis and found 80% were strongly resistant and directly related to the tightness (and integrity) of the husks surrounding the rice kernels. The varieties in which weevil progeny were produced had separated or gaping glumes, or had suffered some form of physical damage. This substantiated the findings of Breese (1960) where infestations did not develop in grain that possess intact husks. Feeding ability of the females and oviposition is directly related in Sitophilus spp., whereby if feeding is inhibited due to the inpenetrable barrier afforded by the husk, then oviposition will likewise be inhibited. Larvae are quite incapable of penetrating or feeding on sound intact paddy grain, even if eggs are deposited loosely amongst the grain. Eggs are normally laid outside the grain by females of Rhyzopertha dominica and Trogoderma granarium, and entry into it is achieved by the 1st instar larva, but in sound paddy, it is only the more well developed larvae that are capable of penetrating the husk. Larvae of Rhyzopertha dominica, as well as exploiting any splits or physical defects, can eventually gain entry via the inflorescence attachment (rachis) into the husk, and is more accessible when the kernels are not fully mature since it is softer and has

less contraction of the pith, but survival is likely to be low. Larvae of Sitotroga cerealella are very adept seed penetrators, even when the husk is complete, but do not pose a serious threat in bulk stored paddy except for surface infestations, because of inability of the larva to penetrate deeply in the bulk, thus restricting its spread. Stored ear corn does not offer the same problem to S. cerealella larvae and is therefore better stored in bulk in shelled form. Many other factors other than genetics can affect the husk condition such as weather, fertilization rate, plant diseases and harvesting machinery. The degree of husk damage, amount of husked and broken kernels, immature, unfilled and shrivelled grains tends to be higher in combine harvested paddy than paddy harvested and threshed by traditional methods. The amount of physical damage to intack hulls and the high percentage of separated and gaping glumes that occurs in HYV's allows S. oryzae and R. dominica to occur frequently and in abundance in stored paddy in Southeast Asia.

Hussain, et.al., (1983) have also shown that S. oryzae has a strong competitive advantage over S. zeamais on paddy based on the averaged index of susceptibility in laboratory trials of 4 HYV's commonly grown in Indonesia (IR 36, IR 42, Cisidini and Cisidane) and confirms the species dominance observed in field surveys (Haines and Pranata, 1982). The most susceptible varieties in paddy form were those displaying widely divergent and gaping hulls.

Cogburn (1977) tested the effects. of R. domonica, S. oryzae and S. cerealella on weight loss and milling yield of six commercial rice varieties. After 3 generations, the lesser grain borer inflicted more weight loss of paddy than the "Angoumois grain moth," while the "rice weevil" inflicted the least weight loss. Milling analyses showed that the "rice weevil" again caused the

least loss, but the grain moth caused a higher loss in total milling yield than the "lesser grain borer" even though both adults and larvae are voracious feeders. This was due to preferential areas of feeding and activity. This study underlines that the loss potential of rice is dependent on variety, storage time and species of insects present.

Rogers and Mills (1974) screened over 1500 sorghum varieties and also found tight glumes covering the kernels conferred almost total immunity to S. zeamais.

Naked barley varieties are also less inhibitory to S. oryzae yielding more progeny than barley with covered kernels. The seed pods of some legumes may also provide an efficient barrier to insect infestation, thus storage should be in unshelled form to take advantage of this relatively "cost-free" form of control.

The seed coat of food grains may also be sufficiently thick and tough so as to inhibit penetration to a degree, even though primary feeders are well adapted to chew into whole undamaged kernels. Certain characteristics of the cellular structure of the seed coats of some cowpea varieties (Vigna unguiculata) partially prevented entry of 1st instar larvae of Callosobruchus maculatus. In highly infestible commodities such as wheat, the absence of broken grain on which adult Rhyzopertha dominica can feed reduces the ovipositing rate to about 12% of the normal rate. The efficiency of penetration in whole grains of wheat is also strongly influenced by the presence of defects in the outer layers of the grain, so that varieties that possess strong, intact outer layers do exhibit a certain level of resistance to Rhyzopertha dominica, perhaps best reflected by the rate of population buildup.

Damaging the pericarp of maize (yellow corn) in some way increases its susceptibility to S. zeamais (Schoonhoven, et.al., 1976) Sitotroga cerealella develop very slowly on pellets composed of pure wheat endosperm, but when a small percentage of pericarp (bran) is added, the rate of development is enhanced, but then retarded and finally unsuccessful when the proportion of bran added was further increased, thus indicating that certain factor(s) in the pericarp offers resistance to entry in whole kernels.

The intack hull character is therefore not likely to be completely effective against all stored grain insect pests. Cogburn (1974) selected kernels of the commercial variety "Belle Patna" microscopically for intack hulls and subjected small samples to S. oryzae, R. dominica, and S. cerealella. S.oryzae adults starved to death and did not reproduce, while immature mortality was 91 % in R. dominica and 71% in S. cerealella. In a large commercial bulk of this variety, there would be an abundance of infestable kernels to allow survival and development of all three species. The rapid multiplication of a population of R. dominica in paddy is dependent on certain varietal characteristics such as the failure of the husk to close properly around the kernel which renders a small proportion of the bulk infestable to both S. oryzae and R. dominica. Also, rapid population growth is dependent on adequacy of food to maintain oviposition in the form of split husk grains, hulled grains, kernel fragments and immature paddy, and in the most part, is dependent on the methods of harvesting and threshing. Parboiled paddy when dried to 14% MC (w.b.) differs little in volume to that of raw paddy (12%), but the husk components once separated by the swelling of the kernel, do not close fully on drying, therefore allowing access by larvae of R. dominica and increasing its infestability. While parboiling allows larvae of R. dominica to gain access and attack the germ,

the separated glumes also facilitates oviposition by S. oryzae, but is generally less infestable than raw polished rice because the endosperm has been toughened or hardened either peripherally or throughout, during the parboiling process.

2) Limited oviposition sites:

Nwanze, et.al. (1975) have found that cowpeas with smooth seed coats were more susceptable to Callosobruchus maculatus than varieties that possessed rough seed coats, the same separation of resistance being displayed by other legume seeds. Nwanze and Horber (1976) suggest that those bruchids that attach their eggs to the external surfaces of legume seeds lay fewer eggs on rough coated varieties than on smooth-coated varieties.

Members of the Bruchidae specialize on feeding on a wide variety of grain legumes with medium-sized, hard seeds. Horber (1983) reporting on work by Jansen (1969) claims that due to natural selection, grain legumes attacked by bruchids have adopted a common strategy of producing smaller seeds per unit of vegetative growth than those species not generally subject to infestation. Larger-seeded varieties of cowpeas (Vigna unguiculata) are better hosts to the cowpea weevil, Callosobruchus maculatus, while fewer progeny emerged from smaller cowpea seeds (Nwanze and Horber, 1975). Thickness of the seed coat in Bengal gram (Cicer arietinum) appeared to influence preference by the pulse beetle, Callosobruchus chinensis, rather than the size of the seeds (Gupta, 1970).

Resistance to the adzuki bean weevil or pulse beetle in chickpeas was attributed to the rough,

nearly spiny seed coat, which effectively inhibited oviposition (Brewer and Horber, 1983). Broadbean (Vicia faba) was not preferred for oviposition by C. chinensis even though it possesses a relatively smooth seed coat, and was shown that most of the larvae died during the first instar due to inability to penetrate the thick seed coat. Brewer and Horber (1983) concluded that larval antibiosis due to mechanical, physical or biochemical factors, and ovipositional antexinosis (properties of the host plant that affect behaviour during search and host selection, such as in the resistant chickpea variety) are assumed to be present. Cowpea (Vigna unguiculata) and lentils (Lens culinarus) were preferred for oviposition, but few progeny emerged, possibly due to high levels of trypsin and chromotrypsin inhibitors in cowpeas.

C. chinensis and C. maculatus both develop much slower on lentils than other members of Leguminoseae. All mungbean (Vigna radiate), pidgeon pea (Cajanus cajan) and adzuki beans (Phaseolus angularus) were susceptible to C. chinensis in this study. Ovipositional preference is not however, an indication of the suitability of the host for insect development. The percentage reduction in germination of infested legumes is also not a good basis for assessing resistance. Germination reduction has been correlated with the number of emergence holes in the seeds, where with more holes, germination was reduced, slower and seedling vigour in those that did succeed in germinating was also reduced. (Southgate, 1979). However Brewer and Horber (1983) were not able to establish any definitive relationship between percent reduction in germination and numbers of insects or weight loss (naturally, a positive correlation existed between numbers of emerged weevils and grain weight loss in the preference for oviposition and feeding damage test!). In preferencial order for the growth and development of C. maculatus, Singh, et.al. (1980), infested green gram, cowpea, red gram,

Bengal gram, black gram, pea and lentil, and reported that lentils increased the developmental period but retarded per cent emergence, size and weight of aduts, fecundity and longevity.

The structural qualities of seed pericarp in legumes have not been fully explored (Horber, 1983). These include the effects of glossiness, stiffness, permeability for gases and water vapour, impact and rupture strength which need to be substantiated with modern equipment to elucidate their protective role in preventing oviposition, penetration and adult emergence (from it).

With respect to cereals, Cogburn (1974) experimented with six commercial rice varieties grown in the United States that differed in their percentage of intact hulls. Female S. cerealella frequently deposits her eggs inside a grain with a broken or gaping hull. Breese (1960) states that the preferred oviposition site is a crevice, especially one in which the surfaces are not smooth, and it maybe for this reason oviposition does not normally occur on milled or polished rice. The husk, although difficult to penetrate, does in fact provide a better attachment for the entrance cocoon of the first instar larva than the smooth exterior of the milled rice kernel. Of the eggs deposited on any variety, the percentage inside glumes was directly proportional to the number of open glumes available, with the variety possessing the most open glumes receiving the most eggs, and the reverse being true for the variety possessing the least number of open glumes. However, the preference of the moth for the varieties for oviposition sites was not directly proportional to the open glumes available and tended to be more or less random between the two extremes.

As previously mentioned, feeding ability and oviposition are almost inseparable in Sitophilus spp., where if females are to oviposit in any grain, it is necessary for her to bore the hole in which the egg willy be laid. Females of R. dominica prefer to oviposit on rough surfaces and has a strong preference for crevices. Few eggs are laid loose according to Birch since many would be destroyed (and in fact, are) by the feeding and wandering activities of the adults. The preferred site in wheat are the cracks in damaged grain, or in the crease or under the loose testa in sound kernels. Unhulled rice is extremely rough, but when intact, may lack the type of crevice favoured by P. dominica for oviposition.

3) Seed hardness:

The hardness of seeds has been demonstrated, especially in cereals, as affecting the successful and rapid multiplication of insect pests. Hard, flinty maize varieties are often more resistant than soft, floury varieties, where those maize varieties that possess the "opaque-2" gene in their genotype are abnormally soft and reflects their high degree of susceptibility to attck by S. zeamais and S. cerealella. The good nutritional characteristics of these particular varieties must be combined and selected for hard kernels to enhance their storability.

Relative hardness of cereal grains has been measured various ways. One method is pearling a grain sample for a specified time and measuring the weight of the abraded material, the less weight loss inferring greater grain hardness. Russell and Rink (1965) found the relative hardness of various sorghum varieties was a dominant factor in affecting the oviposition and adult emergence (survival) rates of both S. oryzae and S. zeamais, where relatively soft

varieties received as much as 2-6x more eggs than relatively hard varieties. Davey (1965) reported that the number of S. oryzaecompleting adult development form "mealy" (soft) varieties of sorghum could exceed the numbers emerging from "vitreous" (hard) varieties by a factor of 20 after only a single generation. A positive relationship exists between weight loss from sorghum samples by pearling and the number of eggs of S. zeamais laid, and the percentage survival of newlyhatched larvae to adulthood in S. cerealella (Rout, 1973).

Relative hardness has also been determined by the depth of impression made by a sharp object such as a diamond point under a constant weight. Hardness may however, not be the only factor affecting penetration, and the toughness and thickness of the pericarp may influence the impression made. A consistent difference was established between 5 isogenic lines of maize with genes for high Iysine content which were softer, and maize varieties with normal endosperm using the same determination for hardness as Rout (Schoonhoven, et.al., 1972). Dobie (1974) reviewed the susceptibility of 25 varieties of maize to S. zeamais utilizing his well known "Index of susceptibility" which takes into account both survival and adult emergence (number of F1 progeny or a measure of productivity) and the speed of development (average developmental period of these progeny). The hardness of the kernels, as estimated by the proportion of floury endosperm was related to susceptibility, and grain hardness was closely related to amylose content of the endosperm. Amylose content may also affect susceptibility via a different mechanism other than grain hardness, and susceptibility in those varieties tested was related to factors operating after oviposition, based on the number of egg plugs between susceptible and resistant varieties.

Rout, et.al. (1976) studied the relative susceptibility of 8 dehusked, high yielding paddy varieties to S. oryzae using the same demographic parameters of developmental tiome and numbers of F1 progeny emerging on each variety as Dobie, and found a positive relationship between grain hardness and resistance with the relatively more susceptable varieties producing heavier weevils. Hardness was estimated by alkali reaction and pressure exertion, where the pressure (in kg/cm) required to cause cracking was taken as a measure of relative harndess.

NUTRITIONAL FACTORS:

Maize with high lysine content has received particular attention as a method of increasing protein quality for people in developing countries that rely on maize as a staple and where other sources of protein are deficient. The study of Schoonhoven, et.al. (1972) as previously mentioned did not demonstrate any correlation between high lysine content in maize lines incorporating the "opaque-2" or "floury-2" genes, and resistance to attack by S. zeamais compared to normal endosperm maize of the same line. Significant differences in the emergence of weevil progeny were noted however, between high lysine maize of different lines, or normal endosperm maize of different lines. Tests preformed by the International Maize and Wheat Improvement Centre in Mexico (CIMMYT) have indicated that as the level of tryptophan and lysine is increased, the numbers and weights of emerging S. zeamais correspondingly increased as well. LeCato and Arbogast (1974) found 3 species of stored products insects multiplied faster on 2 hybrids of high lysine content, but 6 other species did not. Rout, et.al. (1976) also found no consistent relationship between the starch and protein

content of brown rice of different varieties and their relative susceptibility to S. oryzae in their study.

Amos et.al., (1988) studied the development of S. granarius, R. dominica, O. surinamensis and T. confusum on 23 varieties of wheat. Using step wise regression analysis on the data for each moisture content Since each variety was equilibrated to the experimental conditions of 26C and 60-70% RH) significantly influenced th productivity of S. oryzae, S. granarius and R. dominica.

High lysine content cannot be concluded categorically as conferring resistance or susceptibility, since its effect on physical and chemical characteristics of the endosperm have not been elucidated.

CHEMICAL CAUSES OF RESISTANCE:

Toxins:

Most food crops do not contain toxic substances to insects, and if they are naturally present, they probably exist in concentrations that would not significantly affect insect or man. However, certain components that maybe in toxic levels to insects and rendered harmless to man by preparation and cooking are known in legumes or pulses and in some rootcrops, notably cassava.

Cowpeas (Vigna unguiculata) are generally very susceptible to attack by C. maculatus and cause substantial losses. The Grain Legume Improvement Programme of the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria has the global mandate for improving cowpea production, and considerable effort has been devoted to developing resistant varieties in conjunction with the Tropical Development Research Institute (TDRI, formerly TPI) of the Overseas Development Administration (Dobie, 1981). One variety form Northern Nigeria displayed resistance to a strain of C. maculatus emanating from Brazil, which was important if cowpea production was going to be substantially increased in that country. Females of C. maculatus readily laid eggs on the seed surface, and larvae began feeding on the underlying cotyledons, but growth was extremely slow, and eventually most if not all the larvae died. Eighty to ninety-five percent (80-95%) of larvae completed their development on the control susceptible varieties, strongly suggesting the presence of a toxic or antibiotic compound(s). Saponins (triterpenoid glycosides of which 5 aglycones have been isolated in soybeans toxic to Callosobruchus spp.) and lectins (phytohaemag-glutinins found in beans, Phaseolus vulgaris (Jensen, et.al., 1976) were not found, but the resistant variety was shown to contain twice the level of a protease inhibitor (trypsin inhibitor) than the susceptible varieties, and was found to be inheritable, but its stability was unknown (Dobie, 1981). Trypsin is a peptidase enzyme splitting proteins at certain specific peptide links and is secreted as trypsinogen from the mammalian pancreas, occurring in the digestive juices of most animals. Peptides are compounds formed from two or more amino acids by the amino group (NH2) of one joining the carboxyl (COOH) group of the next forming peptide bonds (-HNCO-) with the elimination of water. Peptones are large fragments of peptides formed by the splitting of proteins by

peptidase or protease enzymes. Trypsinogen inhibitors are also known in maize starch.

Most proteinaceous inhibitors have been , orated from the seeds of different members of Leguminosae. Refined extraction methods have isolated three proteinaceous fractions from soybean which strongly inhibit the growth of T. castaneum larvae; and more inhibitors have been isolated from wheat and lima beans (Applebaum and Birk, 1972). Certain cultivars of groundnuts and chickpeas possess higher concentrations of specific J. castaneum protease inhibitors, which would at least confer some partial resistance to damage in storage, and increasing their concentration via breeding programmes would not be detrimental to human and animal nutrition (Hober, 1983).

Apparently, L-canavanine, which may contribute from 8-10% of the seeds dry weight in some legumes of the family Lotoeideae, is toxic to insects and higher animals due to disruption of normal protein synthesis. L-canavanine indescriminately replaces Larginine in structural proteins which are physiologically incompetent.

Amalyase (or diastase) is a group of enzymes which split starch or glycogen variously to dextrin, maltose and glucose and are widely distributed in both plants and animals (such as in malt, pancreatic juices and in microorganisms) and can be purified by Affinity Chromatography.

A glycoprotein from red kidney bean (Phaseolus vulgaris) which inhibits mammalian amylase, has also been shown to inhibit amylase activity in insects such as the yellow mealworm Tenebrio molitor, the larvae of the mediterrancean flour moth, Ephestia kuhniella, and adults

of the confused flour beetle, Tribolium confusum. (Powers and Culbertson, 1982; 1983). The rate at which the bean glycoprotein inhibits insect amylase and therefore their ability to breakdown starch to glucose, is dependent on pH, temperature and ionic strength.

Glycoprotein amylase inhibitors from Phaseolus vulgarus beans have been purified by either conventional techniques or affinity chromotography. Bean amylase inhibitors apparently inhibit mammalian but not microbial or plant amylases.

Bean amylase inhibitors have been suggested as a developed protective agent against insect attack. In the study of Powers and Culbertson (1983), Tenebrio molitor or the "yellow mealworm" was selected as the test insect since its amylase has been previously purified and characterized, and studied with its interaction with wheat amylase inhibitors. The rate of combination for the inhibitor and amylase at 30C and pH 5.4 (optimum for this enzyme) was calculated as a second-order rate constant of 2.7 x 105 per mole per second. At pH below 3.8, very rapid and irreversible loss of enzyme activity was found, and is similar to previous observations of the interaction of bean amylase inhibitor and porcine pancreatic a-amylase, where an increase in inhibition occurs below what is considered optimal for the enzymes pH.

Yetter, et.al. (1979) extracted a-amylase inhibitors from five hard winter wheat varieties and assayed against larval a -amylase of both S. oryaze and J. molitor, with correlation in some varieties between in-vivo inhibition (resistance) and in-vitro inhibition of larval a - amylase by extracted inhibitors. As probably with beam amylase inhibitors, it was concluded that a - amylase inhibitors in wheat could be involved in postharvest resistance to grain insects in

storage.

Differing resistance responses in Phaseolus vulgarus beans to C. chinensis and Acanthoscelides obtectus, the "bean weevil," have been explained by the differential digestion of soluble heteropolysaccharides, which contain arabinose, xylose, rhamnose, glucose, and galactose in molar proportions approximating 9:2:1:1:4. The activity of heteropolysaccharides to C. chinensis (1% soluble fraction) is mainly due to its integral structure rather than component constituents, while insensitivity of A. obtectus ocurrs by effective digestion (hydrolysis) and release of arabinose at the larval midgut (pH of 6.7-6.8) resulting in biological inactivation of the polymer. According to Horber (1983), breeding for higher concentrations of this heteropolyssacharide in beans would be logical to protect them form both bruchid species.

S. cerealella was used as the test insect against 1000 varieties of rice obtained from the World Collection, of which around 20% displayed some level of resistance to moth infestation (Russell and Cogburn, 1977). In subsequent tests, 24 varieties were found to be promising, and three of these varieties were crossed with the very susceptible commercial variety "vista". All 3 crossed varieties displayed resistance through 4 generations, and the resistance was antibiotic, heritable and dominant.

Morallo-Rejesus and Dimaano (1984) compared the susceptibility of 20 varieties of milled rice to S zeamais and T. castaneum. Variable responses were found where some HYV's were more or less susceptible to one species or the other, the differences being attributed to some antibiotic effect on insect development and inhibited oviposition, or a combination of both,

while other varietal characteristics such as protein content were not considered as significant contributory factors to resistance in this study.

LABORATORY METHODS FOR EVALUATING RESISTANCE/SUSCEPTIBILITY:

Mills (1976) has described the methods used to evaluate the resistance or susceptibility profile of different grain varieties being performed by the Department of Entomology at Kansas State University, Manhattan, Kansas, USA.

Samples of each variety, replicated 3-5 times, typically of 50 or 100 kernel size, are placed in small plastic boxes (48 x 48 x 18 mm) with brass inserts fitted into the lids to enable free air interchange. In "nochoice" tests using Sitophilus spp., 6 females and 3 males were commonly used as parental adults, left for 5-7 days for oviposition, and then removed. The relative resistance of each variety is ranked using the Duncans Multiple Range Analysis based on number of emerged progeny (F1) per sample. Resistance may also be conferred based on the speed at which immature development to adult emergence proceeds so an estimate based on mean developmental period for alI F1 progeny should be made. The Index of Susceptibility of Dobie (1974) is simply a ratio of the log of F1 progeny completing development x 100 divided by the mean of the development period where a higher ratio indicates greater resistance relative to other cultivars or varieties under evaluation. In some "no-choice" tests using R. dominica, S. cerealella or T. castaneum a certain number of eggs or newly hatched larvae were used (therefore removing any effects due to oviposition preference) and each sample of each variety evaluated for immature survival and adult emergence. Russell (1975) showed that

varieties of paddy tested against S. cerealella maintain their respective rankings with regard to each other, irrespective of whether the infestation was initiated by adult moths or seeded with eggs. However, the standard error was over 2.5 x greater in analysis of the trials using adult infestations, therefore indicating that less variable results maybe achieved if eggs are used, apparently due to the unavoidable differences in fecundity of the chosen females and lack of any evidence to suggest any direct or deleterious effects on the adults by any of the varieties tested.

"Free-choice,' tests are probably more practical when large numbers of varieties are to be tested, and a larger number of adults are released in the same container, so it is not necessary to exactly determine the number of males and females (sex ratio) as in the "no-choice" tests because of the small sample size and small number of adults used. Free-choice can be conveniently used to eliminate obviously susceptible varieties, although in most cases, when "free-choice" and "no-choice" methods are compared, the rankings according to resistance tend to remain, although the number of progeny may be significantly larger in the "no-choice" tests.

"Free-choice" involves placing any number of samples (dependent on the size of the sample boxes) equidistant from the center of the circular chamber which has a small stoppered opening in the center of the lid, where an appropriate number of test insects can be introduced. Adults were removed after 5-7 days, the sample boxes removed, closed with screened lids, and held in the same constant environment for the emergence of progeny. The regime most commonly used is around 27 Cand 65-70% RH. All grain samples to be tested

should be equilibrated in these layers in this atmosphere for at least 14 days prior to the commencement of the test and introduction of the artificial infestation. Various researchers have shown that the rankings of varieties under evaluation do not alter if the experiment is conducted under a range of different RH levels, even though, as expected, a greater number of progeny are produced at higher relative humidities.

Some research has indicated differences when adults being tested on a particular variety, were previously conditioned on that variety for successive generations prior to the screening test, or if eggs are used as in the case of S. cerealella, paper booklets are exposed to 30-50 adults bred from the appropriate variety and strips of the booklet pages containing the appropriate number of eggs are then seeded on replicates of conditioned rice of the variety in which the adults are bred. Russell (1975) determined that the averaged total emergence from eggs and adults of S. cerealella, over 6 generations on 9 varieties of rice generally increases with each proceeding generation, which could alter the value of any highly resistant variety reported in screening trials. There was greater variation between generations when varieties were "seeded" with adults as compared to egg trials, with the standard error being almost twice as great.

Although the yield of progeny is markedly parallel between generations using eggs or adults, the wide variation between varieties was due to failure of the developing larvae to enter the grain rather than differences in oviposition or survival of the immatures once the kernel had been penetrated.

THE INHERITANCE OF RESISTANCE:

Cogburn (1980) described varieties of rice that originally displayed resistance to S. cerealella that were crossed with "vista" an uncommonly susceptible commercial variety, and progeny evaluated through the F2 generation to establish the heritability of resistance.

When moth survival between 0-35% were plotted against the number of lines that fell in each percentile, survival on vista appeared as a normal curve ranging from 5-35%, while on the resistant variety, 92% of the lines fell in the 0-5% range. If these varieties were crossed, and resistance is determined by a single pair of genes, the cross should exhibit a 3:1 ratio. If many genes were involved (polygenic resistance) than the cross would have exhibited a normal distribution. However, the cross did exhibit data concentrated towards the resistant end of the scale, and therefore did not conform to either genetic convention. When the cross with vista was repeated with another resistant variety, almost identical results were obtained, although Cogburn stated that the meaning of his results at that time were not clearly evident.

Dobie (1981) has described experiments to establish the inheritance of resistance in cowpeas to C. maculatus. Give the varieties he described as A and B, the following crosses were made, the female parent being listed first in all cases.

PARENTS :
A
B

F_1 crosses :

A B
B A

Back crosses:
(A B) A
A (A B)
(A B) B
B (A B)

F_3 cross :

A B

Up to 25 seeds of each material were individually placed at random into glass tubes (5 x 2.5 cm) and a single female C. maculatus adult (0-48 hrs old) was introduced and left to oviposit. Emergence of F1 adults (and therefore, percentage survival since all eggs were counted) demonstrated a large maternal effect in inheritance within the F1 crosses. Resistant seeds come from the cross in which the female parent was the resistant variety, and was further demonstrated in the backcross where only the cross involving the resistant female parent produced resistant seed. A strong negative correlation was found between the percentage of trypsin inhibitor in the seed and the percentage survival of C. maculatus.

Singh and Batia (1978) studied the inheritance of resistance to S. oryzae in reciprocal F1, F2

and F3 generations of two wheat varieties, one of which was resistant, and the other susceptible. No-choice progeny test (3 fertilized female S. oryzae per 20 grain sample, 4 replicates, 7 day oviposition, F1 after 45 days) was used on grain samples of each generation, harvested from the same plot and in the same season, under uniform conditions of temperature, relative humidity and grain moisture. The following observations were made:

i. **The resistance to S. oryzae in crosses of these 2 varieties is heritable and genetically controlled;**
ii. **Resistance is polygenic in nature;**
iii. **"Additive" gene types were more important than "dominant" gene types;**
iv. **Transgressive segregation is evident in cresses involving these 2 varieties:**
v. **Material effects did not have any controlling influence on resistance to S. oryzae;**
vi. **Heritability for resistance is quite high and offers wide scope for further refinement and improvement.**

An analysis of variance showed that the variability of mean F1, emergence from each parent and from each reciprocal F1, cross were not significant ("F" value less than table value) and therefore non-genetic. However, in the segregating generations (the reciprocal crosses in the F2 and F3) were highly significant, therefore due to genetic causes and heritable (i.e., for rice weevil resistance).

The differences in mean F1, emergence of S. oryzae were not significantly different between each reciprocal cross in the F1, and F2 generation, and only significant at the 5% level in the F3

generation. The means were significantly different from the mid parental value (mean of the F1, weevil emergence of both parents), where the F1 and F2 generations tended towards the resistant parent (partial dominance). The F3 reciprocal crosses tended towards the susceptible parent with the F3 means being significantly higher than the F1, and F2 means.

Frequency distributions (based on the number of samples producing the same number of weevil progeny) showed continuous variation in the F2 and F3 generations forming unimodal curves. This indicated that rice weevil resistance was influenced polygenically (multiple gene system). They inferred that resistance in this study was mainly an additive gene effect with duplicate type of epistasis. In other studies, maize weevil resistance in maize was shown to be nonadditive. In this case either dominant or interactive effects were more important than additive types of gene effects, and maternal genotypes did play an important role (as in cowpea resistance to C. maculatus) in determining resistance.

DURABILITY OF RESISTANCE:

Horber (1983) states that resistance remains effective while the resistant varieties are extensively grown in environments favouring insect infestation.

Durability of resistance depends on the genetic control of the resistant factors, such as single gene of multiple gene resistance where each has a small additive effect on the resistant genotype. Polygenic resistance causing antibiosis or antixenosis is preferable (and more stable) than monogenic resistance mechanisms. Because resistance incorporating antibiosis or

antixenosis are exerting a selective influence on insect populations, it is anticipated new biotypes will develop in conditions of prolonged use in isolated populations.

This has become apparent in resistant cultivars developed for pre-harvest pest and disease prevention, but has not as yet been reported in the literature for stored-products insects.

As mentioned previously, genetic uniformity is well recognized in connection with susceptibility to pest and diseases in the field. The lack of genetic diversity means that all major crops are at great risk to large losses after harvest due to the cosmopolitan nature of storage insects. Replacing tight-hulled varieties with loose gaping or thin hulls would be an example where losses would be accentuated. Genetic uniformity can be avoided by using different sources for resistance in the breeding stock.

Natural "gene-pools" carrying the necessary resistant codes are the evolutionary product involving spontaneous mutations, genetic recombinations and natural selection over several millenia. Once lost, they are impossible to replace. More extensive germ plasm collections carrying resistant biotypes from throughout the world, are essental for maintaining genetic diversity, or at least more emphasis on conservation via natural selection.

VULNERABILITY OF RESISTANT VARIETIES IN STORAGE:

As Horber (1983) states, "Nature is the master of safe packaging" whereby seeds are generally well protected and inherently resistant to ensure safe genetic transfer from one generation to

the next.

If resistance is concentrated in the pericarp, resistance could be entirely lost if excessive mechanical damage occurs during harvesting or drying. An undamaged pericarp acts primarily as protective barrier against feeding and subsequent oviposition by female S zeamais in maize kernels.

Germination and fungal infection can also markedly reduce inherited grain resistance. S. zeamais is capable of penetrating tight husks in paddy when these were infected by moulds.

The harder the seed, the less the pericarp is open to penetration, so for full expression of resistance in the genotype, moisture absorption by the grain during storage or inadequate drying prior to storage, must be avoided. Grain hardness becomes an integral component of an IPM strategy, since it reduces breakage during storage and handling, reduces risk of high percentage of dust and brokers and the infestation by insects and infection by moulds in bulk storage, that would otherwise increase safety and health hazards.

PLANT BREEDING PROGRAMMES:

Crops that have been subjected to plant breeding to develop new and improved varieties have become increasingly important in the productivity programmes of developing countries. They have been developed with high yielding characteristics in mind. Usually they are resistant to one or several of the more common and destructive preharvest pests and diseases, combined

with desirable agronomic characteristics such as a reduced growing period which enables multiple cropping systems to be realized. However, these HYV's and hybrids are generally very susceptible to attack by storage insects, which effectively reduces their impact in enhancing overall food utilization. The number of international and national breeding programmes that have been devised to improve the genetic characteristics of crops to post-harvest insect attack are very limited (Dobie, 1984). T.D.R.I. have done considerable work with maize. Collaborative work with IITA, (Ibadan, Nigeria) and the University of Durham (U.K.) has developed cowpea varieties (an inherently susceptible crop of immense value in terms of protein supplement) that display resistance to a range of Bruchid pests. The International Rice Research Institute has performed limited screening evaluations of the IR varieties, in conjunction with the Department of Entomology, University of the Philippines at Los Banos (UPLB).

Morallo-Rejesus, et.al. (1981; 1982) investigated that IR 24 was the most resistant paddy variety (among the 6 paddy varieties screened) to infestation by S. cerealella and R. dominica at 15 and 18 weeks storage. A further 23 IR varieties of paddy were screened against S. cerealella, and those that were found least damaged were; IR8, IR20, IR24, IR26, IR28, IR32, IR38, IR42, IR44, IR46, IR52 and IR54 based on comparative Indices of Susceptibility.

Morallo-Rejesus, et.al. (1981 + 1982) investigated the physiochemical properties of 15 varieties of brown rice to S. zeamais, T. castaneum and R. dominica, again using the Index of Susceptibility analysis. Susceptibility to S. zeamais was negatively correlated with amylose content, protein content, alkali spreading value, and significantly with grain hardness, while positive correlations were found with grain width and weight. The waxy varieties (high

amylose content) combined with lowest hardness indices were consistently the most susceptible to S. zeamais, while IR24 (the lowest amylose content) was the most resistant (as well as IR 3800-10). Weight loss of bran to R. dominica was positively correlated to alkali spreading value and susceptibility to T. castaneum was positively correlated to amylose content, which underlines the fact that differing characteristics of the grain affect storage insects differently in their ability to feed, oviposit, survive, emerge and breed on different varieties.

Juliano (1981) in a review of rice grain properties and resistance to storage insects, also claims that studies on postharvest resistance is still essentially a neglected approach with regard to correlating varietal resistance with the grain's physiochemical properties. Research has shown that breeding for characteristics such as intact, tight hulls; high degree of grain hardness; high amylose content; high endosperm starch gelatinization temperature; low oil content; small and light weight grain proportions; and low moisture content, contribute to impeding the development of a variety of storage insects such as S. oryzae, R dominica, S. cerealella, T. confusum, P. interpunctella, T. castaneum and E. cautella. Juliano suggests that future research be conducted on the interrelationship between grain hardness and grain quality (such as protein content, amylose content, alkali spreading values and gel consistency) and that varietal resistance to the major storage insects be investigated using pairs of sister lines differing in the property under review.

Mills (1976) suggests that insects themselves can be used to select and increase resistance, a method traditionally used by farmers for centuries when selecting seed for planting. Exposing

bulk seeds to insect attack and planting those that survived, showed a reduction of about 50% in emergence of S. zeamais from 50-kermel lots of sorghum after 2 years of continuous selection. A similar resistance to R. dominica was found when sorghum was replanted from seeds that initially survived unscathed during storage.

Cogburn (1980) also described attempts to intensify resistance in crosses of paddy varieties between two resistant varieties and "vista". Seeds from the three parents and from the two crosses were separated and duplicated, one lot being frozen for one year, while the other lot were infested. Each lot will be grown in a bulk planting and when harvested, the procedure is repeated. Simultaneously seed is tested for resistance to several species of storage insects, and the whole procedure is repeated for several years, which in theory, should select out susceptilbe seeds and leave only resistant seeds for planting.

Varieties of rice are likely to display variable resistance when grown under different geographical and agronomic conditions. This simply means that varieties that were shown to display resistance in screening tests might be susceptible under different circumstances of production, and therefore growing rice in different environments strongly affects their infestability. Resistant displayed in screening trials using small grain samples may also not be repeated when infested by the same test insects in a bulk storage situation. Mills (1976) mentioning work done by Diaz (1969) found that maize from tropical lowlands were more resistant to S. zeamais than from other areas, and this was explained by the fact that continuous weevil pressure in storage and field infestations naturally selected for resistance. This in part has been attributed to the resistance in local, indigenous varieties, being grown

under traditional, nonimproved systems in developing countries as opposed to the recently introduced, improved varieties.

Release of resistant varieties may cause the selection of biotypes which in time will be able to feed and develop on the variety, such as C. maculatus on cowpea varieties with high trypsin inhibitor concentrations.

In order to anticipate such an eventuality, research must continue in order to identify alternative sources of resistance, such as resistance to oviposition, or resistance of seed pods to larvel penetration which can then be incorporated in the resistant variety by suitable breeding programmes.

USING RESISTANCE:

Although it is not necessary to know what causes resistance to use it, and this is the case in many situations, research must continue to more fully identify the mechanisms of resistance, and an interdisciplinary research programme comprising of physiologists, biochemists, geneticists, ecologists, entomologists and plant breeders is necessary. Total resistance against all storage insects is not possible, but any level of resistance is considered useful and virtually free, once it has been incorporated in agronomically and socially acceptable varieties. Most crop species that have been investigated have displayed a certain amount of variation in resistance to major storage pests. Breeding programmes should be designed to exploit such variation and produce resistant varieties, which will enable advances to be made in the

reduction of losses, especially at subsistence farmer level, where investment capital in pest control is generally lacking.

Resistant varieties therefore does not require the introduction of technology which may not be suitable/inappropriate and therefore unacceptable for small scale farmers, and perhaps could also reduce the demands made on conventional chemical control techniques at all levels of crop storage.

CONCLUSIONS:

- **Resistance located in pods, hulls or seedcoats acting as a physical barrier are removed during processing for food.**
- **Antibiotic mechanisms in the germ and endosperm may require inactivation or extraction prior to processing and consumption.**
- **Physical causes imparting resistance are small kernel size, kernel hardness, closed and perfect hulls and tightly fitting husks in maize.**
- **Sound pericarp in sorghum and maize constitutes an important physical barrier to damage.**
- **Bruchids display strong preferences for large and smooth-coated legume seed for successful oviposition.**
- **The development and use of artificial diets in insect rearing have identified nutritional requirements and identify feeding stimulants, repellents and inhibitors.**
- **Cereal starches, dextrins, amylopectin and glycogen are a better diet for S. oryzae than**

amylose, cellulose, lignin both mono-and polysaccharides.

- **Legumes have more potential in developing chemical resistance than cereals.**
- **The digestibility of heteropolysaccharides determines host specificity to bruchids.**
- **Amylose is of poor nutritional quality for bruchid pests.**
- **Alpha amylose inhibitors confers resistance to post-harvest infestation of wheat and beans, while most protease inhibitors have been isolated from the Leguminosae.**
- **Increase of protease inhibitors confer resistance without detriment to the nutrition of humans or farm animals.**
- **Polygenic resistance involving several additive genes controlling factors causing antibiosis and antixenosis is more preferable, stable and durable compared to monogenic forms of inheritance.**
- **A pedigree system or recurrent selection procedure is recommended for breeding insect resistance.**
- **Lack of genetic diversity places maize in high risk category when local flint varieties are replaced by dent varieties.**
- **High Iysine maize is increasingly susceptible to stored-grain insects. Triticale (a cross between wheat and rye) combines the high protein content of wheat and high Iysine content of rye, but is highly susceptible to storage insects when compared to wheat, barley and maize. Triticale is adaptable to adverse environmental conditions especially in marginal areas where sorghum and millet are grown. When grain is harvested and marketed quickly, S. oryzae on triticale could be easily controlled, but severe problems remain for small scale farm storage, and in warm humid areas. Susceptibility to s. oryzae**

should be screened before cultivars are released.

- **Mechanical damage during harvesting and handling seriously undermines resistance if located in the pericarp. The engineering technology of improved harvesting and handling equipment must take this into account.**
- **Storage conditions can also dramatically modify the expression of resistance, and is related to grain hardness and the effects of moisture content, germination and fungal infection.**
- **Varietal resistance to stored grain insects provides the basis on which to build an IPM programme. It will be most beneficial when used in combination with improved and acceptable cultural, chemical, physical, and biological control methods.**
- **New crop varieties must be screened before ultimate release, to ensure storage characteristics are acceptable and if not better, than preceeding varieties.**
- **Further efforts should be directed towards intensified research for resistant germ plasm. (In both local and imported varieties), studies on the structural and chemical causes of resistance; studies on inheritance and genetic engineering to facilitate hybridization, and implementation of pest management and control strategies including the incorporation of suitable resistant cultivars.**

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Botanical insecticides for control of storage insect pests

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by Adelaida C. Quiniones

Chemical control of stored product insect pests has been the most efficient and effective means of protection of stored produce. However, with the increasing cost of inorganic chemicals and their known hazards to the environment an integrated means of control has been widely adopted. The search for botanical insecticides could supplement the expensive petroleum based chemicals. Botanical preparations have long been used for protection of stored produce by small scale farmers in oriental countries such as India where the neem tree has been used extensively. The success of these small farmers serve as the impetus for exploring the utilization of indigenous resources for small scale product protection and for possible industrial scale product protection. Several species of plants are now being screened for source of active ingredients against stored product insect pests (Table 1) commonly used are made of polyethylene or polyvinyl chloride film having a thickness of 0.1 mm (.004 inch) weighing 100 grams per square meter.

Table 1 List of plants studied for insecticidal activity on stored product insects

Table 1 List of plants studied for insecticidal activity on stored product insects - continue

After the completion of the fumigation it is necessary to collect the tablet residues or expended sachets. These materials can be disposed properly by swamping it in a soapy water until bubbling ceases (in open air) and then buried at least 30 cm below the soil surface. Under no circumstances should large quantities of expended residues be placed in a heap prior to being buried, because of the possible danger of ignition.

Formulation Available and dosage Rate

Phosphine (PH_3) is commonly available in the form of tablets, pellets and sachets containing aluminum phosphide.

- **Each tablet weighs 3 g and after complete decomposition 1 g of phosphine is released.**
- **Each pellets weighs 0.6 g i.e., 1/5 of tablet weight) and on complete decomposition, 0.2 g of phosphine is released.**
- **Each sachet weights 34 g and on complete decomposition 11 g of phosphine is released.**

The following equivalents, based on commercial formulations containing aluminum phosphide as the phosphine source are given to simplify dosage calculations.

1 g phosphine = 1 tablet = 5 pellets = 1/11 sachet

Under Philippine conditions, the following rates of phosphine application are recommended.

15-45 tablets*	2-5 tablets per	15-20 tablets

per 100 cu. ft	metric ton	per 1000 cu ft
or	or	or
3-5 sachets **	1 sachet per	2-3 sachet per
per 1000 cu ft	1-3 metric ton	1000 cu. ft.

* Recommended exposure time 3-5 day when tablet is used
** Recommended exposure time 3-6 days when sachet is used

Identification of Active Principles

Neem leaves and fruits

The constituents of the neem leaf powder were obtained by Tirimanna (1985) using two-dimensional thin layer chromatography. As a result of a strong saponification procedure, it is only the very stable compounds, which were observed on the chromatoplate. The compounds violaxanthin, butoin, 3carotene, zeaxanthin , ant heroxanth in, cryptoxanth in and neoxanthen were identified.

Seven epoxy compounds have also been isolated from fruits of Melia azedarach (Kraws et al., 1981). These compounds were rather stable and maybe of significance to scientists in search of a stable compound having insecticidal properties. This is relevant in view of the fact the neem leaf loses insect repellent property with age.

One sterol and 3 stable ketones were found, together with six very stable phenolic compounds. The occurrence of phenolic compounds in the neem plant is already well documented (Sengupta et al., 1960). Phenolic constituents are also recorded as antihelminthic factors (Taniguchi, 1960). The identification of quercetin in the neem leaf accounts for its antibacterial and antifungal properties and hence the curative properties of leaves in cases of sores and scabies (Basak and Chakraborty, 1968).

T. diversifolia (Wild Sunflower)

The active fraction D from this plant contains alpha - lactone with a hydroxyl group attached either to the lactone ring or to the alkyl substituent (Caring and Morallo-Rejesus, 1982). The unsaturation may be present in the ring itself or alpha to the carbon as indicated by the strong IR absorption at 1670 cm' and by the failure of the sample to decolorize bromine in carbon tetrachloride.

Studies of the characterization and identification of active insecticidal principles of the other plants listed in Table 1 are being continued at the University of the Philippines at Los Banos.

Insecticidal Activity

Black pepper (Piper nigrum) and red pepper (Capsium frutescens)

The insecticidal activity of these two species of plants against eight species of stored product

insect pests listed in Table 1 was determined by Javier and Morallo-Rejesus (1968). They found the crude and semi-purified extracts of black pepper topically applied on the insects toxic. The admixture treatment of grains showed the ground black pepper (GBP) more toxic than the semi-purified and crude extract as residual contact and stomach poisons against the corn weevil (Javier and MoralloRejesus, 1982). It was also found resisually toxic for 2 months on the saw-toothed grain beetle, lesser grain borer and corn weevil.

The ground red pepper (GRP) and GBP were residually toxic to the bean weevils for 2 and 6 months, respectively.

The effectiveness of red pepper on bag rice under warehouse condition (natural infestation) at average temperature of 25.70-27.90°C was further evaluated. The major stored product insect pests were corn weevil and red flour beetle. Three methods of applications were done: mixing powdered and whole fruit of RP with rice grain or in sachet with doses of 1200 ppm or 1800 ppm and spraying the bag with either 5, 10 or 20% of the extract. All methods of application and dose levels effectively protected the bagged rice from infestation for no longer than two months storage.

Neem (azadirachta indica A. Juss)

The repellent and antifeedant effect of seed powder and oil of neem on five species of stored product pests was investigated by Akou-Edi (1985). Laboratory trials in Togo using red corn treated with neem oil at concentrations of 1, 2, 4 & 8 ml/kg or with seed powder at 20, 40, 80

g/kg infested with confused flour beetles and corn weevils showed significant difference between the treated and untreated samples (Tables 2 & 3). As a rule, the effect of neem oil and need powder increased with higher concentrations but difference between concentrations was not significant on both test insects.

Atudy on the effect of neem seed kernel and leaf powder on the development of Callosobruchus maculatus (F.) on 3 bean seed species treated at the rate of 0.5, 0.1 and 2.0 parts per 100 parts of seeds showed reduced oviposition capacity, lengthened larval and pupal period, reduced percent adult emergence, longevity and growth index (Quiniones and Thawatsin, 1987). Among mungbean, bush bean and cowpea bean, the latter was found to be the most preferred food for the development because it supported normal development and enhanced population growth. Tables 4 and 5 showed reduced oviposition and percent egg hatched as an effect of neem leaf and seed kernel powder.

Studies on the effect on neem kernel extract (NSKE) on metamorphosis of Ephestia kuehniella (mill moth) and which established a concentration effect curve were undertaken by Maurer (1985). A methanolic extract of sundried neem seeds was applied at a concentration of 4 ppm on 4-5th instar larva. The stage of development that a larva had reached was determined by measuring the size of the head capsule. The average size of the head capsule at each larval stage was determined in a preliminary experiment on untreated larva under the same conditions (30°C, 82-86% RH). A 4 ppm concentration and less resulted in the insertion of an additional larval instar between the fourth and fifth larval stages. Hence, metamorphosis was prolonged after treatment with neem seed kernel extract. The mortality induced by

concentrations of 4 ppm NSKE or higher, occurred first during the molting phase between different larval stages. No feeding deterency was observed.

Table 2. Repellant effect of neem oil on adults of two stored product insect species

INSECT SPECTES	**NEEM OIL CONCENTRATION (ml/kg)**	**AVG. NUMBER (+ SD) OF INSECTS COUNTED AFTER 7 DAYS**		
		CONTROL	**TREATED**	**P**
Tribolium	1	17.25 ± 5.70	7.74 ± 2.12	0.01
confusum	2	18.25 ± 4.56	6.75 ± 2.82	0.0001
	4	17.75 ± 4.33	7.25 ± 2.32	0.0001
	8	23.37 ± 2.53	2.63 ± 1.85	0.0001
Sitophilus	1	15.37 ± 4.60	9.63 ± 1.92	0.01
zeamais	2	16.37 ± 3.20	8.63 ± 3.34	0.01
	4	15.25 ± 5.80	9.75 ± 2.57	0.05
	8	17.38 ± 3.42	7.62 ± 3.54	0.01

Table 3. Repellant effect of neem seed powder on two stored product insect species

	NEEM SEED	**AVG. NUMBER (I.S.D.) OF INSECTS**

INSECT SPECTES	POWDER CONCENTRATION (ml/kg)	COUNTED AFTER 7 DAYS		
		CONTROL	TREATED	P
T. confusum	20	18.50 ± 3.89	6.50 ± 2.78	0.0001
	40	21.87 ± 4.79	3.13 ± 3.09	0.0001
	80	21.87 ± 6.11	2.75 ± 2.55	0.0001
Sitophilus	20	14.87 ± 4.94	10.13 ± 5.67	n.s.
zeamais	40	16.62 ± 2.62	8.38 ± 3.38	0.01
	80	17.62 ± 7.23	7.38 ± 4.53	0.01

Table 4. Mean eggs of C. maculatus on three varieties of bean seeds treated with neem powder

TREATMENT	BEAN SEED VARIETY			MEAN
	MUNGBEAN	BUSH BEAN	COWPEA	
Control	32.78	33.67	37.60	34.74^{a}
Leaf powder, 0.5%	21.17	21.57	24.40	22.38^{b}
Leaf powder, 1.0%	15.67	16.13	18.27	16.69^{c}
Leaf powder, 2.0%	14.97	15.30	17.37	

Seed kernel powder, 0.5%	15.63	16.03	18.18	15.88^{c} 16.61^{c}
Seed kernel powder, 1.0%	7.37	7.63	8.33	7.78^{d}
Seed kernel powder, 2.0%	6.60	6.83	7.50	$6\ 97^{d}$
SEED MEAN	16.31^{b}	16.77^{b}	18.80^{a}	

Mean with the same letter superscript is not significantly different at 1% level (DMRT).

Table 4a. Analysis of variance on mean eggs of C. maculatus

SV	**Df**	**SS**	**MS**	**Fc**
Seed type(A)	2	74.24	37.12	43.087**
Neem application (B)	6	4772.06	795.34	923.203**
A x B	12	18.62	1.551	1.800^{ns}
Error	42	36.185	0.8615	
TOTAL	62	4901.011		

**highly significant

^{ns}not significant

CV = 5.36%

Table 5. Percent hatching in three varieties of bean seeds treated with neem in days

TREATMENT	BEAN SEED VARIETY			MEAN
	MUNGBEAN	BUSH BEAN	COWPEA	
Control	100.00	100.00	100.00	100.00^{a}
Leaf powder, 0.5%	79.31	81.51	84.41	81.74^{b}
Leaf powder, 1.0%	69.68	69.37	73.22	70.75^{c}
Leaf powder, 2.0%	66.29	67.35	71.12	68.25^{c}
Seed kernel powder, 0.5%	71.75	71.44	75.26	72.82^{c}
Seed kernel powder, 1.0%	48.87	49.42	51.09	49 79^{d}
Seed kernel powder, 2.0%	45.55	46.36	48.36	46.76^{d}
SEED MEAN	68.78^{b}	69.35^{b}	71.92^{a}	

Mean with the same letter superscript is not significantly different at 1% level (DMRT).

Table 5a. Analysis of variance on percent hatching

SV	**Df**	**SS**	**MS**	**Fc**
Seed type(A)	2	117.687	58.843	4.047*
Neem application (B)	6	17,980.10	2,996.68	206.138**
A x B	12	35.156	2.929	0.201^{ns}
Error	42	610.562	14.537	
TOTAL	62	18,743.50		

***highly significant**
CV = 5.44%
nsnot significant

According to Zehrer (1985) one of the traditional preservatives in Togo for protecting cowpea (Vigna unguiculata) from Callosobruchus maculatus infestation in storage is neem oil. Cowpea is Togo's most important pulse crop. It is already infested in the field by bruchids, especially C. maculatus, hence, signs of natural infestation can already be seen before storage. A study to compare efficiency of other traditional preservatives with that of neem oil was conducted. Cowpeas were treated with 0.5% V/V neem oil and peanut oil. Monthly observations on the insect and its damage on the stored produce were carried out. The untreated seeds retained

only 60% of the original weight after 6 months and 30% of it after 10 months. The damage was caused by bruchids, mostly C. maculatus and T. confusum. On the other hand, neem oil protected the cowpeas throughout the storage period but their taste war slightly bitter. The use of neem oil would be feasible and appropriate for subsistence farmers since it is available throughout the country and no additional equipment is required for the production of neem oil.

Lageundi (Vitex negundo)

The protectant effect of whole and powdered leaves of lagundi on stored corn against corn weevil was evaluated by Bhuijah (1988) for a period of 6 months. Two level of dosages were used (1 & 5%) for both whole and powdered leaves on 5 kg of corn. After 30 days of storage, the protectant effect of 5% lagundi leaf powder and 1% whole leaf was lost as shown by increased infestation of all lagundi treated samples (Table 6). Only neem seed oil treated corn seeds remained protected and had low percent infestation throughout the study. Apparently the insecticidal constituent of the lagundi leaves were not stable, hence the repellent property were readily lost.

Vegetable Oils Controlling Storage Insect Pests The practice of adding a little vegetable oil to stored rice or legumes for protection against stored-insect pests is well known and well established in oriental countries like China, India and Indonesia. Recently the practice of protecting stored produce with oil has spread and has been adopted in Africa and South America. Recently Van Rheenen (1983) pointed out the applicability of this method of

protecting storage grains to supplement safe chemical formulations.

The mode of action, appropriate dosages and duration of efficacy of oils have been investigated by various workers on storage insect pests (de Oca et al., 1978, Pandey, et al, 1976, Sangappa, 1977 and Singh et al., 1978). Differences between crude and purified oil have been studied and crude oil has been found to be a better protectant (Van Schoonhoven, 1978) while the triglyceride oleic acid combination was found the most effective (Schoonhoven and Hill (1981).)

The amount of oil needed for the control of most storage pests vary from 2 cc/kg seed (Magoya et al., 1982) to 15 cc/kg seed (Cruz and Cardona, 1981) depending on the level of infestation. Table 7 & 8 list the various vegetable oils controlling corresponding pests and pests of crop species in storage.

Table 6. Percent infestation of treated corn grains at different storage periods at room temperature

TREATMENTS	**DAYS**					
	30	**60**	**90**	**120**	**150**	**180**
Control	2.04a	10.60a	22.91a	61.97a	80.27a	88.51a
1% lagundi leaf powder	1.67a	11.24a	18.26a	58.51a	78.11a	90.22a
5% lagundi leaf powder	0.90b	6.79a	14.42a	65.73a	77.51a	90.15a

1% lagundi whole gried leaf	1.52ab	5.48ab	15.69a	62.20a	74.79a	38.58a
5% lagundi whole dried leaf	2.55a	10.80a	19.05a	57.12a	70.73a	88.81a
Actellic	0.38bc	0.33b	0.17b	5.45b	5.24b	14.19b
Neem seed oil	0.25c	0.06b	0.00b	1.30b	14.14b	31.75b

Means in a column followed by a common letter are not significantly different at the 1 % level by DMRT.

Table 7. Vegetable oils controlling storage pests

OIL	STORAGE PEST	AUTHOR (S)
Castor	C. chinensis	Sangappa, 1977
Coconut	C. maculatus	Varma and Pandey, 1978
Cotton seed	C. chinensis	Sangappa, 1977
	C. maculatus	Pandey, et al., 1981
	S. oryzae	de Oca et al., 1978
	S. granarius	Yun-Tai Qi and Burkholder, 1981
Groundnut	S. cerealella	de Oca et al., 1978

	C. maculatus	IITA, 1976
	S. granarius	Varma and Pandey, 1978
		Yun-Tai Qi and Burkholder, 1981
Maize	C. maculatus	Akelo-Tsegah, 1976
		Singh, et al., 1978
	A. obtectus	Magoya et al., 1982
		van Rheenenet al., in press
	C. chinensis	Cruz and Cardona, 1981
	S. granarius	Yun-Tai Qi and Burkholder, 1981
	S. oryzae	de Oca et al., 1978
	S. cerealella	de Oca et al., 1978
Mustard	C. chinensis	Sangappa, 1977
	C. maculatus	Varma and Pandey, 1978
Neem	C. chinensis	Pandey et al., 1976
		Sangappa, 1977

Palm	S. oryzae	de Oca et al., 1978
	S. cerealella	de Oca et al., 1978
Rice	C. maculatus	Pandey et al., 1981
Sunflower	C. chinensis	Sangappa, 1977
	C. maculatus	Pandey et al., 1981
Sesame	C. maculatus	Varma and Pandey, 1978
Soybean	C. chinensis	Cruz and Cardona, 1981
	S. granarius	Yun-Tai Qi and Burkbolder, 1981
	S. oryzae	de Oca et al., 1978
	S cerealella	de Oca et al., 1978
Sunflower	A obtectus	Magoya et al., 1982
		van Rheenen et al., in press
	C. chinensis	Sangppa, 1977
	C maculatus	Varma and Pandey, 1978
Paraffin	C. maculatus	Calderon, 1979

Table 8. Storage pests controlled by vegetable oils

OIL	**STORAGE PEST**	**AUTHOR (S)**
Cajanus cajan	Callosobruchus chinensis (L.)	Sangappa, 1977
Cicer arietinum	C. chinensis (L.)	Pandey et al., 1976
	C. maculatus (F.)	Calderon, 1979
Phaseolus vulgaris	A. obtectus (Say)	Magoya et al., 1982
		van Theenen et al., in press
	Zabrotes subfasciatus (Boh.)	Hill and van Schooven, 1981
		van Schooven, 1978
Sorghum vu/gare	Sitotroga cerealella (Ol.)	de Oca et al., 1978
	S. oryzae (L.)	de Oca et al., 1978
Triticum vu/gare	S. cerealella	de Oca et al., 1978
	S. granarius (1.)	Yun-Tai Qi and Burkholder, 1981
Vigna radiate	S. oryzae (L.)	de Oca et al., 1978
	C. maculatus (F.)	Pandey et al., 1981
		Varma and Pandey, 1978
Vigna unguiculata	C. chinensis (L.)	Cruz and Cardona, 1981
	C. maculatus (F.)	Akelo-Tsegah, 1976

		IITA, 1976
		Singh et al., 1978
Zea mays	S. cerealella (Ol.)	de Oca et al., 1978
	S. oryzae (L.)	de Oca et al 1978

Prospects of Insecticides from Plants

Acceptance of botanical insecticides for the control of storage insect pests by smal scale farmers is influenced by the following parameters: availability, safety, quality and cost. Application of the control methods is simple and does not need sophisticated equipment add to the desirability of the method. The search for more plant species with insecticidal properties is being pursued by scientists from all over the world. However, a systematic evaluation and identification of chemical and physical characteristics of active constituents should be accelerated. It is on this basis where knowledge on their efficacy in time, various levels of dosages and knowledge on toxicity on target pests will be valuable in field application.

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Entomopathogens for the control of storage pests

by Adelaida C Quiniones

Several disease pathogens from insect pests of stored products have been studied. These diseases are caused by pathogenic bacteria, viruses, fungi, nematodes and protozoa and are frequently fatal to these pests especially during the larval stage. The young larvae are the most sensitive to the pathogens.

Insect pathogens can be isolated from infected insects and then cultured in the laboratory

except for the viruses and protozoa which can only be cultured in living insects. Dust preparations or aqueous suspension of these pathogens can be applied to stored products in bulk in much the same way as conventional insecticides.

Another way of application of the pathogens is through food baiting with peromone or other attractants. The baited food may be placed close to the stored products. This method was effective for dissemination of pathogenic protozoa to kill beetles (Burkholder and Shapes, 1979) and also viral and bacterial pathogens of moths. Shapes et al., (1977) suggested, however, that to be very effective, the contaminated insects must leave the source of attractant and spread the pathogen within the pest population. This could be achieved by using a pheromone that fades out quickly and where there is little or no shelter so that the infected pesta are inclined to move out after feeding.

In this method, transmission of the pathogens may occur in one of these ways: 1) larval eating on cadavers on infected larvae or adults, 2) consumption of infected stored food, 3) contamination during mating, and 4) infection from the female during oviposition to its progeny.

General considerations:

In the considering the potential use of microbial insecticides, it may be generalized that the mode of infection or mode of action of entomogahhogens or their toxic by-products may be divided into two groups; according to their natural port of entry into their hosts.

The first group includes bacteria, protozoa and viruses. These pathogens must be ingested in order to cause infection and later on mortality. Viruses are quite specific in their sites of development and multiply only in certain tissues within the body of their host. Bacteria may multiply throughout the tissues and body fluids of the host causing septecemia. The crystalliferous bacteria (Bacillus thuringiensis) may kill their hosts purely on the basis of the activity of their associated toxins.

The second group includes the fungi and nematodes which enter their host through their integument. These microorganisms are more subject to regulations by the environment. If the environment favors them, they multiply tremendously and easily colonize their hosts. Physical factors like temperature and humidity affect their survival and/or ability to cause infection. These will also affect the progress of infection and the susceptibility or resustance of the host.

Bacteria:

Bacillus thuringiensis Berliner, the most common bacteria used for the control of stored insect pests, was isolated from diseased larvae of the Mediterranean flour moth, Ephestia knehniella Zeller in 1911. It has been tested on a wide range of storage moths: Plodia interpunctella Hubner, E. elutella, E. cautella, S. cerealeela (Oliver) and Corcyra cephalonica (Stainton). It was effective against all the above species except S. cerealella. In this species the larva spends its life cycle within the single grain and hence probably does not have sufficient contact with the pathogen when applied on the surfaces (McGaughey, 1976).

In bulk wheat and corn infested by P. interpunctella and E. cautella, it has been demonstrated by McGaughey (1976, 1978) that good control (at least 92%), may be obtained if only the surface layers of the bulk are treated with dust or aqueous suspension of B. thuringiensis. The recommended depth of treatment was 100 mm at least and the pathogen had to be well-mixed to ensure an even treatment within this layer. The recommended dose rate for this particular preparation was 125 mg/kg.

Admixture to surface layers of bulk grain during grain transfer and after the silo have been filled were found eaually effective. Viability of B. thuringiensis is slightly reduced after one year storage (Kisinger and McGaughey, 1976).

Viruses:

A nuclear polyhedrosis and a granulosis viruses have been isolated from E. cautella. Both severely infect P. interpunctella; whereas granulosis virus from P. interpunctella does not cross-infect E cautella (Hunter, 1973). Another nuclear polyhedrosis virus has been isolated from C. cephalonica (Rabindra and Subranabian, 1973) but it is not known whether it will cross-infect other moths. In all cases the young larvae are the most susceptible stage.

Granulosis virus controlled effectively moth on bulk wheat and corn when surface layer of bulk was treated with aqueous suspension or dust of the virus to a depth of at least 100 mm. The suspension or dust both contained 3.2 x 103 virus capsules/mg and the grain was treated at a rate of approximately 1.9 mg/kg.

The application of an aqueous suspension of the granulosis virus of P. interpunctella has also been found to be an effective protectant for stored in-shell almonds (Hunter, et al, 1973), and stored raisins (Hunter et al, 1979). High storage temperature, however, reduces the viability of the virus and hence lowers the efficiency of this method of control. At 27°C, the control, after 5 & 6 months, remained high at 93% and 77%, respectively. In contrast, at 32°C, control after four months was 87% but this dropped to 34% and then 16% after 5 & 6 months.

Protozoa:

Several protozoa are known to be severe pathogens of the Coleopteran. The following protozoans were tested: Nosema whitei for Tribolium castaneum (Herbs") and T. confusum J. du Val (Burges et al, 1971), N. whitei and N. oryzaephili for Oryzaephilus surinamensis (Linn) (Burgess et al, 1971) and Mattesia trogoderma for Trogoderma spp. (Schwalbe et al, 1974). Another species, Nosema plodiae Kellen and Lindegreem, has been isolated from the moth Q interpunctella and its mode of transmission investigated (Keller and Lindegren, 1971). Its use in control has not been studied.

The admixture of protozoa spores in particular, N. whitei to stored grain has been discussed by Burges (1973). He suggests that it would be necessary to admix a pathogen cocktail to control the range of Coleoptera that might be encountered in a particular storage situation.

Fungi:

There are many fungi (hyphomycetous species) which grow on insects. Some are saprophytic while others are parasitic. Those most widely encountered are members of the genera Beauveria, Metarhizium and Isaria, and to a lesser extent Aspergillus, Cephalosporium, Sorosporella, and Hirsutella.

The commonest species is Metarhizium anisopliae (Metch) (Sor., the cause of the green muscardine disease of river' insects). This fungus has been seen to penetrate the integuments of several insects. In a recent study by Quiniones (1986) on the control of stored pests of copra, particularly the copra beetle, diluted spores of M. anisopliae up to 4.8 x 10-6 when topically brushed on the surface of stored copra, was effective to control this beetle.

Table 1. Microbial pathogens of some stored pest product insects

INSECT SPECIES	**PATHOGEN**	**REFERENCE**
Ephestia cautella Hubner	B. thuringiensis Berliner	Burger & Hurst 1977
Plodia interpunctella Hubner		
E. elutella (Hubner)		
Sitotroga cerealella (Oliver)		
P. interpunctella Hubner	Granulosis virus (GV)	Hunter et al 1975
P. interpunctella Hubner	Nuclear polyhedrosis virus (NPV)	Hunter et al 1975

E. cautella (Huhner)		
E. cautella (Hubner)	NPV	Rabindia & Subranabiam 1973
Tribolium castaneum (Herbs")	Nosema whitei	Burges et al 1971
T. confusum J du Val		
Oryzaephilus surinamensis L	Nosema oryzaephili	Burges et al 1971
Trogoderma spp	Mattesia trogodermae	Schwalbe et al 1974
T. glabrum (Herbs")		Shapes et al 1974
P. interpunctella	Nosema plodiae	Kellen and Lindegren, 1971
Tribolium castaneum	Metarhizium anisopliae (Metch) Sor.	Quiniones, In press
Necrobia rufipes De Geer		
Oryzaephilus surinamensis L.		
Tenebrio molitor	Beauveria bassiana Vuill.	Masera, 1936
	Serratia marcescens Bizio	

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