A STUDY OF THE EFFECTS OF STORAGE ENVIRONMENTS AND OF RICE WEEVIL *(SITOPHILUS ORYZAE L.)* ON SEED DETERIORATION IN MAIZE *(ZEA MAYS)*

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Rice weevil adult emerging from maize seed.

ABSTRACT

Seed of maize variety XL45 was adjusted to three different initial moisture contents (12.4, 15.1 and 18.5%) and stored under four different environments (20 C - 40% RH, 20 C - 65% RH, 30 C - 40% RH and 30 C - 65% RH) for 20 weeks. Half of the seed samples were inoculated with adult rice weevils (*Sitophilus oryzae*). The remaining seed samples were stored free of insects. Sampling was carried out after 2, 4, 6, 8, 12, 16 and 20 weeks storage. Measurements of seed moisture content, germination, storage fungal development and changes in insect population number and survival were made at each sampling time. Assessments of the number and categories of abnormal seedlings present in laboratory germination tests and internal seed damage caused by insects were also carried out.

The changes in initial seed moisture content in response to the relative humidity level in the storage environment were rapid. All seed samples reached equilibrium moisture contents within the first four weeks of storage irrespective of initial moisture level. Equilibrium moisture content in all cases was below the 15% considered safe for short term storage of maize. In the absence of insects, and in environments involving a 40% level of humidity, no extensive reduction in germination percentage generally occurred. However, a relatively small drop in germination capacity was observed late in the storage period in the most extreme combination (initial moisture content 18.5%, 30 C, 65% RH storage environment, 16-20 weeks storage).

Major deterioration in seed quality occurred only in those storage environments suitable for rice weevil development i.e. 20 C - 65% RH, 30 C - 65% RH. In particular, loss of germination and increase in both the number and categories of abnormal seedlings were apparent in these treatments. X-ray photography of seeds from different storage environments showed the internal damage caused by rice weevils

and this was related to seedling development in sand tests and to normal and abnormal seedling production in standard laboratory germination tests.

Under favourable conditions (20 C - 65% RH and 30 C - 65% RH) rice weevil numbers increased dramatically. This increase was greatest at 30 C and also resulted in an increase in seed moisture content. The other storage conditions (20 C - 40% RH and 30 C - 40% RH) were unfavourable for insect survival. This was a direct effect of the low level of relative humidity which resulted in the death of adult rice weevils and prevented the development of larval populations.

Studies on the rate and extent of internal seed damage using X-ray techniques showed that germination did not deteriorate until larvae had eaten sufficient of the endosperm to prevent adequate food reserves being available for the seedling. The level of damage to seed viability by *Sitophilus oryzae* was clearly a function of the size of the insect population and the time over which it persisted.

The maize seed used in this study was substantially infected by storage fungi initially. However, despite the provision of high temperature (30 C) and a moderate humidity level (65%) for up to 5 months storage fungi did not cause total loss of germinability despite some increase in the levels of *Aspergillus spp.* and *Penicillium spp.* Apparently, even the most extreme environments used in this study were relatively unfavourable for storage fungal development.

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INTRODUCTION

Maize (*Zea mays*) is the third most important crop in the world after rice and wheat. It is important in warm temperate regions as well as in the humid and sub-humid tropics. In 1982 world production reached 448 million tons from an estimated land area of 134 million hectares (FAO 1982). Maize is a valuable crop for human and animal food as well as the raw material for many industrial products.

Associated with this large annual production of maize is the need for adequate provision for seed storage. This must be accompanied by a clear understanding of the determinants of seed quality (Moore 1963, Delouche 1971), the processes involved in seed deterioration (Helmer, Delouche and Lienhard 1962, Delouche 1963, 1964, 1965, 1969, Delouche and Baskin 1970, 1971), the principles of environmental engineering (Sijbring 1963, Munford 1965, Welch and Delouche 1967, Matthes, Welch, Delouche and Dougherty 1969) and data on local climatic conditions. In addition, provisions for seed storage must include procedures for preventing, minimising or controlling the incidence and severity of damage by external agents such as insects and storage fungi.

In Pakistan, the ambient temperature and relative humidity levels are usually considered to be unfavorable for safe long term storage of seed for planting. This is particularly the case during the wet (monsoon) season from July to September. It is during this time of the year that seed deterioration due to loss of viability caused by moisture absorption by the seed and the deleterious activities of insects and fungi can seriously reduce seed quality.

Rice weevil (*Sitophilus oryzae*) is considered to be the most destructive insect pest of stored cereals in Pakistan. Its rapid rate of multiplication and its capacity to

damage seed makes it a particularly dangerous pest. The main direct effects of its activity include loss of seed weight and germinability, reduction in palatability and nutritive value and spontaneous heating which may cause mould development.

Population increase and extent of damage caused by *Sitophilus oryzae* are greatly modified by the storage environment, particularly temperature, relative humidity and seed moisture content. Certainly the combined effect of these factors can dramatically enhance the activity of this insect and consequently the level of seed damage in storage. These same variables also have a direct effect on the rate of seed deterioration due to respiratory losses and on the activity of species of storage fungi such as *Aspergillus and Penicillium*.

The present study was conducted at the Seed Technology Centre, Massey University, its main objectives being:

1. To study the influence of different storage environments on the viability of maize seed.
2. To evaluate the effects of storage conditions on the development of rice weevil populations in maize seed.
3. To evaluate the effect of different rice weevil populations on seed viability.
4. To determine the effect of storage environments on the development of storage fungi in maize and the effect of storage fungi on seed viability.

CHAPTER I

LITERATURE REVIEW

1.1 SEED STORAGE

The amount of literature available on the effects of seed storage environment on viability is voluminous. For this reason, and to assist with both clarity and brevity, only work related to the present study will be considered in this section of the review. In particular reference will be made to published work on the influence of those parameters considered in the present experiment i.e. initial seed moisture content and changes in seed moisture content (during storage), temperature, relative humidity and storage microflora. In addition, this review will be generally confined to work relating to maize, quoted work on other species only being included for emphasis or because of its particular relevance.

1.1.1 Influence of Storage Environment on Seed Viability

The storage environment i.e. moisture content of seed, temperature and relative humidity, affect the storability of seeds. However, Barton (1961) regarded moisture content as being of the utmost importance. Certainly, seed deterioration increased as moisture content increased. Roberts (1972) presented formula and described the relationship of temperature and moisture content to the period of seed viability of certain crop species. The absorption equilibrium values for corn and wheat were found to be approximately 1.6% higher than desorption values at 32% and 22% relative humidity, respectively (Hubbard et al 1957).

The storage life of most crops also decreased with increasing temperature (Justice and Bass 1978). Seed viability and vigour are reduced as temperature is increased. When the relative humidity remains constant the moisture content

of wheat decreases approximately 0.6 to 0.7% per 10°C rise in temperature (Oxley 1948a).

The two critical atmospheric conditions i.e. temperature and relative humidity were studied in relation to the safe storage of seeds by Harrington (1960). He described "rules of thumb" for safe storage and states that the numberical sum of the percent relative humidity plus the temperature in degrees Fahrenheit should not exceed 100. He also indicated that the period for which seeds may be stored without a significant decline in germination doubles for each 1% reduction in seed moisture and for each 5-6°C (10 F) drop in temperature. These rules are both logarithmic and act independently. For example, seed of 6% moisture and at 10°C will remain viable 128 times as long as seed at 10% moisture and stored at 25°C ($2^4 \times 2^3 = 2^7 = 128$).

Relative humidity and temperature of the storage environment are thus the most imporatant factors influencing the maintenance of seed quality (Barton 1961, Delouche 1968b, James 1967, Litynski 1957 and Owen 1956).

Toole (1957) suggested that the relative humidity of the air should not exceed 60% for seed stored at 21°C. Also, the relative humidity should not be higher than 70% for seeds at 4 to 10°C. Many crop seeds can be stored for 10 years or longer at 5°C and 45-50% RH. Similarly, Toole (1950) stated that the viability of most crop seeds is lost rapidly when the relative humidity of stored seeds becomes 80% and temperature lies between 25°C and 30°C. She determined that seeds could be kept for 10 years or longer at a RH of 50% or less combined with a temperature of 5°C or lower.

Relative humidity affects the physiological quality of seed in two ways:

a. Seed moisture content is a function of ambient relative humidity

b. The infestation, growth and reproduction of both storage insects and fungi is drastically affected by the relative humidity of the micro-environment in the seed mass (Delouche 1973).

Seeds are hygroscopic in nature. In storage they absorb or lose moisture until the vapour pressure of seed moisture and atmospheric moisture attain equilibrium. At a particular temperature and pressure the vapour pressure of atmospheric moisture is a direct function of the degree of saturation or relative humidity (Delouche 1973). When different kinds of seeds are subjected to different levels of atmospheric relative humidity, the seeds attain specific moisture contents. The moisture content attained by seeds under these conditions is known as the equilibrium moisture content or hygroscopic equilibrium. Delouche (1968b) found that the hygroscopic equilibrium of seed at a given relative humidity decreases slowly with increasing temperature and increases slightly with increasing deterioration of the seed. For successful storage of seed the maximum level of relative humidity depends on the kind of seed, the length of the storage period and the temperature.

Seed moisture content, relative humidity and temperature are the interrelated factors affecting the longevity of seed in storage. Field crop seeds with a high moisture content (14 to 16%) can be stored for a year or more at a temperature of $10^{o}C$ or lower, while low moisture seed (10% or less) can tolerate temperatures in the range of 30-$34^{o}C$ for the same period without appreciable loss of viability (Delouche 1968b).

High humidities and high temperatures are disasterous for viable seed storage. Substantial decreases in seed viability and vigour occur in most kinds of seed during even six months of storage at $30^{o}C$ and 75% relative humidity (Delouche 1973).

Delouche (1973) recommended several different conditions (i.e. variables of temperature and relative humidity, and moisture content of seed for different storage periods) which can be used to maintain seed viability in storage. These are briefly described as follows:

1. Short term storage
   Conditions that will maintain seed quality from harvest to the next planting season (1-9 months)
   a. 30°C and 50% relative humidity (seed moisture content ranging from a maximum of 12% for cereal seed to 8% for oil seeds)
   b. 20°C and 60% relative humidity (seed moisture contents ranging from a maximum of about 13% for cereal seed to 9.5% for oil seeds)
   c. Other combinations of temperature and relative humidity as favourable as those above

2. Intermediate term storage
   Conditions that will permit carry over storage of the more elite classes as a hedge against seed production failure (18-24 months)
   a. 30°C and 40% relative humidity (seed moisture content ranging from a maximum of approximately 10% for the cereals to 7.5% for oil seeds)
   b. 20°C and 50% relative humidity (maximum seed moisture content ranging from 12% for cereals to 8% for oil crops)
   c. 10°C and 60% relative humidity (maximum seed moisture content ranging from about 12% for cereals to 9% for oil seeds)
   d. Other combinations of temperature and humidity as favourable as those above.

3. Long term storage
   Conditions suitable for the long term storage of breeder's seed, genetic stocks and the more costly and scarce vegetable, ornamental and field seeds (3-10 years storage)

The quality of seed can be maintained for a longer time in cold and dry conditions in storage. For 3-5 years of storage, 10°C and 45% relative humidity are satisfactory for most kinds of field crop seeds. Successful storage for 5-16 years can be secured under conditions of 0 to 5°C and 30-40% relative humidity (James 1967). These levels of temperature and relative humidity can only be maintained with heavy-duty refrigeration and dehumidification systems.

Harrington (1963) determined that if dried seeds are stored in open storage in less humid climates, the seeds will reach an equilibrium of approximately 10-12% moisture which is safe for most types of seed for at least one year. However, in most humid and sub-humid parts of the world seed moisture content will rise to 14-16% with a resultant rapid deterioration of seed viability due to the development of micro-organisms.

The phenomenon known as "hysteresis" also affects the equilibrium moisture content of seeds. Due to its influence the equilibrium moisture content of the same type of seed is not always the same at a given relative humidity. When wet seed attains equilibrium moisture content at a given relative humidity, the equilibrium moisture content of the seeds will be higher than if dry seeds are allowed to gain moisture to reach equilibrium at the same relative humidity. For corn and wheat a maximum hysteresis effect occurs between 12% and 44% humidity and amounts to 1.6% moisture difference (Hubbard et al 1957).

Douglas (1975) reached the following conclusions concerning different seed moisture contents.

Seed moisture content above:		
	40-60%	Germination occurs
	18-20%	Heating may occur
	12-14%	Mould grows on and in seeds
	10-12%	Sealed storage is unsafe
	8-9%	Insects become active and multiply

A study on the thermal death point of maize under low temperatures (below -23.3^{o}C) was conducted by McRostie (1939). He determined that seeds above 15% moisture content deteriorated severely and loss of viability occurred, but such losses were higher at fluctuating temperatures compared to constant temperatures.

The effect of different temperatures and relative humidity levels on the germination of sweet corn seed was examined by Boswell et al (1940). The seed was stored for 110 days at 26.6^{o}C with 78, 66 and 44% relative humidity resulting in final values of 15, 68 and 70% germination respectively. The seeds stored for the same duration at 10^{o}C and with 81, 66 and 51% RH gave 65, 70 and 72% germination respectively. It is evident that the lowest temperature and relative humidity combinations are most suitable for maintaining seed viability.

The seeds of two corn hybrids, Embo-260 (Yellow dent) and Poey T-66 (Yellow flint) were stored by Gill and Delouche (1973) under different environmental conditions for 18 months. They found no significant reduction in germination in the dent corn hybrid at 7^{o}C - 50% RH and 30^{o}C - 32% RH storage conditions. The germination of flint corn hybrid, however, was reduced significantly by 41% at the first count and by a further 12% at the final counting at 30^{o}C - 32% RH after 16 months storage. At 30^{o}C - 55% RH all seeds lost their viability completely after 18 months storage. At 30^{o}C - 75% RH complete seed deterioration occurred after only 12 months.

Moisture equilibrium values in relation to mould formation of seeds of several grasses and small seeded legumes was studied by Dexter (1957). The relative humidity used ranged from 55 - 85%. Seeds of common grasses and legumes were stored at these relative humidities until equilibrium was reached. Grass seeds reached equilibrium rapidly although a marked difference was observed in moisture content at different relative humidities in various species. Greater difference in the steepness of the equilibrium moisture/relative humidity curves was not noticed. Legume seeds reached equilibrium very slowly. However, mould development was more rapid in grass than in legume seeds which was due to the rapidity of establishment of hygroscopic equilibrium.

Seed reaches a peak of vigour and germination at full maturity. However, loss of vigour and germination occurs at high moisture contents. If the seeds are dried to moisture levels in equilibrium with 15 to 20% relative humidity and stored to maintain these moisture levels, then the seed may be kept for many years without a significant loss of germination (Harrington 1971).

The equilibrium moisture contents of different seed species at the same relative humidity will not be the same. This occurs because there is variation in the chemical composition of seeds. Oils or lipids do not absorb water. However, protein absorbs the most water per unit of weight while starch absorbs less but still considerable amounts. Corn containing low lipid will have a higher moisture content than cabbage seed containing high lipid when in equilibrium with the same relative humidity. The equilibrium moisture content of corn seed is 14% at 60% relative humidity, while the equilibrium moisture content of cabbage seed (containing 35% lipid) is only 7% (Harrington 1973).

Even in one kind of seed the size of the seed, the thickness of the seed coat and the nutrition of the mother plant

will all influence the relative amounts of various chemicals in the seed. Thus, lots of the same kind of seed may also differ in equilibrium moisture content at the same relative humidity by as much as 1% (Harrington 1973).

Temperature also affects the equilibrium moisture content of the seed because water molecules are less active at lower temperature and tend to cling more readily to the macro-molecules. As the temperature decreases the equilibrium moisture content for a given relative humidity increases (Harrington 1973).

In wheat if the moisture content is constant in the range of 10 to 20%, about a 3% increase or decrease in equilibrium relative humidity may occur for every $10^{o}C$ rise or fall in temperature (Ayerst 1965b, Pixton and Warburton 1971).

It has been determined that the viability and vigour of seed is reduced with increasing temperature, exposure to high temperature for a longer time and an increase in moisture content of seed. At a given temperature damage is reduced as the seed moisture content is reduced (Justice and Bass 1978).

The seed coat, endosperm and embryo constituents of different species vary in their permeability to moisture. As a result the time for a given species to reach equilibrium moisture content will differ. Pixton and Warburton (1968) reported that 90% equilibrium moisture content in wheat was reached after 5 - 14 days by absorption and 2 - 9 days by desorption. The equilibrium moisture content of seed therefore depends upon seed characteristics and the temperature and relative humidity of the storage environment. The attainment of complete equilibrium moisture content may take from a few hours to several days and in some cases up to 60 days. Similarly, the time required for moisture to enter the seed is only a small fraction of the

time required for moisture to move through a mass of seed (Justice and Bass 1978).

The moisture content of seed influences the respiration rate and chemical deterioration rate of corn seed. Samples of dent corn were conditioned to six moisture levels ranging from 13% to 18% and stored for 14 days, by Olafson, Christensen and Geddes (1954). They found that the seed respired slowly at a constant rate for a period of 14 days when the moisture level of seed was below 14.5%. The respiratory rate however, increased rapidly in seed with 15.2% and 17.0% moisture contents and high mould development was observed in seed with high moisture levels. In respiring seeds the fat acidities were higher and non-reducing sugar and viability decreased.

The moisture content, temperature and the chemical composition and soundness of seed influence the rate of seed respiration. Soft, starchy wheat for example, has been shown to respire faster than hard, vitreous wheats containing the same percentage moisture content. At 14.5% moisture content the rate of increase is gradual and uniform, but when the moisture content exceeds 14.5% the rate of respiration is accelerated (Baily 1918). At moisture contents above 14.0% shrivelled wheat respired two to three times more rapidly than plump wheat. Such differences were not observed in shrivelled or plump wheat at moisture levels below 14% (Baily 1918). Increasing temperature enhances the rate of respiration until 55^{o}C is reached. With the increase in temperature the diastatic action on starch increases until enzyme activities are stopped. Spontaneous heating caused by biological oxidation of dextrose and similar sugars in the germ embryo of the kernel also cause seed to deteriorate in storage (Bailey 1918).

Pfeffer (1878) also reported that the intensity of respiration increases with increasing temperature, while Hoff (1896) stated that the rate of respiration increases two

or three times for each 10 degree rise in temperature according to the usual rule of chemical reactions.

Different combinations of temperature and moisture content cause seed deterioration due to self-heating, the activity of moulds, insects and mites, and by loss of germination and baking quality. Theimer (1978) suggested that grain could be safely stored up to 14^{o}C with a moisture content not exceeding 15%, the only spoilage risk being a slight possibility of mite infestation.

Studies on the influence of high temperature and high humidity on the storability of wheat, soybean and maize, were carried out by Rengifo and Pfost (1976). They found that there was no significant loss in test weight between aerated and unaerated grain. Mould development occurred at high storage temperatures and at high moisture content/ relative humidity equilibrium. High storage temperature accompanied by high relative humidity also decreased germination and artificial drying increased grain damage. They suggested that currently recommended safe moisture contents for stored grains require more research.

1.2 INSECTS AND SEED STORAGE

1.2.1 Introduction

The rice weevil (*Sitophilus oryzae (L)*)is an insect of significant importance whose pest status has been recognised in most parts of the world. Although fragmented biological studies on species of *Sitophilus* have been published, a recent comprehensive study on the biology of *Sitophilus oryzae and Sitophilus granarius* was conducted by Longstaff (1981). Khan (1948) has published a description of the morphology and anatomy of *Sitophilus oryzae* (rice weevil) and Ricahrds (1947) has examined its reproductive biology.

The rice weevil is one of the most destructive pests of stored cereals throughout the world. It attacks both cereal grains and cereal products and causes millions of dollars of loss each year. It is also a major pest of stored grain throughout the warmer and humid regions of the world and is a serious pest of grain in China, South America, Pakistan and India where maize is a staple food.

The storage environment (moisture content of seed, temperature and relative humidity) influences the development of insect pests and fungi. These agents together with rodents are primarily responsible for the deterioration of stored grain. A favourable storage environment may enhance the development of insects and fungi to a stage which can render tremendous losses to stored grain in a short time.

1.2.2 Biology and Classification of Rice Weevil

The rice weevil (*Sitophilus oryzae*) belongs to the order Coleoptera and genus *Sitophilus*. The genus is part of the sub-family Calandrinae. Important stored product insects belong to five orders. Among these the Coleoptera (beetles) and Lepidoptera (butterflies and moths) are of prime importance.

Rice weevil is restricted in its habitat and breeding to cereal grains and partially processed cereal products. Champ and Dyte (1976) reported that it is one of the most important insect pests of cereals. The strain of insect and grain varietal difference are known to influence reproduction to a considerable extent. The range of grain moisture content for breeding of *Sitophilus oryzae* is restricted from 10% to 16%. The species occurs in the tropical, sub-tropical and warm temperate regions of the world but very high summer temperatures are not favourable for its survival. Data collected from different sources (Richards 1944, Birch 1944, 1946, 1954 and Kono 1954) show that *Sitophilus oryzae* is viturally cosmopolitan in

distribution. The female makes a hole in the grain into which an egg is deposited. The egg is white and oval in shape and may be laid anywhere in the grain though few are laid in the embryo. Richards (1947) reported that the majority of eggs in wheat are placed at the end farthest from the embryo. In a single grain more than one egg may be laid although only one larvae develops to maturity. Four larval instars occur within the grain. The larvae is white in colour and about 4 mm long when mature. Before pupation it assumes an inactive prepupal form for a short period and later becomes dark brown (Hill 1975).

The insect completes its life cycle in about 5 weeks at 30°C and 70% RH. However, the length of the life cycle is extended by lower temperatures, development occurring only slowly below 17°C (hill 1975). Khan (1948) indicated that at temperatures ranging from 15 to 30°C and a range of humidity from 50-90%, *Sitophilus oryzae* had a slightly shorter developmental period than *Sitophilus granarius (L)*.

The adult rice weevil is small, 3.5 mm long, brown to dark brown with a long snout or rostrum. It has elbowed antenae which end in a distinct club. The punctures on the thorax are round and the lines of punctures on the elytra are narrowly separated. This latter characteristic allows distinction to be made from the closely related *Sitophilus granarius*.

Tsai and Chang (1935) reported that the range of relative humidity for oviposition was 60 to 100%, but Reddy (1950) concluded that egg laying could also occur at RH as low as 52%. Mathleen (1938) and Mouitia (1942) reported that rice weevils can develop in grains having a moisture content as low as 8.5%. However, Davidson (1940), Harris (1943) and Birch (1945) have all recorded 10% moisture content as the minimum requirement for rice weevil development.

Baker and Mobie (1973b) found that increasing the casein level of grain from 0% to 20% increased the survival of rice weevil adults although no effect was observed upon growth rate of the larvae

The life span of adult *Sitophilus oryzae* depends upon several factors such as temperature, humidity, moisture content of seed and dietary requirements. However, the adult normally lives for about 5 months. Howe (1952) found that weevils breeding at low humidities produced a large proportion of infertile females compared with weevils bred at high humidities.

1.2.3 Effect of Storage Environment on Rice Weevil Development, Survival and Population Growth

Reddy (1950) investigated the effects of temperature, relative humidity and moisture content of wheat on the development of rice weevil. Development was completed in wheat seed with a moisture content of 10.2% at 25°C, 26.6°C and 30°C but not at 32°C. He reported that the weevils developed in the shortest time in wheat with a moisture content of 17.6%. He also noticed that optimum conditions for the development of weevils involved a temperature range of 28°C to 30°C, relative humidity levels between 75 and 90% and a seed moisture content between 13.5 and 17.6% in wheat.

It has been generally recognised that the development of most seed storage pests is slow at temperatures below 20°C, although at this temperature *Sitophilus spp.* has been shown to complete its life cycle in 120 days in wheat at a relative humidity of about 60% (Oxley and Howe 1950).

Cotton et al (1960) reported that rice weevils were unable to reproduce in grain with a moisture content below 9% and the adults soon died in dry grain. They also determined that rice weevil adults survived for only one week in 8%

moisture content wheat at 29.44°C (85°F) and in 9% moisture seed about 70% were dead after three weeks, though a few lived for seven weeks. Certainly it has been shown that the activity of most stored insect pests decreases rapidly as relative humidity drops below 50% and reproduction stops altogether at less that 35% relative humidity (Cotton 1963).

Howe (1952) investigated the effects of 21°C and 25°C storage temperatures and relative humidities ranging from 50 to 100% on egg production of rice weevil in wheat. He found that while high egg production was obtained at high humidities the rate of oviposition was very low at low humidity levels. At 21°C and 50% RH, eggs were noticed in only one grain compared with 31 out of 40 grains at 100% relative humidity. In addition, the daily egg laying rate recorded was 0.03 and 1.48 per female insect respectively. At 25°C the number of eggs laid per female per day was 2.75 at 100% relative humidity and 2.55 at 90% relative humidity.

The influence of different temperatures on oviposition rate by rice weevil was also assayed by Howe (1952). The temperatures used were 17°C, 21°C and 25°C and the relative humidity was 100%. He found that the daily oviposition rate per female average 1.3 at 17°C, 2.5 at 21°C and 3.4 at 25°C with an approximate ratio of 3:6:8.

Howe (1952) also found that at 21°C and 100% relative humidity females laid fewer eggs when they were placed in a "competitive" situation where there were many females but few grains. He also noticed that the presence of males reduced oviposition rate considerably. Maximum egg laying was not secured unless many grains per female were available for oviposition. This was also confirmed by work of Birch (1945) who studied the influence of temperature, humidity and insect density on the oviposition of a small strain of Calandra (*Sitophilus oryzae*). He found that highest oviposition rate was obtained at a density of one

female to approximately 50 grains at 25.5°C. Birch (1945) also found that while rice weevil females were capable of laying eggs in wheat with 10% moisture content, no eggs were observed in grains with a 9.5% moisture content. Certainly fewer eggs were laid at 12% moisture content than at 14% but very little difference was observed in the number of eggs laid in seed with 11% compared to 12% moisture content.

Reddy's (1950) work with wheat on the oviposition of *Sitophilus oryzae* using constant temperatures from 13°C to 35°C and a range of relative humidities from 30% to 99% showed that the highest number of eggs laid and percentage of hatching occurred at 30°C and 99% relative humidity. A relative humidity of 73% or less was unfavourable for egg laying and no eggs were laid at 30% relative humidity.

Birch (1944, 1953, 1954) has investigated the influence of temperature, moisture and food source on the capacity for increase and survival of Calandra (*Sitophilus oryzae*) in wheat and maize. Certainly the insect is sensitive to both temperature and relative humidity levels in the storage environment. In particular, relative humidity because of its effects on dessication of eggs causing mortality and because of its influence on seed moisture content is a particularly important parameter in the storage environment. Birch also concluded that the higher the temperature the higher the mortality at a particular relative humidity value.

Birch (1954) investigated the survival of the immature stages of Calandra (*Sitophilus oryzae*). The range of temperatures used was 13°C to 35°C and that of seed moisture content 8% to 14%. He found that the maximum temperature for survival was 34°C. A minimum temperature of 15.2°C and a seed moisture content of 9% was necessary for survival. When the moisture content of seed declined below 14% insect mortality increased rapidly. Mortality

was lowest at 30°C at all moisture contents. Most mortality during the immature stages occurred in the first larval stage.

Khan (1948) also worked on the mortality of rice weevil at 15, 20, 25 and 30°C and at RH levels of 50, 70 and 90% in both wheat and maize. He determined that 50% RH irrespective of temperature was unfavourable for larvae and that a temperature of 15°C was fatal for this insect. Wheat with 19% moisture content in eqiulibrium with 90% RH was invaded by mould even at low temperatures. Such fungal invasion stopped the growth of rice weevil larvae and insect mortality increased. High mortality was observed in maize at 70% RH.

Studies on insect population growth by Birch (1953) found that the population of rice weevil at 15.2°C doubled after 10 weeks. However, in the same time at 29.1°C the population was 2000 times greater than at 15.2°C. Such dynamic changes in insect populations are obviously environmentally controlled. However, Hardman (1977) worked on the environmental changes associated with the growth of a population of *Sitophilus oryzae* in wheat found that seed moisture content increased from 13.4% to 19.7% after 60 days storage, that rice weevils raised seed moisture content by 7% during a 112 day period, and that high populations also increased the temperature by 7°C. This effect of population increase in changing the storage environment has been observed by a number of workers. Howe (1943) for example, conducted an investigation of the changes occurring in a bin of stored wheat infested by rice weevil. The wheat (12.0% moisture content) showed the development of a high population of insects. This development caused the grain to heat over five months. A steep temperature gradient between the infested grain and the air cooled surface and sides of the storage container increased the water content of the grain. Due to the production of high concentrations of carbon dioxide at the bottom of the bin, conditions then became unsuitable for the survival of the insects.

1.2.4 Damage Caused by *Sitophilus oryzae* in Stored Grain

Koura and El Halfawy (1972) evaluated the weight losses in grain infested with insects under natural temperature conditions. Wheat, maize and sorghum samples were collected from warehouses in the summer and the infested grains were separated. The percentage weight loss was calculated by comparison of the weight of infested grains with that of a similar number of sound grains. They reported that weight loss averaged 32% in wheat, 22.2% in maize and 33.8% in sorghum.

In a laboratory experiment by the same authors (Koura and El Halfawy 1972) several varieties of wheat, rice and barley were fumigated and the seeds then inoculated with adults of *Sitophilus oryzae*. The adults were removed after two days and the grains were stored at 25°C and 70% RH to allow insect development. The emerged larvae and adults damaged the grains and weight loss was recorded. The maximum percentage weight loss was 37% for wheat, 84% for rice and 69% for barley. In a more controlled study, Stoyanovic (1966) investigated the loss of weight in stored wheat infested with rice weevil at initial population densities of 1, 2 or 3 adults per 0.5 kg of grain. The influence of temperature on weevil activity at 10°C and 20°C was also studied. He determined that after 200 days the percentage weight loss in wheat grains was only 5-14% at 10°C but rose to a maximum of 71% at 20°C over the same storage period.

Golebiowska, Pradzynska and Nawrot (1977) worked on the feeding capacity of some species of storage insects. Rice weevils were confined in association with other storage insects in wheat, rye and maize grains under a range of temperatures (20-29°C) and humidities (60-94%). They found that most species, including rice weevil, fed most frequently on the germ end of the grain and less frequently on the brush end. At higher humidities in particular, insects confined their attack to parts other than the germ.

The resultant damage had a major effect in reducing seed germination and subsequent seedling growth capacity. Similar findings have been recorded by Kamel and Zewar (1973) in work on the rate of loss of weight in maize and millet grains infested with rice weevil and *Rhizopertha dominica*.

Singh, Sinha and Wallace (1977) studied changes in oxygen, carbon dioxide and microflora levels induced by weevils in stored wheat. The lowest oxygen concentration (35.2 mg/litre, 2.8% by volume) and the highest carbon dioxide concentration (370 mg/litre, 21.7% by volume) were observed at the bottom of storage bins associated with a 10% infestation of grain with rice weevils. It was determined that both at 5% and 10% infestation levels the rate of development of rice weevils was higher than that of granary weevil. *Aspergillus* and *Penicillium* storage fungi were also found to have a more severe effect on the viability of grain samples taken from the bottom layers than from the top layers of the storage container.

1.3 FUNGI

About 150 species of fungi have been isolated from cereal seeds. According to their behaviour they have been classified into two main groups, field fungi and storage fungi. Field fungi comprise a wide range of species including *Alternaria*, *Cladosporium*, *Helminthosporium* and *Fusarium* species (Christensen and Kaufmann 1965). In comparison the storage fungi consist of members of only two genera, *Aspergillus* and *Penicillium*. They can grow in grains and seeds whose moisture contents are in equilibrium with relative humidities of 70-90%. Storage fungi are commonly found on different organic and inorganic materials, food products, fabrics and insulating materials made of plant fibre, paints, leather goods and glues. These fungi exist everywhere and contaminate all kinds of

grains and seeds. In this latter case they influence the grade, conditions, keeping quality and storability of seeds (Christensen and Kaufmann 1965).

It has been reported that a few *Aspergillus* species invade and destroy stored cereal grains (Christensen and Kaufmann 1969). *Aspergillus amstelodami*, *Aspergillus chavalieri*, *Aspergillus repens*, *Aspergillus restrictus* and *Aspergillus ruber* grow in seeds with a moisture content of 13-15%. Other species such as *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus tamarii* and *Aspergillus versicolour* are found in cereal seeds with a moisture content above 15% (Christense 1957). By comparison the *Penicillium* species are found in stored cereals with a moisture content above 16% and often at relative low temperatures (Christensen and Kaufmann 1965).

1.3.1 Influence of Environment on the Development of Storage Fungi

The storage fungi develop rapidly at about $30^{o}C$ - $32^{o}C$ and their growth rate declines with reduction in temperature. However, the development of a few strains of the *Aspergillus glaucus* group are capable of very slow growth at $35^{o}C$ - $40^{o}C$ and some species of *Penicillium* can grow at a temperature several degrees below freezing (Christensen and Kaufmann 1965).

Corn seeds at 15% moisture content which are free of storage fungi, sound and in good contion can be kept at a temperature of $45^{o}F$ - $50^{o}F$ for 9-12 months without any deterioration. However, if the grain has already been contaminated with storage fungi and stored at 15% moisture content and a temperature of $45^{o}F$ - $50^{o}F$, the development of fungi may occur and the seed may deteriorate within 6 months (Christensen and Kaufmann 1968).

Aerated and non-aerated samples of corn were stored by Bottomly, Christensen and Geddes (1952) at 30°C at moisture contents between 19 and 31%. Mould growth and acidity increased, while viability and non-reducing sugar levels decreased with increasing moisture content and time of storage. *Aspergillus glaucus* was involved in a rapid loss of non-reducing sugar and a slight increase in fat acidity. But *Aspergillus flavus, Aspergillus candidus, Penicillium* species and *Fusarium* species were associated with marked increases in fat acidity. In non-aerated samples slight changes in fat acidity or non-reducing sugars were observed and a decline in germination also occurred (Bottomly, Christensen and Geddes 1952).

Hard wheat free of fungi was inoculated with *Aspergillus candidus, Aspergillus repens* and *Aspergillus restrictus* and stored for 7 months in dessicators at 25°C by Papavizas and Christensen (1957). Humidifying solutions were used to maintain seed moisture content at levels from 14.7 to 20%. The uninoculated controls were also stored under the same range of storage conditions. It was shown that while the controls decreased in germination slightly, the inoculated seeds lost germination drastically during the storage period. At 14.7 to 14.9% moisture content *Aspergillus restrictus* caused a more rapid decline in seed germination than *Aspergillus repens* or *Aspergillus candidus*. At 15 to 16% moisture content both *Aspergillus candidus* and *Aspergillus restrictus* caused a rapid decrease in seed viability. It is considered that fungal invasion of stored seed is a common cause of "sick" wheat in commercial storage (Papavizas and Christensen 1957).

Corn seed was stored at different moisture contents by Bottomly, Christensen and Geddes (1950) to study the effects of variation in temperature and oxygen concentration on fungal growth, viability and biochemical properties. The moisture contents chosen were in equilibrium with relative humidities of 75 to 100% and ranged from 17.4 to 31.2% (dry weight basis). Temperature ranged from 25-45°C

and oxygen concentration from 0.1 to 21%. The variation in relative humidity affected fungal growth and biochemical properties but had little influence on atmospheric composition. When the relative humidity of the storage atmosphere was increased from 75 to 100% the amount of fungus infection increased logarithmically. Internal fungal infection and fat acidity increased rapidly and a slight increase in total and water soluble nitrogen occurred. Reducing sugar levels also increased. Conversely a reduction in non-reducing sugars, total dry matter and viability of grain was observed. The variation in temperature also affected fungal growth. Highest mould counts were recorded at 25°C and at 40°C the highest fat acidity level was noted. With a reduction of oxygen content in the storage atmosphere from 21% to 0.1%, fungal growth was four times greater (Bottomly, Christensen and Geddes 1950).

The two main genera of fungi differ in their response to different storage environments. For example, corn seed in equilibrium with a relative humidty of 80%, develops mainly *Penicillium* at 25°C, mainly *Aspergillus* at 30-35°C and mainly *Mucor* species at 45°C. However, *Penicillium spp* and a few *Aspergillus* species including *A. candida pseudo-tropicalis* were highly tolerant to low oxygen tensions (Bottomly, Christensen and Geddes 1950).

Samples of corn were collected by Christensen (1948) from commercial bins to study fungal growth at low seed moisture levels. The seeds were stored in vacuum bottles at different moisture contents. As a result of increase in the seed moisture content the fungal population and temperature also increased (Christensen 1948).

In bulk storage situations there is also the possibility that some of the grains may be moist enough when stored, or acquire high moisture as a result of moisture migration. This situation provides favourable conditions for the

development of *Aspergillus restrictus* and *Aspergillus glaucus*. *Aspergillus restrictus* is slow growing and generally does not cause hot spots or increase moisture content. However, *Aspergillus glaucus* develops rapidly and increases the temperature of grains to 35-40°C. When the moisture content of grain exceeds 15.0% to 15.5%, *Aspergillus candidus* growth begins, which can increase the temperature and moisture content of grains very rapidly if optimum conditions prevail. As the moisture content of the grain reaches equilibrium with a relative humidity of 85% (18.5% moisture in cereals) *Aspergillus flavus* can grow. *Aspergillus candidus* and *A. Flavus* together may then increase the temperature of the grains to 55°C and maintain it at this level for several weeks (Sinha and Wallace 1965). Under adiabatic conditions the temperature and respiratory increases in moist corn samples are directly correlated to fungal development until the temperature reaches 52-55°C, where fungi are killed, respiration stops and heating ceases. At higher moisture contents bacterial development may heat the seed to the bacterial thermal death point ranging from 68-70°C. Due to non-biological oxidation the spontaneous heating of seed may continue under controlled adiabatic conditions (Milner, Christensen and Geddes 1947).

It has been shown that in starchy cereal seeds stored at moisture contents between 14.0 and 15.5% and in soybean seed at between 13.0 and 14.0%, a difference of only 0.2% in moisture content may influence the rate of fungal growth and the extent of damage in a given length of time (Christensen and Kaufmann 1965).

Seeds of cabbage, cucumber okra, onion, pepper, radish, salsify, spinach and turnip were inoculated with spores of *Aspergillus amstelodami* or *Aspergillus flavus* and stored at 85% RH and 22-25°C for 30 days, by Kulik (1973). Non-inoculated seeds served as control. He found that at the end of 30 days these fungi had invaded only a small number of seeds of cabbage, cucumber, radish and turnip. More seeds of okra, onion, pepper, salsify and spinach

were invaded by either or both *Aspergillus amstelodami* and *Aspergillus flavus* but fungus invasion did not appear to cause a decline in germinability.

Heydecker (1972) stated that temperature, as well as affecting the number germinated and the rate of germination, can also affect the uniformity of germination. Germination reduced with rise in temperature. At low temperatures the rate of germination is very low, hence the potential for spread of the distribution of germination if time is great.

Generally it seems that seeds are softer and are more easily damaged at high moisture content but at very low moisture content, in wheat, the bran becomes brittle and breaks easily. In these circumstance, the endosperm is hard and the soft embryo may be eaten cleanly out of every wheat kernel and even *Sitophilus spp*. lay their eggs in the scutellum around the embryo (Howe 1973).

CHAPTER II

MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN AND METHOD

The present investigation was designed to determine the influence of selected storage environments on rice weevil (*Sitophilus oryzae L.*) infestation and their effect on maize seed deterioration. The study involved a randomised block design incorporating three initial seed moisture contents (18.5, 15.1 and 12.4%), two storage temperatures (30°C and 20°C), two storage relative humidities (65% and 40%) with three replicates of each treatment. Half of the seed samples were stored directly under these conditions. In the other half of the seed samples rice weevil adults were introduced immediately prior to storage. This provided the opportunity to evaluate seed deterioration and insect effects under a common set of environments.

Sound and untreated maize seed of variety XL45 was used in this experiment. This seed had a pre-storage germination of 92% and an initial moisture content of 13.4%. The seed moisture content was adjusted to different desired moisture contents (12.4, 15.1 and 18.5%). To obtain the lowest moisture level 32 kgs of seed from the initial lot was artificially dried using a "kiwi" minidryer. The changes in moisture content were monitored using the high temperature air oven method (ISTA 1976). The drying and mixing of seed by hand was continued until the required moisture content of 12.4% was secured. To obtain medium and high levels of seed moisture i.e. 15.1% and 18.5%, 32 kgs of maize seed for each moisture content was taken from the bulk sample and spread in thin layers in a plastic container. A calculated quantity of water was sprayed in a thin mist on to the seed. After spraying, the seed was thoroughly mixed by hand. The seed samples in plastic containers were completely covered with a plastic sheet to reduce evaporation. The mixing of seed was carried out

twice each day. Moisture tests were taken at intervals until the prescribed moisture levels of 15.1 and 18.5% were achieved. The seed samples were kept for five days in a controlled temperature room at 5°C.

The seeds at each initial moisture content were each thoroughly mixed by passing them through a soil divider. This mixing was repeated three times to obtain a uniform seed sample.

The experiment was conducted in two parts. In the first part a series of 504 one litre capacity glass jars were used. Approximately 120 grams (about 300 seeds) of maize was placed in each jar. Half of the jars were inoculated with 60 adult rice weevils each. Insect counting was carried out using a vacuum counter developed at the Seed Technology Centre (Plate 1). This initial insect population represented one adult weevil per two grams of maize seed by weight. The jars containing insects were covered with open mesh gauze. Jars not containing insects were covered with open weave nylon material (Plate 2). All the jars were stored in the appropriate temperature and humidity treatment of 30°C - 40% RH, and 20°C - 65% RH.

In the second part of the experiment the seed was stored at either 30°C - 65% RH or 20°C - 40% RH. In order to obtain the required levels of humidity in this part of the experiment it was necessary to store the jars in large airtight containers (Plate 3) each containing controlled humidity solutions prepared by mixing the required proportions of water and glycerine as employed by Hill (1965). The 65% RH atmosphere at 30°C was obtained by mixing glycerine and water in a 64 ml:36 ml ratio. The 40% RH atmosphere at 20°C was obtained using pure glycerine. Such mixtures were used to maintain the required relative humidity levels of 65% at 30°C and 40% at 20°C within $\pm$1%.

In this part of the experiment the internal atmosphere in the plastic containers was circulated during the storage

Plate 1: Vacuum Insect Counter

Plate 2: Glass Jars Used for Seed Storage

Plate 3: 110 Kgs Plastic Container Used For Controlled RH (40% and 65%)

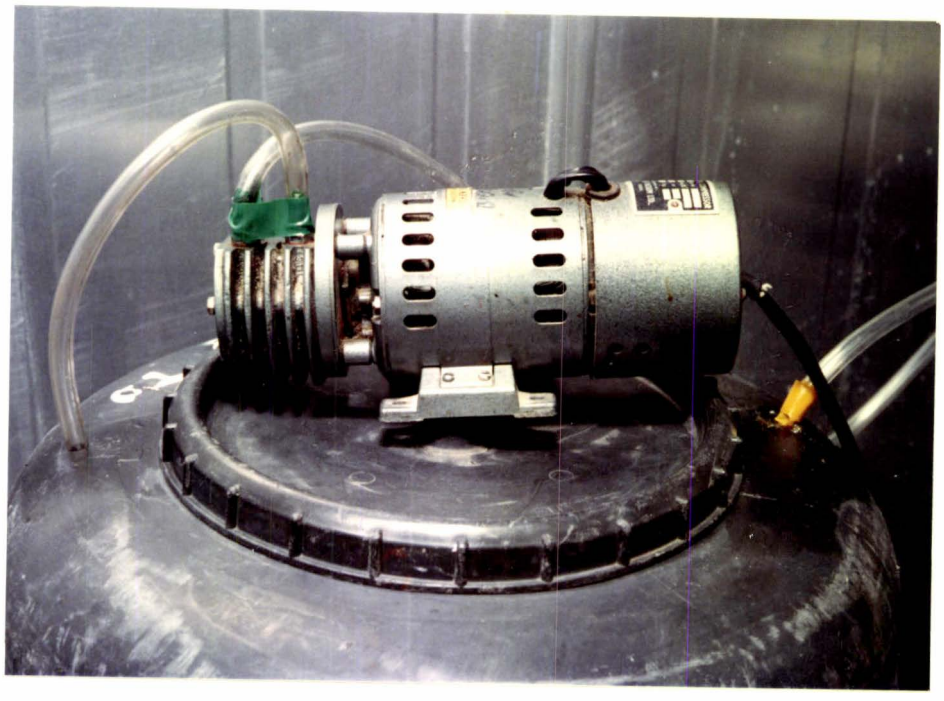


Plate 4: Electric Air Pump Used for Aeration

period using an electric air pump. All the plastic containers for any particular RH level at either 20°C or 30°C were interconnected with plastic tubing. An electric motor was fitted to a time clock. The plastic tubing was connected in a circuit with the electric motor (Plate 4). The internal atmosphere in the plastic containers was aerated twice every 24 hours. This aeration procedure was continued throughout the storage period to ensure a uniform relative humidity level in each container. The RH was checked twice a week by wet and dry bulb thermometer (Plate 6).

In both parts of this experiment samplings were carried out after 2, 4, 6, 8, 12, 16 and 20 weeks storage. At each sampling time measurements of seed germination, seed moisture content and the incidence of storage fungi were carried out. The development and mortality of rice weevils in each treatment was also recorded.

2.2 MEASUREMENTS

2.2.1 Germination of Seed

The ultimate object of testing the seed for germination is to gain information with respect to its field planting value (ISTA 1976). The germination test for each treatment was conducted on 3 x 100 seeds counted randomly. The rolled paper method was used at a temperature of 25°C. The first seedling count was recorded on the sixth day and the final count on the ninth day.

The percentage of normal seedlings was recorded. The cirteria for normal corn seedling development comprised a well developed primary root system or with adventitious and lateral roots with adequate length and vigour. Also a well developed intact epicotyl with well developed primary leaves emerging through the coleoptyl (ISTA 1976).

The percentage of abnormal seedlings was also noted. Abnormal seedlings were classified as those which did not

show the capacity for continued development into normal plants when grown in good quality soil and under favourable conditions of water supply, temperature and light (ISTA 1976). Damaged, deformed and decayed seedlings and seedlings showing any other defects when tested on artificial substrata were classified as abnormal. The percentage of ungerminated and dead seeds was also noted.

2.2.2 Moisture Content Estimation

Moisture is the main factor affecting seed quality and the activities of fungi and insects. Moisture tests were conducted at every sampling time. Estimation of moisture content was made by loss in weight when seed samples were ground and dried in an air oven at a high constant temperature as recommended in the ISTA Rules (1976). Two replicates, each of 5 grams, were ground and kept for four hours in an air oven at $130^{o}C$. The samples were shifted to a dessicator for 30 minutes after drying to cool. The percentage moisture loss in drying was calculated as follows:

$$M2 - M3 \times \frac{100}{M2 - M1}$$

Where

M1 = is the weight in grams of the container and its cover.

M2 = is the weight in grams of the container, cover and its contents before drying.

M3 = weight in grams of the container, cover and contents after drying.

2.2.3 Determination of Storage Fungi

The agar plating method was used to detect storage fungal development in maize seed. The growth and development of fungus was assayed in all treatments at each sampling time.

100 seeds from each treatment were tied in a muslin cloth and immersed for one minute in a 0.1% mercuric chloride solution. The seed samples were then washed in running water for five minutes. Surface sterilised seeds were plated onto malt-salt-agar (MSA) medium. The petri dishes were kept at 30°C for two weeks. Seeds with fungal invasion were then counted. The malt-salt-agar used comprised the following ingredients:

Malt extract, Difco	= 30.0 grams
NaCl	= 75.0 grams
Bacto agar	= 15.0 grams
Distilled water	- 1.0 litre

2.2.4 Internal Seed Damage Assessment

Since *Sitophilus oryzae* completes its life cycle inside the maize grains, the damage rendered by larvae and adults could not always be evaluated by visual examination. Attempts were therefore made to assess the amount of internal damage and also to examine the possibility of preferential feeding by rice weevils on different parts of maize grains. A simple X-ray photography system was employed using a Faxitron 43804N X-ray machine (Plate 5) with exposure onto Polaroid film. Photographs of maize grains from seedlots prior to storage and at regular intervals during the storage period allowed comparison to be made between treatments in the rate and extent of insect damage to internal seed tissues. Following X-ray photography the seeds from each sample were sown in sand in the same sequence. This allowed a comparison to be made between the type of internal seed damage shown by X-ray analysis and the capacity of seeds to develop into normal seedlings in a standard sand test. At the end of the germination test photographs of different seedling categories (normal and abnormal seedlings and dead seeds) were taken.

Plate 5: Faxitron X-ray System Model 43804 Used for Taking X-ray Photographs of Maize Seed

Plate 6: Wet and Dry Bulb Thermometer Used for Determination of Relative Humidity

2.3 STATISTICAL ANALYSIS

Analysis of results showing change with time, while important in itself, was considered to be less meaningful because of the vast differences and the small error values in germination results presented early in the experiment compared with the much larger values occurring later. For this reason, and because the main comparative requirement was within time and between treatment comparison, only within sampling time analysis was carried out.

CHAPTER III

RESULTS

The results of this study are presented in three sections. The first (3.1) considers the effect of the storage environment on seed moisture content, seed germination and abnormal seedling development. The second section (3.2) considers the performance of seed stored under the same conditions as those described in the first sections but with the additional effects of insect populations and insect damage on seed quality. The final section (3.3) looks specifically at changes in storage fungus development and the effects of *Aspergillus* and *Penicillium* species on seed quality during storage.

3.1 SEED STORAGE EFFECTS

3.1.1 Initial Seed Quality

Parameters of initial seed quality, after adjustment of seed moisture content, are set out in Table 1. Conditioning of seed to the desired experimental percentage moisture content had no deleterious effect on germination or other determinants of quality.

TABLE 1: INITIAL LEVELS OF MAIZE SEED QUALITY

	Initial Seed Moisture Content		
	12.4%	15.1%	18.5%
Normal Germination (%)	92.5	92.3	92.0
Abnormal Germination (%)	4.3	5.0	5.3
Dead Seed (%)	3.2	2.7	2.7
Field Fungi (%)	1.0	2.0	4.0
Storage Fungi (%)	34.0	36.0	38.0

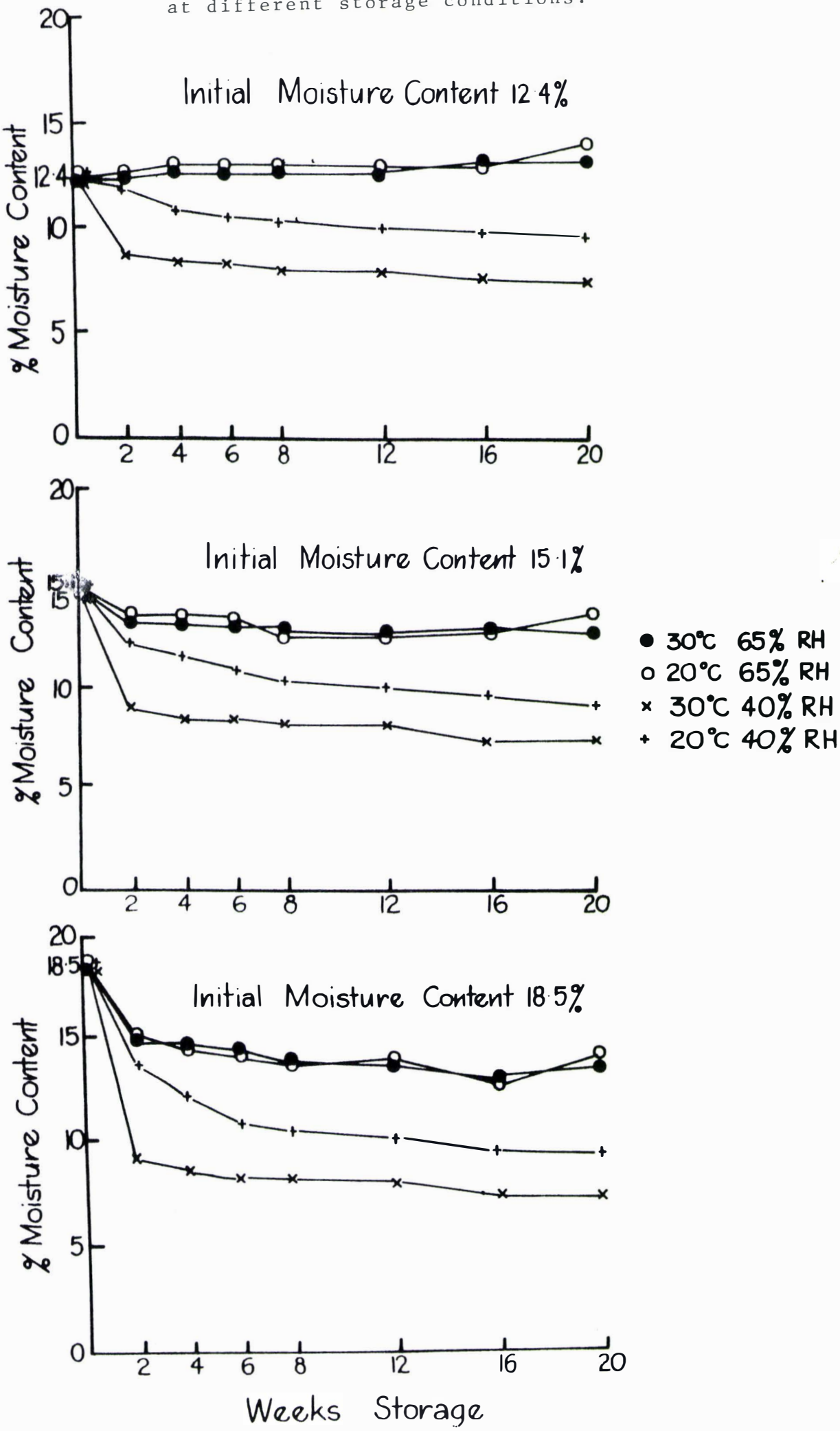
3.1.2 Changes in Seed Moisture Content

The maize seed used in this experiment was stored initially at three different seed moisture contents (18.5, 15.1 and 12.4%). The results presented in Figure 1 clearly show that both storage temperature and relative humidity had rapid and significant effects on seed moisture content. In all treatments the initial moisture content of the seed was increased or decreased until it reached equilibrium with the storage environment. This absorption or desorption of seed moisture with time clearly demonstrated the hygroscopic nature of the seed. In addition, it stressed the dramatic role the relative humidity of the storage environment plays in altering seed moisture content. Storage temperature also had a smaller influence on both the rate and extent of moisture change.

In the case of seed stored at $20^{o}C$ or $30^{o}C$ accompanied throughout with a 40% relative humidity, seed moisture content fell in all treatments to an equilibrium moisture content of between 7.4% and 9.3% after 20 weeks storage. The rate of moisture movement from the seed into the storage atmosphere was initially rapid, particularly over the first 2-4 weeks. Subsequently the rate of moisture change was considerably slower, with often only 1.0 to 1.5% seed moisture loss occurring over the last 12-14 weeks of storage. The rate and extent of moisture loss in 40% RH storage treatments was greater at $30^{o}C$ than at $20^{o}C$. High temperature had its greatest effect in speeding up the rate of initial moisture loss, particularly during the first two weeks of storage. It also resulted in seed reaching a lower equilibrium moisture content (E.M.C) at the end of the experiment. Seed stored at $30^{o}C$ and 40% RH reached an E.M.C. of 7.4-7.6% compared to a 9.1-9.4% E.M.C. in seed stored at $20^{o}C$ and 40% RH.

A similar trend occurred in seed stored at either $20^{o}C$ or $30^{o}C$ under 65% relative humidity. However, the initial moisture content of the seed determined whether the seed

Figure 1: Changes in moisture content of maize seeds stored at different storage conditions.



absorbed moisture from the storage environment as occurred in the 12.4% initial moisture content treatment or desorbed moisture as occurred in the seed stored at initial moisture contents of 15.1% or 18.5%.

The results in Figure 1 show that the rate and extent of moisture absorption in the 12.4% initial moisture content treatment was slow, rising to 13.2% in the 30°C environment and 13.9% in the 20°C storage environment after 20 weeks. In the two higher initial moisture content seedlots desorption of moisture occurred very rapidly over the first 2-4 weeks. Thereafter the rate of change was much slower, a loss of less than 1% occurring in most cases over the remaining 16-18 weeks storage.

Again, temperature played a minor but significant role in the level of equilibrium moisture content reached. The higher storage temperature (30°C and 65% RH) resulted in a lower E.M.C. value (13.1-13.5%) after 20 weeks while the 20°C and 65% RH environment resulted in a 13.9-14.2% E.M.C. value by the end of the experiment.

It is interesting that the initial moisture content of the seed, which ranged from 12.4% to 18.5%, had only a very short influence on seed moisture content in storage. Certainly after 4 weeks in all treatments all of the maize seed had a moisture content below the 15% considered safe for short term storage of mazie seed, irrespective of the initial moisture content. This shows that the influence of pre-storage moisture content was only transitory, 18 out of the 20 weeks storage being influenced most strongly by the storage environment generally, and by the relative humidity in the storage environment in particular. By the end of the experiment there was no significant difference in the E.M.C. of seed attributable to initial seed moisture level (Appendix 1).

3.1.3 Germination

The level of initial seed moisture content i.e. 12.4, 15.1 and 18.5% had no effect on the germination of seed in all storage conditions up to 8 weeks of storage (Table 2). Subsequently, however, a slight decline in germination occurred at all storage conditions after 12 weeks of storage, but the decrease in germination was greatest at the high initial moisture content treatments associated with storage of seed in combination with high temperature ($30^{o}C$) and the higher level of relative humidity (65% RH). At the end of the experiment the reduction in germination at $30^{o}C$ and 65% RH was 11, 14 and 28% in seed with initial moisture contents of 12.4, 15.1 and 18.5% respectively. These results show that the significant reduction in germination at the higher moisture content i.e. 18.5%, was due to the direct influence of relative humidity, temperature and period of storage. The deterioration of seed at this moisture content and storage conditions was gradual and only after 16 and 20 weeks storage did the effect of storage environment become evident (Appendix 3).

It is interesting that each individual seed storage parameter (initial seed moisture content, storage temperature and storage relative humidity) had no major effect on seed germination during 20 weeks storage at their lowest levels i.e. 12.4% and 15.1% initial seed moisture content, $20^{o}C$ storage temperature and 40% storage relative humidity. The most significant reduction in germination capacity occurred at the higher levels of each parameter and then only during the later stages of the storage period. This effect is shown by most significant reductions in germination being recorded in 18.5% initial moisture content seed, at $30^{o}C$ storage temperature and 65% relative humidity. These results suggest that these three storage parameters can be "ranked" in order of severity of influence on seed deterioration rate as high initial moisture content, storage relative humidity and storage temperature.

Table 2: Effect of Moisture Content, Temperature and Relative Humidity on the Germination of Maize Seed. Figures in the Body of the Table are Percentage Germination.

Storage Periods (weeks)	Initial Moisture Content 12.4%				Initial Moisture Content 15.1%				Initial Moisture Content 18.5%			
	20°C		30°C		20°C		30°C		20°C		30°C	
	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH
0	92.5a	92.5a	92.5a	92.5a	92.3a	92.3a	92.3a	92.3a	92.0a	92.0a	92.0a	92.0a
2	92.3a	92.0a	92.0a	92.0a	92.3a	92.3a	91.7a	92.3a	92.0a	92.0a	91.7a	92.0a
4	92.0a	91.7a	91.3a	92.0a	92.3a	92.3a	91.0a	92.0a	92.0a	91.7a	91.0a	92.0a
6	91.3a	89.7a	90.7a	91.3a	91.3a	90.0a	90.3a	91.0a	91.7a	90.0a	89.7a	90.0a
8	90.7a	89.7a	89.7a	90.0a	90.3a	88.3a	90.0a	90.3a	90.3a	87.7b	89.7a	90.0a
12	90.0a	87.3b	88.7ab	89.7a	90.0a	86.3bc	89.3a	89.3a	90.3a	87.0b	89.0a	85.0c
16	84.7bc	85.7b	88.3a	83.0c	84.3bc	85.3b	88.0a	81.3c	82.0c	84.3bc	88.3a	75.0d
20	84.0b	84.7b	89.3a	81.3c	83.3bc	85.3b	87.7ab	78.3d	80.0cd	84.3b	86.0ab	63.7e

Unlike letters in each row show significant differences at $p \leq 0.05$.

The strong effect of high initial seed moisture (18.5%) was surprising since the results in Figure 1 have shown that the change in seed moisture content from 18.5% to a safe level of less than 15% generally occurred during the first 2 weeks of storage. Presumably this was still sufficient time for seed deterioration to occur. This deterioration was reflected in reduced storage longevity of seed, as shown by a significant reduction in germination capacity at 12 weeks storage.

Despite the effects of initial moisture content, storage temperature and relative humidity the greatest drop in seed germination capacity was only 28%. This occurred in the most extreme treatment (18.5% IMC, 30°C - 65% RH). In all other storage treatments the drop in germination from a common level of 92% was small and was generally of the order of 8-12%.

3.1.4 Abnormal Seedling Development

The results in Table 3 show that initial moisture content and storage conditions did not affect abnormal seedling percentage to a great extent. However, in some treatments a slight effect was observed on abnormal seedling percentage. This increase in abnormal seedling percentage was pronounced after 16 and 20 weeks of storage. The most marked increase in abnormal seedlings was at 18.5% M.C., 65% relative humidity and 30°C storage temperature. The abnormal seedling percentages at this storage condition increased 4.5, 2.3 and 5.5 fold for seed at 12.4, 15.1 and 18.5% initial moisture content respectively.

The individual effects of initial seed moisture content and storage temperature and relative humidity on abnormal seedling percentage were generally quite small. Significant differences in abnormal seedling percentage generally occurred only towards the end of the storage period, usually after at least 12 weeks storage.

Table 3: Effect of Moisture Content, Temperature and Relative Humidity on Abnormal Seedling Development of Maize Seed (Without Insects). Figures in the Body of the Table are Percentage Abnormal Seedlings.

Storage Periods (Weeks)	Initial Moisture Content 12.4%				Initial Moisture Content 15.1%				Initial Moisture Content 18.5%			
	20°C		30°C		20°C		30°C		20°C		30°C	
	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH
0	4.3a	4.3a	4.3a	4.3a	5.0a	5.0a	5.0a	5.0a	5.3a	5.3a	5.3a	5.3a
2	3.7ab	3.3b	3.0b	5.7a	4.7a	5.0a	4.7a	3.7ab	4.7a	5.7a	5.3a	3.7ab
4	3.7ab	3.0ab	5.3b	3.3ab	6.0a	4.3a	2.3b	4.5a	4.0a	2.3b	2.0b	4.0a
6	5.0b	3.3c	7.3a	5.7ab	3.7bc	3.7bc	6.0a	3.7bc	4.7b	7.0a	6.7a	4.7b
8	5.7a	5.7a	3.3b	6.7a	6.3a	5.0a	6.0a	4.7a	6.7a	5.0a	5.3a	5.3a
12	4.0b	6.0a	6.0a	5.0b	5.0b	3.7b	3.3b	7.7a	7.0a	4.7b	6.3a	8.3a
16	9.3c	6.0d	2.7e	14.0a	9.3c	4.3e	5.0e	7.7d	14.7a	6.3d	3.7e	12.3b
20	15.0bc	7.0d	5.0d	18.0b	10.3c	4.3d	6.3d	11.0c	13.0bc	9.0cd	8.0cd	28.7a

Unlike letters in each row show significant differences at $P \leq 0.05$.

These results show that the treatments used in this study had a relatively small effect on the percentage of abnormal seedlings. However, there were some variations in the types of abnormalities in seedling development which were observed.

In a germination test at each sampling time the determination of abnormal seedlings was considered to be a useful criterion for indicating seed deterioration. Certainly the level of abnormal seedlings was directly related to percentage normal germination. The analysed data with respect to abnormal seedling development is given in Appendix 4.

Types of Abnormal Seedlings

A number of different categories of abnormal seedlings became apparent in germination tests carried out at different storage intervals. While the level of seedling abnormality was generally rather low four main types were apparent. These included abnormal coleoptile and root development, seedling decay and a general category which included weak or unbalanced seedlings and seedlings with missing structures. The proportion of abnormal seedlings in each category varied with different storage treatments, highest numbers of abnormals occurring in the most severe storage environments.

Normal seedling development (Plate 7) and seedling abnormalities were recorded and identified according to categories prescribed in the ISTA Rules (1976). Different types of abnormalities were recorded from 2-20 weeks of storage. The main types of abnormal seedlings (without insects present) are shown in Plates 8-11. Changes in the abnormal seedling percentage should be taken into consideration along with the fluctuating numbers of dead seeds and normal seedlings in evaluating the influence of a particular treatment. Details of the occurrence of

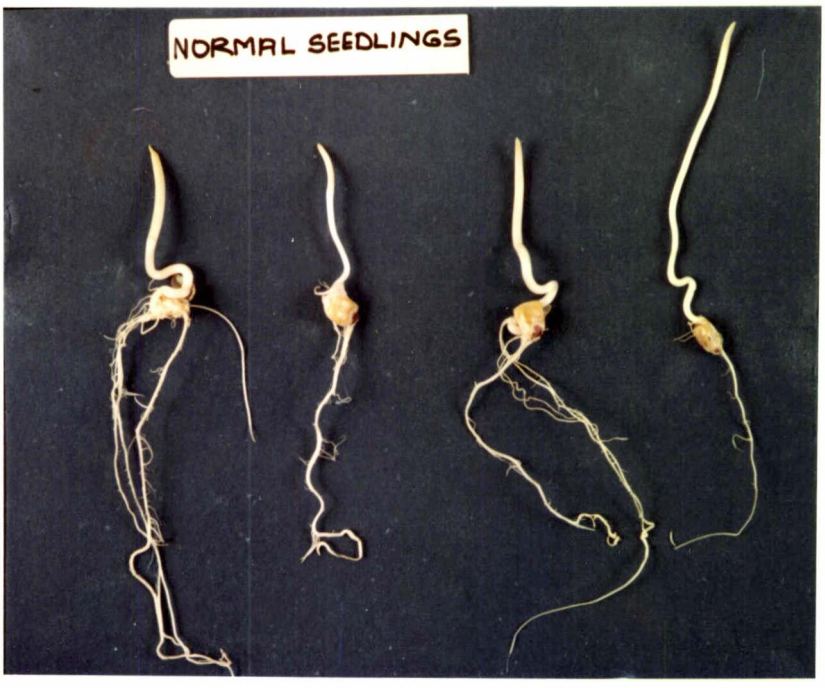


Plate 7: Normal Seedlings With Well Developed Primary Roots and Adventitious Roots and Well Developed Shoots

the different categories of abnormal seedlings after 12 and 20 weeks storage are given in Figure 4 (page 56).

Types of abnormal seedlings recorded were:

1. Split Coleoptile
   Primary leaves split longitudinally and coleoptile with a split easily visible to the naked eye (Plate 8). Coleoptile abnormality was particularly apparent after 8 weeks storage, especially in the 30°C - 65% RH storage treatment. In all other storage treatments the level of coleoptile abnormality was generally less than 1%.

2. Abnormal Roots
   These seedlings showed weak and short primary roots, with a few adventitious roots but with well developed shoots (Plate 9).

3. Decay
   Seedling decay became more obvious as a cause of abnormal development in the 65% RH storage treatments. The most common type of decay involved necrosis of the plumule and root tissue (Plate 10).

4. Other Abnormalities
   Other abnormalities, including weak or unbalanced development and weak or missing plumules occurred generally at low levels (2-3%) in all storage treatments. Generally such abnormal seedlings showed no plumule, or weak plumule development (Plate 11).

The level of total abnormal seedlings present in germination tests carried out at different times was consistent with the rate of general seed deterioration which occurred during the total storage period of 20 weeks.

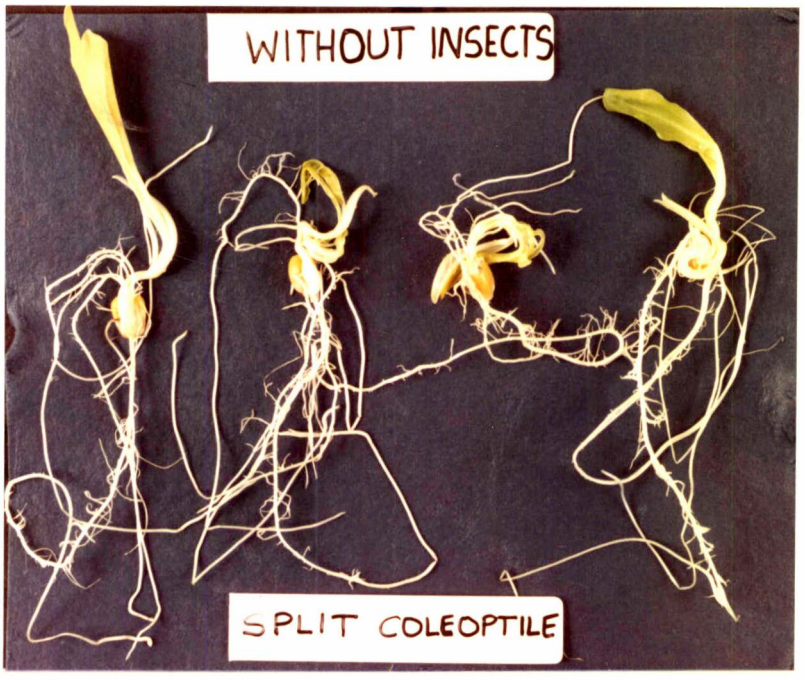


Plate 8: Abnormal Type 1 - Longitudinally Split Coleopitle

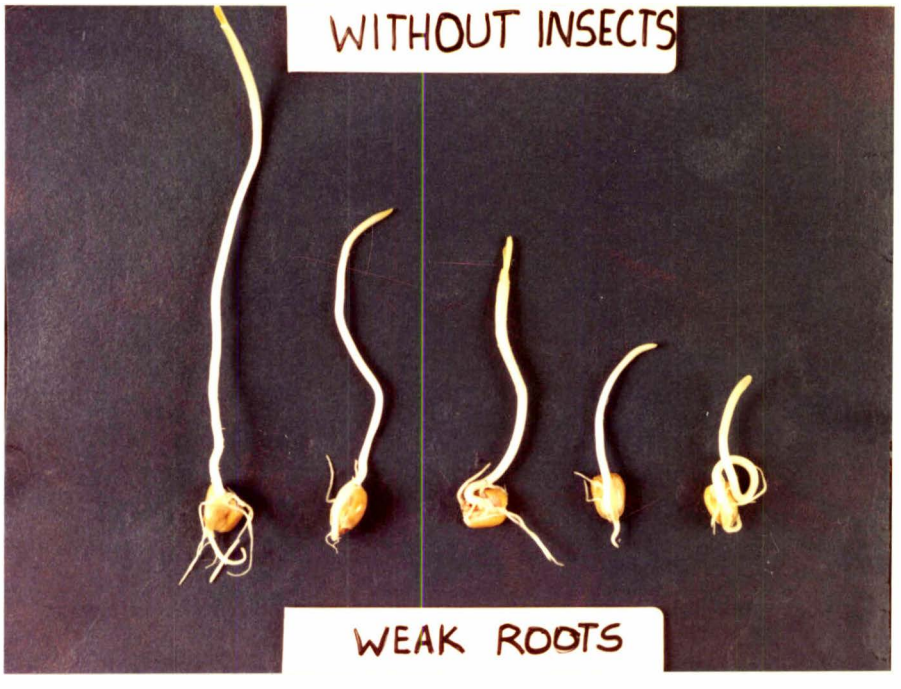


Plate 9: Abnormal Type 2 - Primary Root Weak and Short, With a Few Adventitious Roots

Plate 10: Abnormal Type 3 - Decayed Primary Roots

Plate 11: Abnormal Type 4 - Weak Plumule

3.2 EFFECTS OF INSECTS ON STORED SEED

The results obtained with insects in the presence of stored seed are considered under two headings; the effect of storage condition on insect populations (3.2.1) and the effects that insect populations had on (a) seed moisture content (3.2.2) and (b) seed germination and abnormal seedling development (3.2.3).

3.2.1 The Effects of Storage Conditions on Insect Populations

The effects of seed storage conditions on survival of introduced adult insects and on overall population development are shown in Figure 2a, b, c and Appendix 2.

Mortality of introduced adult insects and overall population development were greatly influenced by storage conditions. At 40% RH the number of introduced adult insects declined under all initial seed moisture contents and both temperatures. Mortality was greater at lower initial seed moisture contents than higher and was also higher at 30°C than at 20°C.

Mortality of introduced insects was 100% after 6 weeks storage at 30°C and initial moisture content of 12.4%, but it took 8 weeks and 12 weeks respectively at 15.1% and 18.5% initial moisture content before all introduced insects were dead.

At 65% RH little mortality of introduced insects occurred after 6 weeks storage regardless of initial seed moisture content and temperature. In contrast, numbers of adult insects increased in all seed moisture contents and both storage temperatures after 6 to 12 weeks storage. This increase in numbers was most rapid at the higher temperature and at the higher initial moisture contents. The effect of initial seed moisture content in this respect

Figure 2.a: Changes in Rice weevil populations in seeds with 12.4% moisture content stored under different storage conditions.

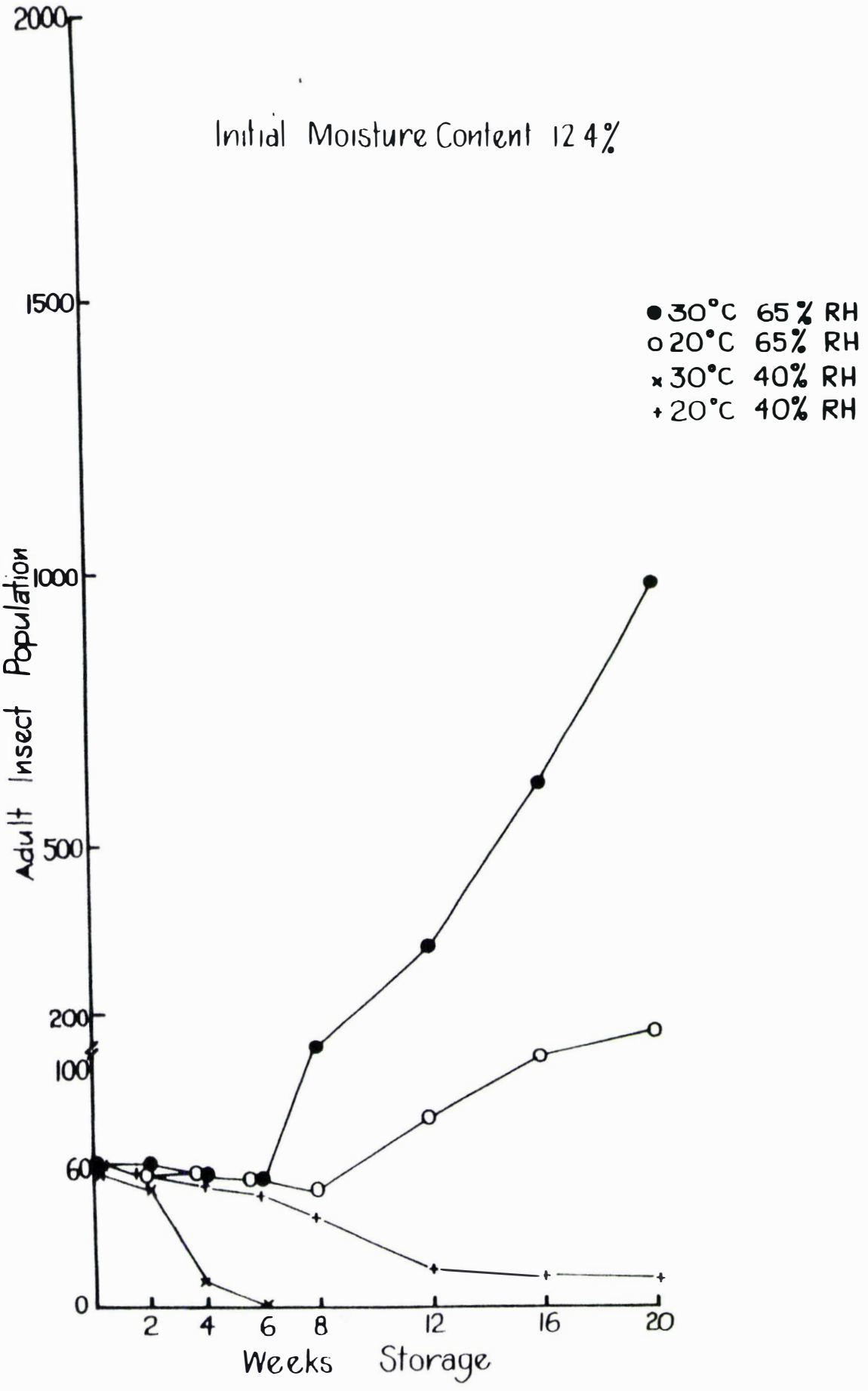


Figure 2.b: Changes in Rice weevil populations in seeds with 15.1% moisture content stored at different storage conditions.

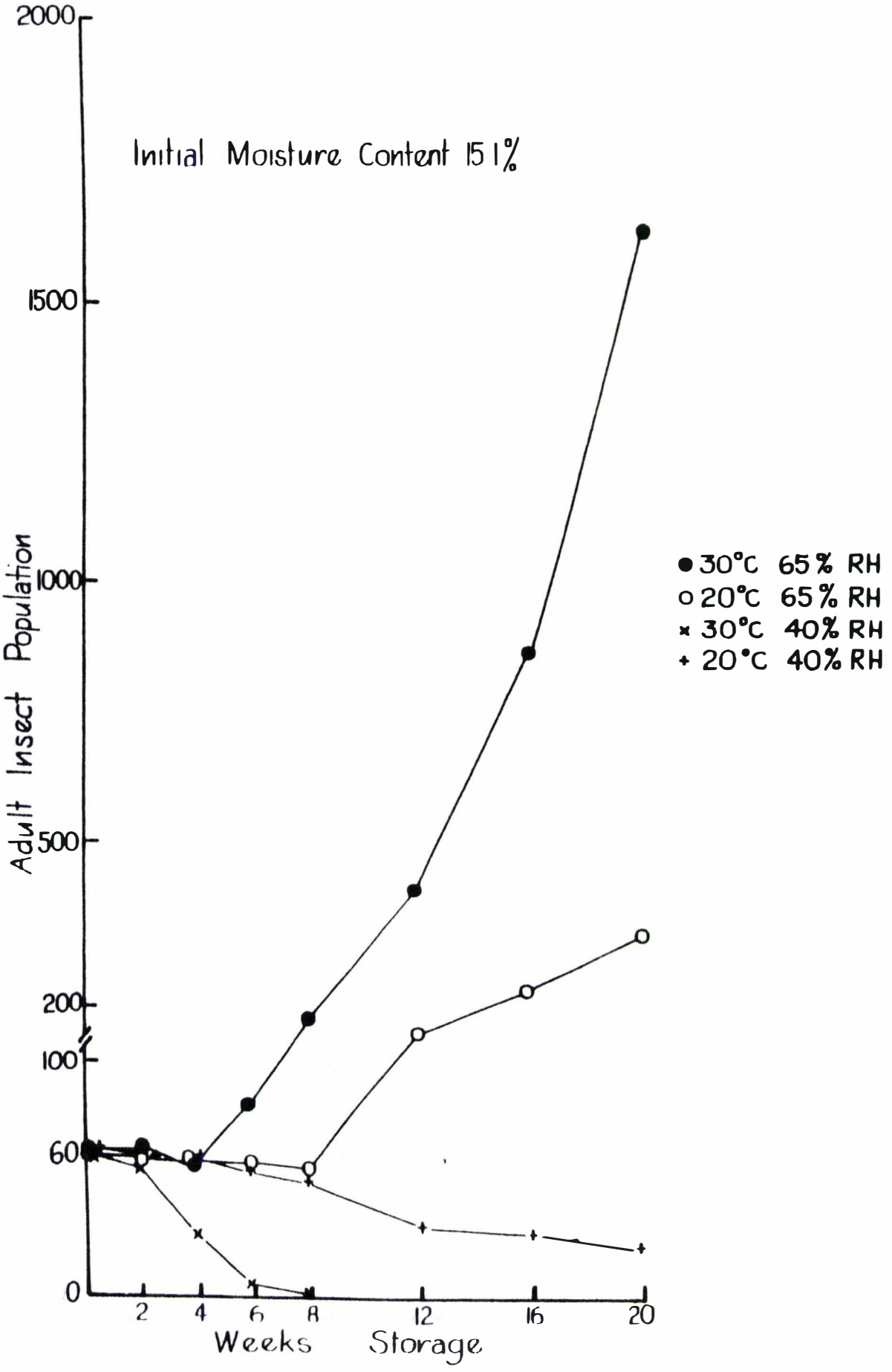
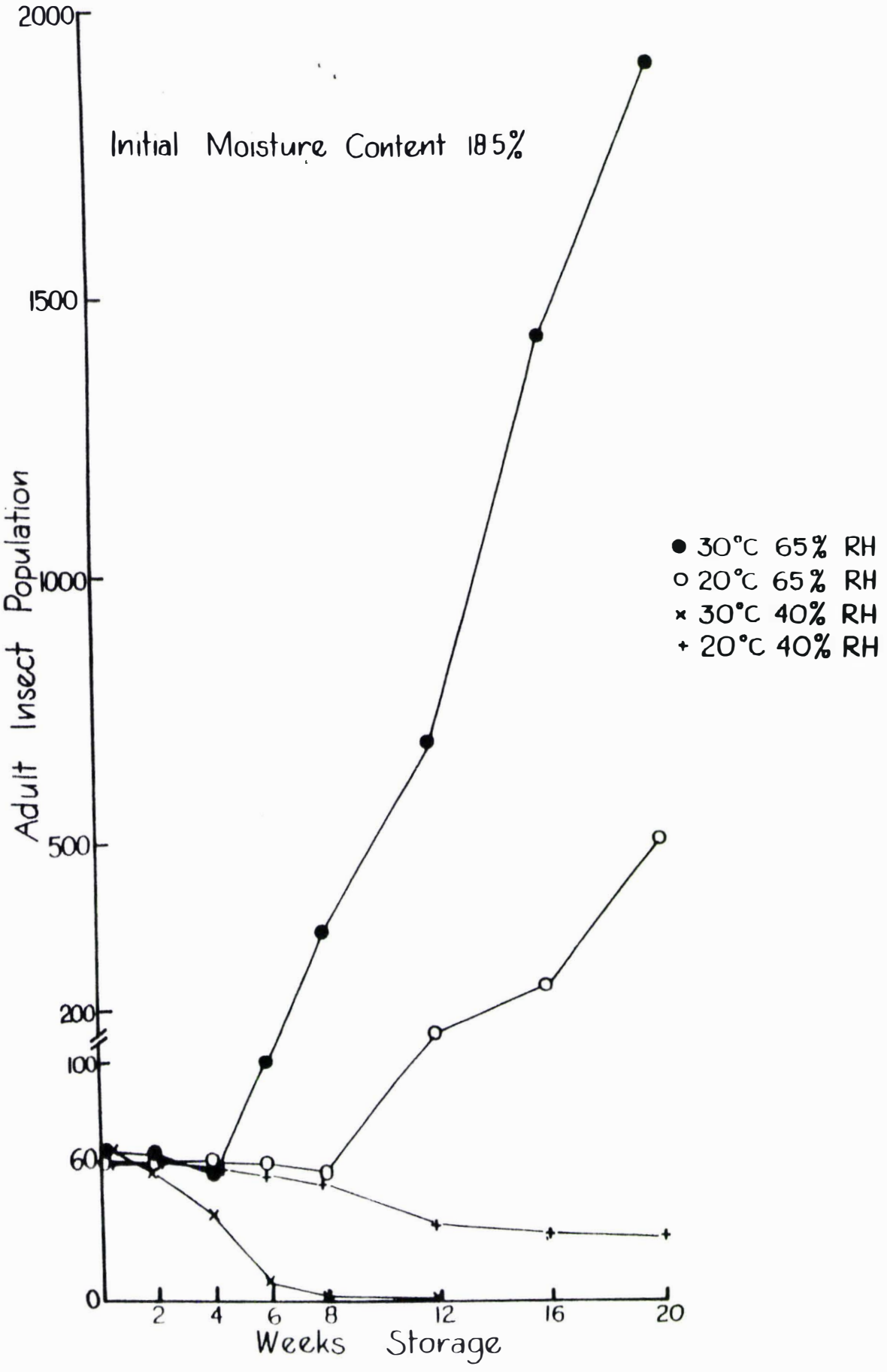


Figure 2.c: Changes in Rice weevil populations in seeds with 18.5% moisture content stored at different storage conditions.



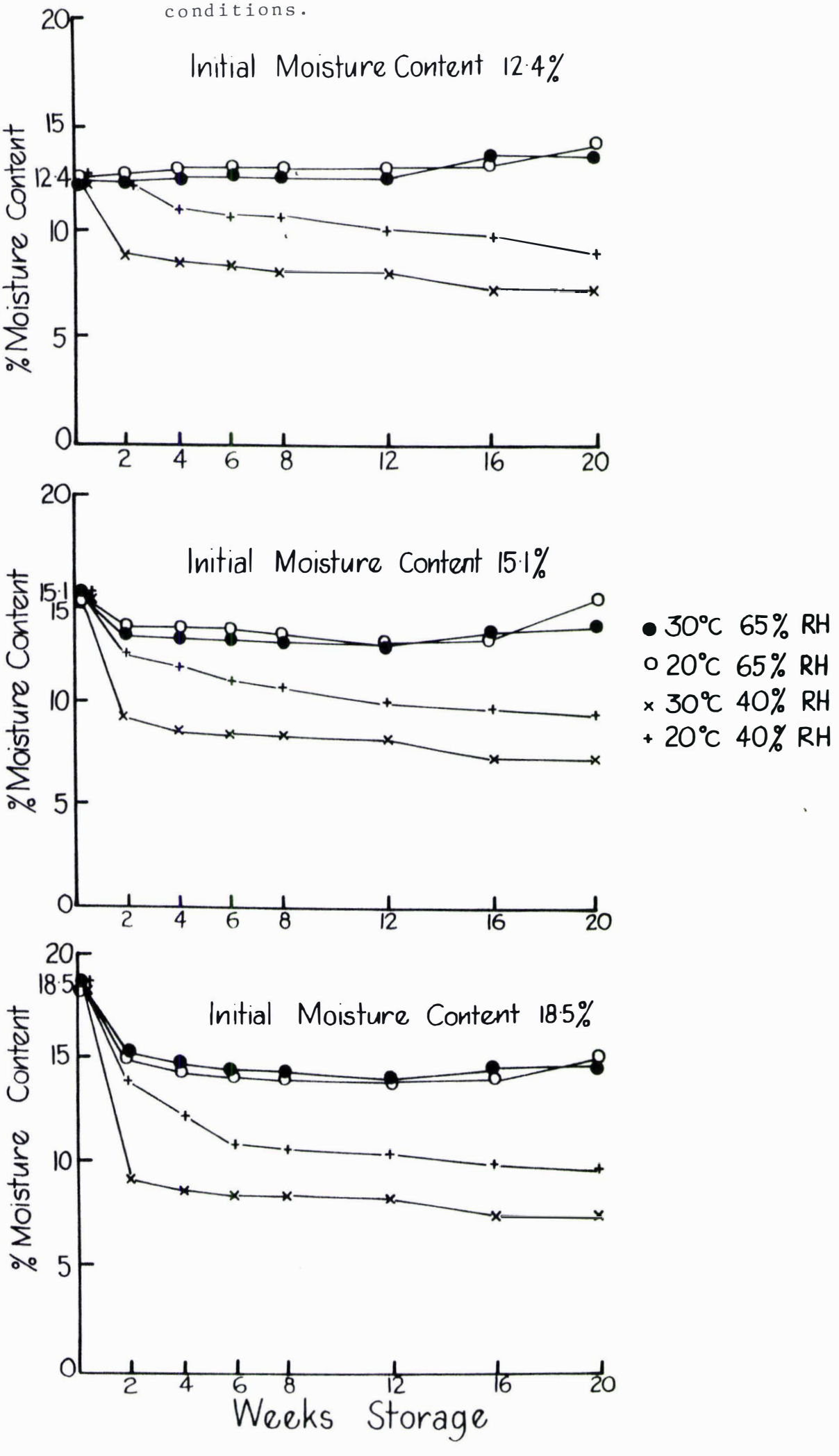
is interesting as it could only have acted over the first two weeks of storage as after this period all seed moisture contents were similar (see Section 3.1 and Figure 1). At the end of the experiment (20 weeks) the adult insect population had increased to 1000 (x16) to 1659 (x27.5) and to 1908 (x31.8) in seed stored at 30°C and with initial moisture contents of 12.4, 15.1 and 18.5% respectively. At 20°C the corresponding increases were only to 181 (x3.0), to 351 (x5.8) and to 529 (x8.8) respectively, showing the important effect of temperature. From published informatiom it seems likely that rice weevils completed three life cycles at 30°C during the period of the experiment but only two life cycles at 20°C.

The over-riding factor determining adult insect survival and ability to reproduce was clearly humidity. 40% RH caused direct mortality of introduced insects and prevented reproduction, whereas 65% RH caused little mortality and enabled the population to increase.

3.2.2 The Effect of Insect Populations on Seed Moisture Content

Changes in the moisture content of seed stored at 40% RH with introduced insects, were closely similar to those of seed stored without insects (Figures 3 and 1). At this low humidity mortality of introduced adults insects occurred as previously discussed and no reproduction occurred. In contrast, at 65% RH the insect population increased, the increase being most marked at high temperature and high initial seed moisture content. These insect populations caused no change in seed moisture content for the first 12 weeks of storage but after that time, up to the termination of the experiment at 20 weeks, seed moisture contents increased in the presence of insects compared with seed stored under the same conditions without insects (Figures 3 and 1). It may be postulated that increased

Figure 3: Changes in moisture content of maize seeds (with insect) stored under different storage conditions.



respiratory activity associated with the higher insect numbers accounted for the observed increase in seed moisture content.

3.2.3 The Effect of Insect Populations on Germination and Abnormal Seedling Development

Changes in percentage germination of seed stored with and without insects are shown in Tables 4 and 2. It will be seen that seed stored at 40% RH, in which introduced insects did not survive and in which reproduction did not take place, showed no reduction of germination in the presence of insects. This indicates that any feeding which introduced adult insects may have undertaken had no detectable effect on the seed. In contrast, however, all treatments stored at 65% RH, under which conditions insect populations increased, showed a marked decline in germination commencing from about 8 weeks of storage and continuing at a progressive rate through to the termination of the experiment. The degree of decline in germination was closely correlated with increase in insect numbers. At 30°C and 65% RH germination percentage was reduced to, or close to, zero after 20 weeks for all initial seed moistures. The fact that no decline in germination was detected until at least one generation of weevil reproduction had been completed (6-8 weeks depending on temperature) suggests that all significant injury to the seed was caused by feeding larvae rather than by adults.

Abnormal Seedling Development

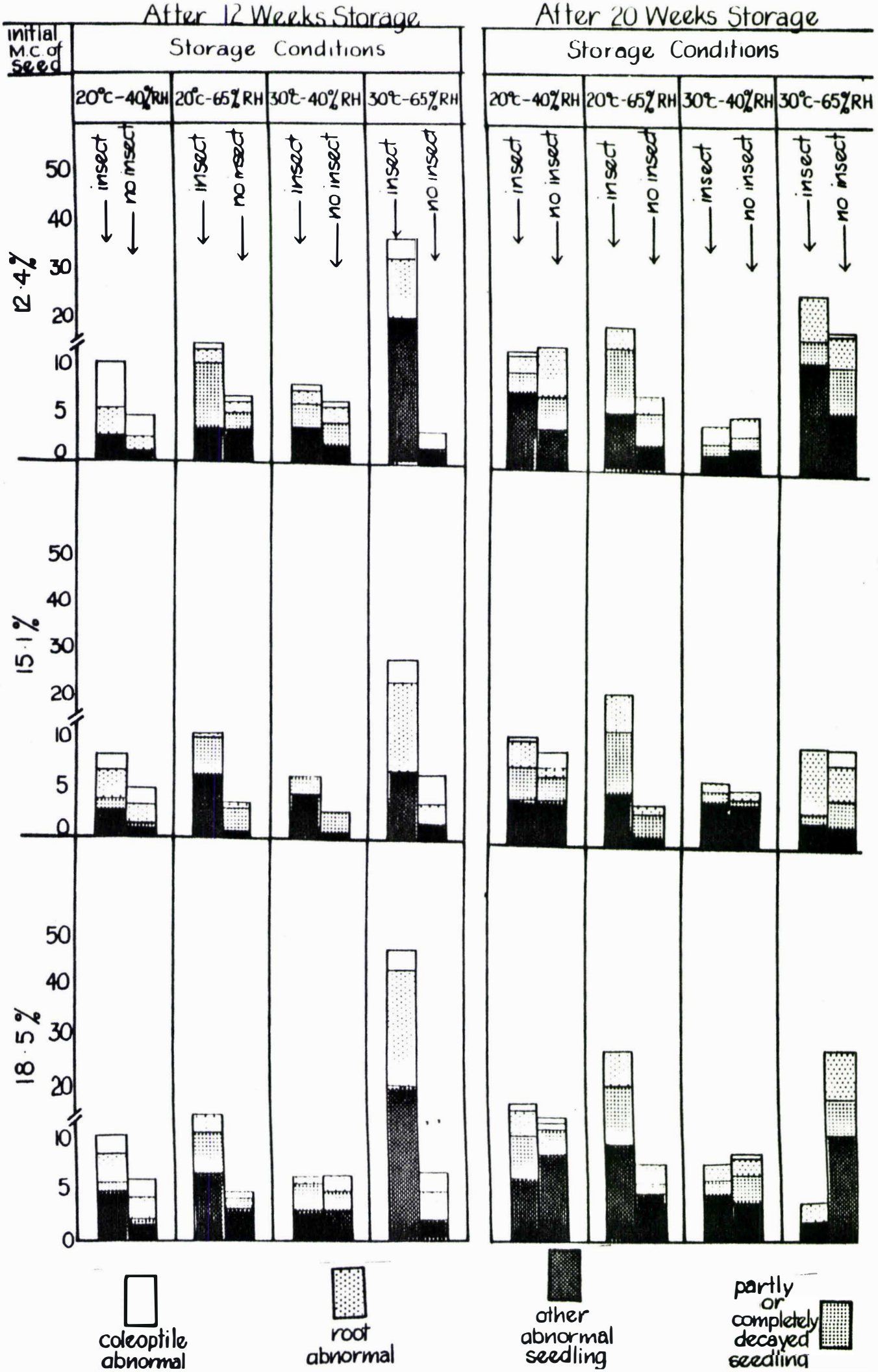
Abnormalities of seedlings in insect treatments were generally greater than occurred in seed storage without insects and were pronounced after 6 weeks of storage. The same four main types of abnormalities occurred as with seed stored without insects (Figure 4), (coleoptile abnormality, abnormal root development, partly or completely

Table 4: Effect of Moisture Content, Temperature and Relative Humidity on the Germination of Maize Seed (With Insects). Figures in the Body of the Table are Percentage Germination.

Storage Periods (weeks)	Initial Moisture Content 12.4%				Initial Moisture Content 15.1%				Initial Moisture Content 18.5%			
	20°C		30°C		20°C		30°C		20°C		30°C	
	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH
0	92.5a	92.5a	92.5a	92.5a	92.3a	92.3a	92.3a	92.3a	92.0a	92.0a	92.0a	92.0a
2	92.0a	91.7a	92.0a	91.7a	92.3a	91.7a	92.3a	92.0a	92.0a	91.7a	91.3a	89.0b
4	92.0a	91.7a	91.0a	91.7a	92.0a	90.7a	90.3a	90.0b	92.0a	90.0b	90.7a	87.7c
6	90.0a	89.3a	90.0a	89.0a	90.3a	88.3ab	89.3a	84.3c	90.7a	86.7b	89.0a	80.3d
8	90.0a	84.0b	88.7a	81.3b	88.3a	82.3b	89.0a	80.7b	87.3a	73.0c	87.7a	63.3d
12	88.7a	73.0ab	88.7a	67.0c	86.7a	72.0b	87.7a	52.0d	85.0a	67.0c	88.3a	25.0e
16	82.3b	68.0c	88.3a	34.7e	81.0b	62.3d	87.3a	28.3f	79.0b	61.0d	86.0a	7.0g
20	81.3b	67.0e	88.3a	8.0h	77.0c	49.0f	86.7a	2.0i	71.7d	19.0g	86.3a	0.0i

Unlike letters in each row show significant differences at $P \leq 0.05$.

Figure 4: Effect of different storage conditions on the percentage of abnormal seedlings and the development of different types of abnormalities in maize seed during germination tests after 12 and 20 weeks storage.



decayed seedlings and other miscellaneous abnormalities which comprised weak seedlings with weak or missing structures (plumule or root)).

The extent of abnormalities depended upon the insect population. Thus in favourable conditions for insect development i.e. 30°C - 65% RH and 20°C - 65% RH, abnormalities were correlated with the insect population. (Details are given in Table 5)

Abnormal Seedling Categories

Coleoptile abnormalities occurred after 6 weeks of storage and were highest in the most favourable conditions for insect development (2-3%). At the end of the experiment the percentage of these abnormalities was reduced, presumably because many affected seeds had by then decayed and died. Such types of abnormal seedlings showed the primary leaves to be split longitudinally (Plate 12). In other cases the coleoptile was physically broken (Plate 13) and was easily visible to the naked eye.

Root abnormalities were recorded in all treatments. After 12 weeks storage highest root abnormalities occurred in seedling from the 30°C - 65% RH storage environment at a rate of 12.4, 19.2 and 22.6% at initial moisture contents of 12.4, 15.1 and 18.5% respectively. However, after 20 weeks of storage the occurrence of such abnormalities was also reduced. Such types of abnormal seedlings showed weak and short primary roots, but with well developed shoots (Plate 14).

Some decay of seedlings occurred in most of the treatments but was most pronounced after 12, 16 and 20 weeks storage. The highest level of seedling decay of 11.2% occurred in seed stored at 20°C - 65% RH. These abnormalities generally showed complete decay by the end of a normal germination test (Plate 15).

Table 5: Effect of Moisture Content, Temperature and Relative Humidity on the Development of Abnormal Seedlings of Maize Seed (With Insects). Figures in the Body of the Table Are Percentage Abnormal Seedlings.

Storage Periods (weeks)	Initial Moisture Content 12.4%				Initial Moisture Content 15.1%				Initial Moisture Content 18.5%			
	20°C		30°C		20°C		30°C		20°C		30°C	
	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH
0	4.3a	4.3a	4.3a	4.3a	5.0a	5.0a	5.0a	5.0a	5.3a	5.3a	5.3a	5.3a
2	3.3c	4.3bc	4.0bc	4.7bc	5.3b	4.6bc	5.7b	4.7bc	4.7c	5.7b	5.0b	8.0a
4	3.7b	3.0b	6.3a	5.3ab	3.3b	5.0ab	4.0ab	5.0ab	3.0b	4.3ab	5.0ab	6.0a
6	7.0b	3.7c	6.0bc	6.0bc	6.3bc	7.7ab	8.0ab	10.0a	6.0bc	11.3a	7.0b	12.7a
8	8.0c	7.7c	5.0c	13.7b	6.7c	7.3c	5.0c	16.7b	8.7c	14.0b	8.3c	25.0a
12	9.3ef	12.7e	7.0f	25.7c	10.0ef	12.7e	6.7f	31.7b	12.7e	18.0d	6.0f	47.7a
16	11.7d	16.0cd	3.7e	26.3b	14.3cd	13.3d	3.7e	43.3a	14.3cd	18.7c	5.7e	24.0b
20	12.7d	18.7c	5.7e	26.3a	11.3d	23.0ab	6.7e	10.3d	21.7b	26.7a	7.3e	4.0e

Unlike letters in each row show significant differences at $p \leq 0.05$.

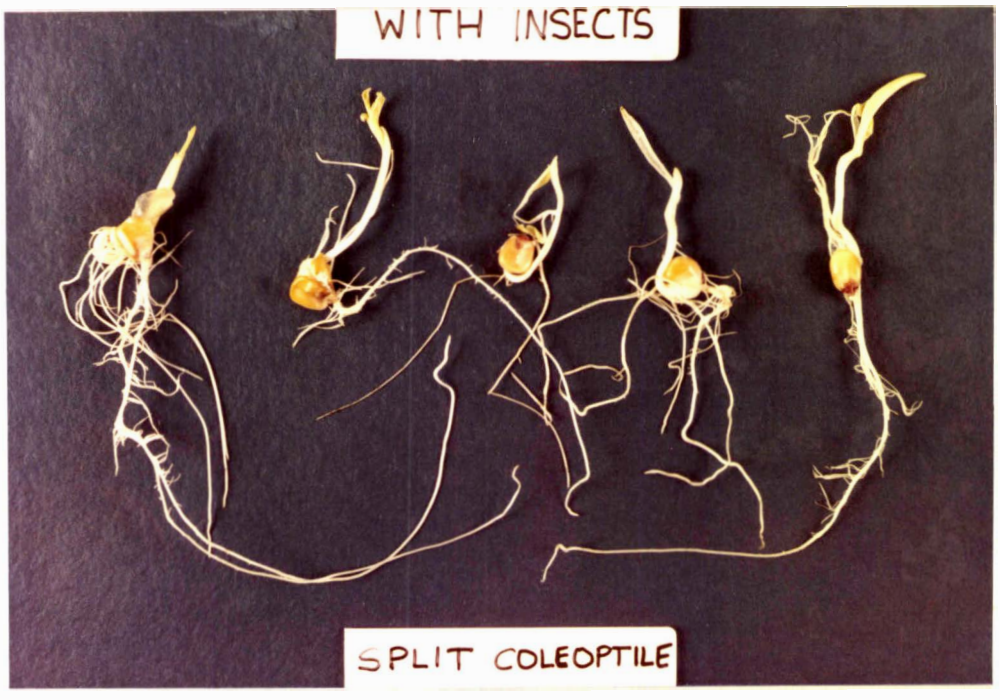


Plate 12: Abnormal Type 1 - Longitudinally Split Coleoptile

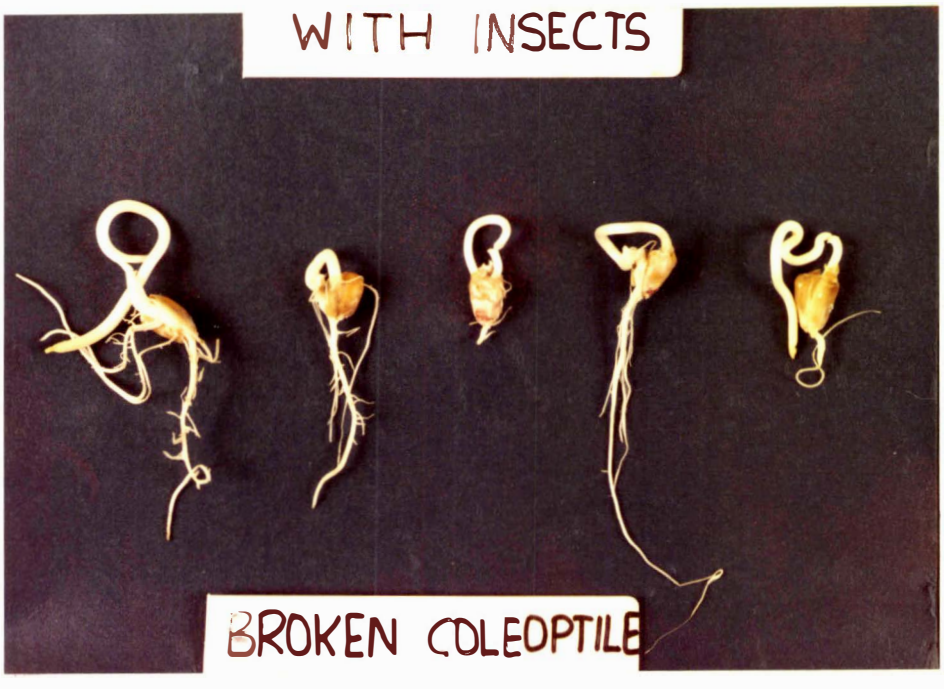


Plate 13: Abnormal Type 2 - Broken Coleoptile

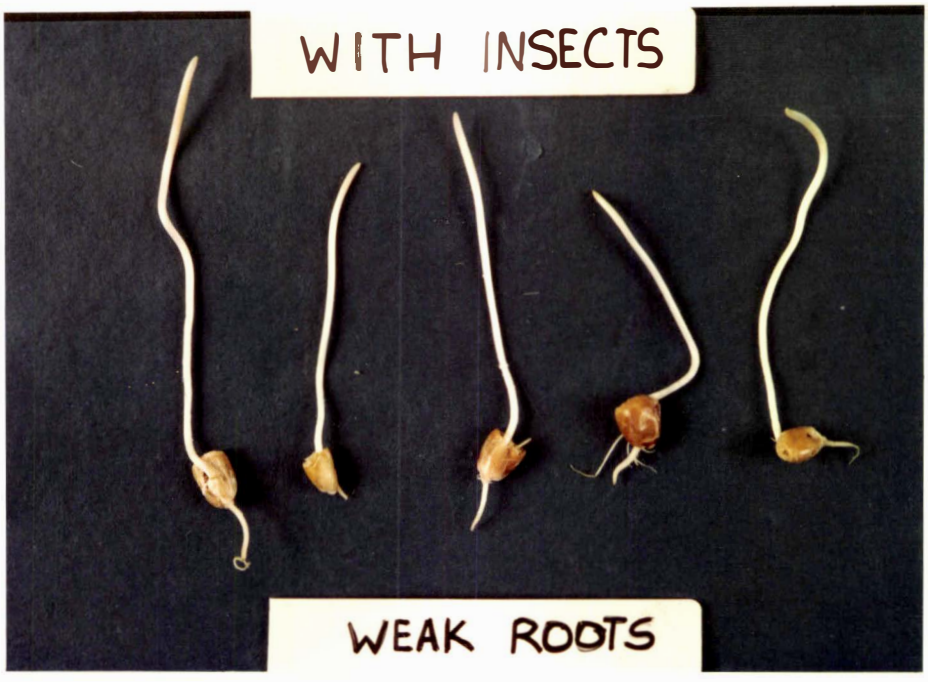


Plate 14: Abnormal Type 3 - Primary Root Weak or Short

Plate 15: Abnormal Type 4 - Decayed Seedlings

Other abnormalities generally included weak seedlings, and seedlings showing unbalanced development of the plumule and roots (Plates 16 and 17). Such abnormalities reached a peak after twelve weeks of storage but had almost disappeared by the end of the experiment.

The maximum percentage of abnormal seedlings was 47.7% after 12 weeks storage in the 30°C - 65% RH treatment compared with 26.7% after 20 weeks storage in the 20°C - 65% RH treatments. These levels reflected the damage to seed caused by increasing insect populations. Certainly, in storage treatments where insects did not cause seed damage, (i.e. 20°C - 40% RH, 30°C - 40% RH) abnormal seedling percentages were generally low within the range 5.7-12.7% after 20 weeks storage. However, in the 18.5% initial moisture content 20°C - 40% RH treatment the abnormal seedling percentage reached a maximum of 21.7%. The decline in abnormal seedling percentage after it had reached a maximum reflected a continuation in seed deterioration and was due to a corresponding increase in the percentage of dead seeds (Appendix 4).

3.3 INSECT DAMAGE ASSESSMENT

Plates 18-20 show three degrees of external visible damage to maize seed caused by rice weevils, ranging from no visible damage in Plate 18 to severe damage in Plate 20. In Plate 19 the first row of seeds appears sound with no visible signs of external damage. In the other four rows holes in the starchy and embryo parts of the seed are conspicuous. In the third row of the same plate most of the grains show damage near the embryo region of the seed. In Plate 20, most of the grains show damage both to the starchy as well as to the embryo parts. Plates 21-23 are X-ray photographs of the same groups of seeds as in Plates 18-20. Corresponding photographs of the seeds after tray germination are shown in Plates 24-26. Finally,

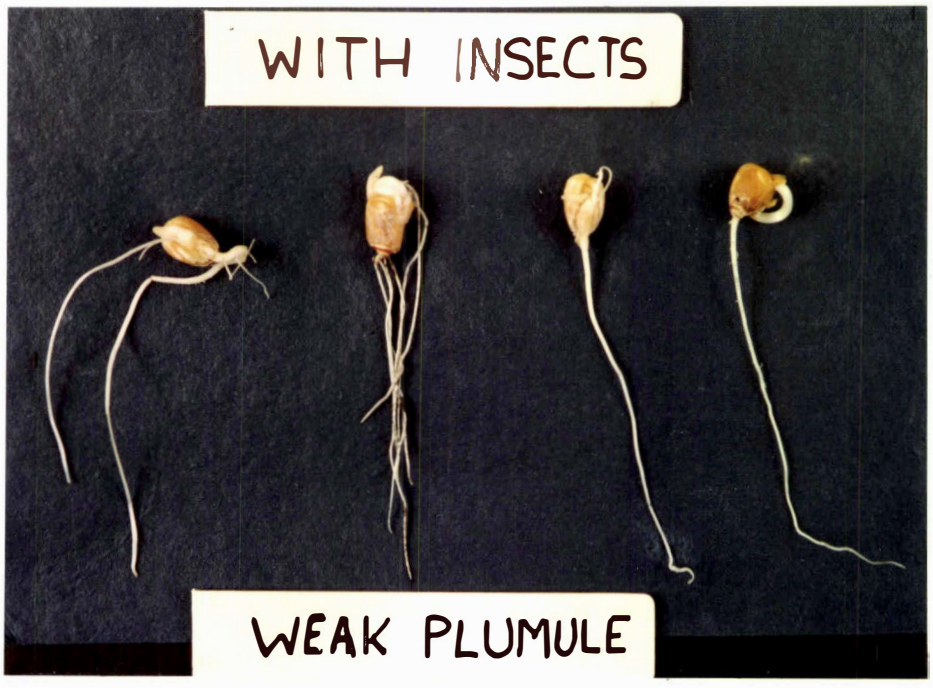


Plate 16: Abnormal Type 5 - Weak Plumule

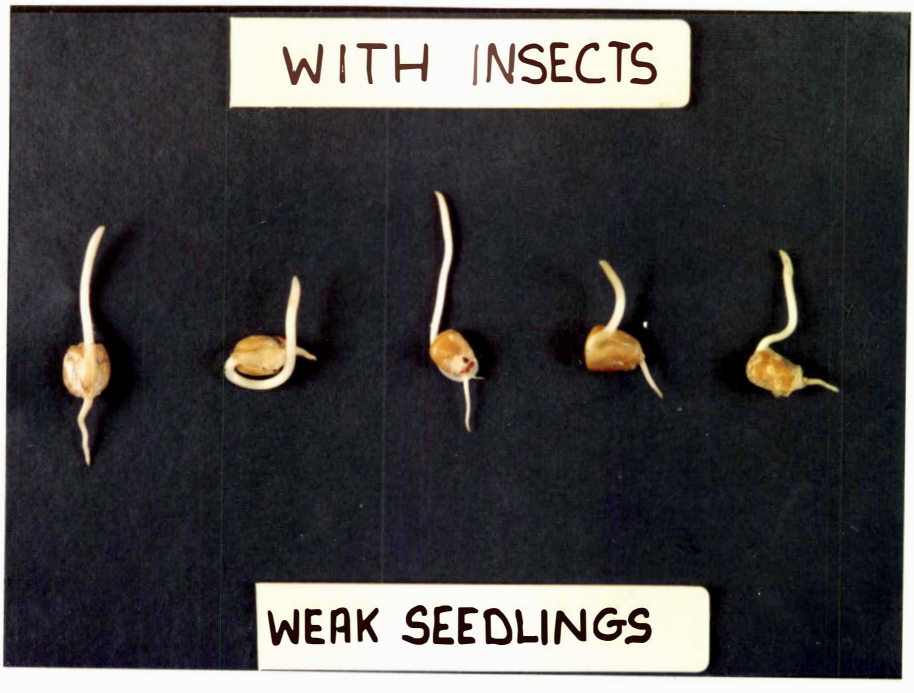


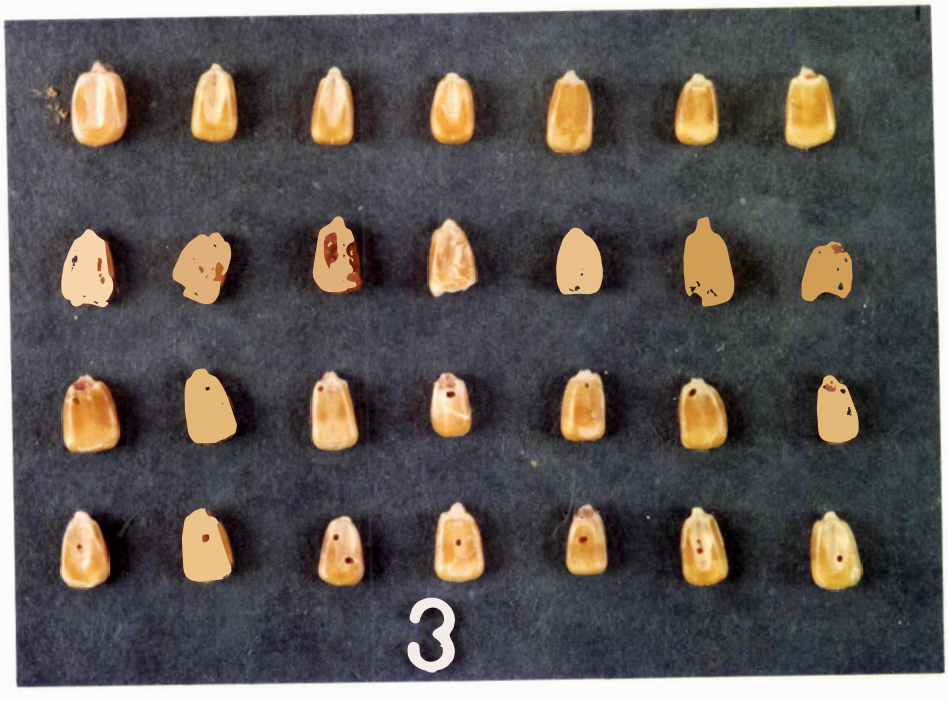
Plate 17: Abnormal Type 6 - Weak Seedlings

Plate 18: Seeds Without Visible External Damage

Plate 19: Seeds With Medium External Damage

Plate 20: Seeds With Severe External Damage


16 1


3


16 2

Plate 21: X-ray Photographs Showing the Seeds With No Internal Damage

Plate 22: X-ray Photograph Showing the Seeds With Medium Internal Damage

Plate 23: X-ray Photograph Showing the Seeds With Severe Internal Damage

Plate 24: Sand Germination Test of Maize Seeds Without Damage

Plate 25: Sand Germination Test of Medium Damaged Maize Seed

Plate 26: Sand Germination Test of Severe Damaged Maize Seed

Plates 27-29 show groups of seeds with similar levels of injury after roll germination tests.

The seeds in Plate 21 show no evidence of internal injury from the X-ray photograph. The same seeds when germinated produced a high germination percentage of normal seedlings (Plates 24 and 27). However, several seeds from Plate 19 which had no visible external damage showed internal injury when X-rayed (Plate 22; 1st, 2nd and 6th grains in the first row) and the presence of a larva in each grain. In the rest of the grains more than one larva is visible. The area of internal damage is from the starchy to the embryo region. The germination percentage for these seeds was low with many abnormal seedlings (Plate 25).

The photo X-ray of severe external damage (Plate 23) reveals that all the grains in this case are infested with larvae. Most of the grains show severe internal damage. Both the starchy and embryo part of the grains have been damaged. Germination in this case was practically zero (Plate 26).

Roll germination tests for three similar groups of seeds (Plates 27-29) show the same pattern of increasing numbers of dead seeds with increasing insect injury to the seed and a high percentage of abnormal seedlings with intermediate insect injury.

3.4 FUNGAL DEVELOPMENT

Changes in the extent of fungal infection occurring in maize seeds during 20 weeks storage are shown in Figures 5 and 6 with and without insect treatments respectively.

The initial seedlot used in the experiment had 34, 36 and 38% infection with storage fungi in the 12.4, 15.1 and 18.5% initial moisture content samples respectively. At the beginning of the experiment, and at the sampling after 2

Plate 27: Showing Normal Seedlings, Abnormal Seedlings and Dead Seeds. Planted Without Damaged Maize Seed

Plate 28: Showing Normal Seedlings, Abnormal Seedlings and Dead Seeds. Planted With Medium Damaged Maize Seeds

Plate 29: Showing Normal Seedlings, Abnormal Seedlings and Dead Seeds. Planted With Severe Damaged Maize Seeds


16—1


3


16 2

Figure 5: Changes in percentage of fungal development in maize seeds (with insect) at initial moisture contents of 12.4%, 15.1% and 18.5%, stored under different storage conditions.

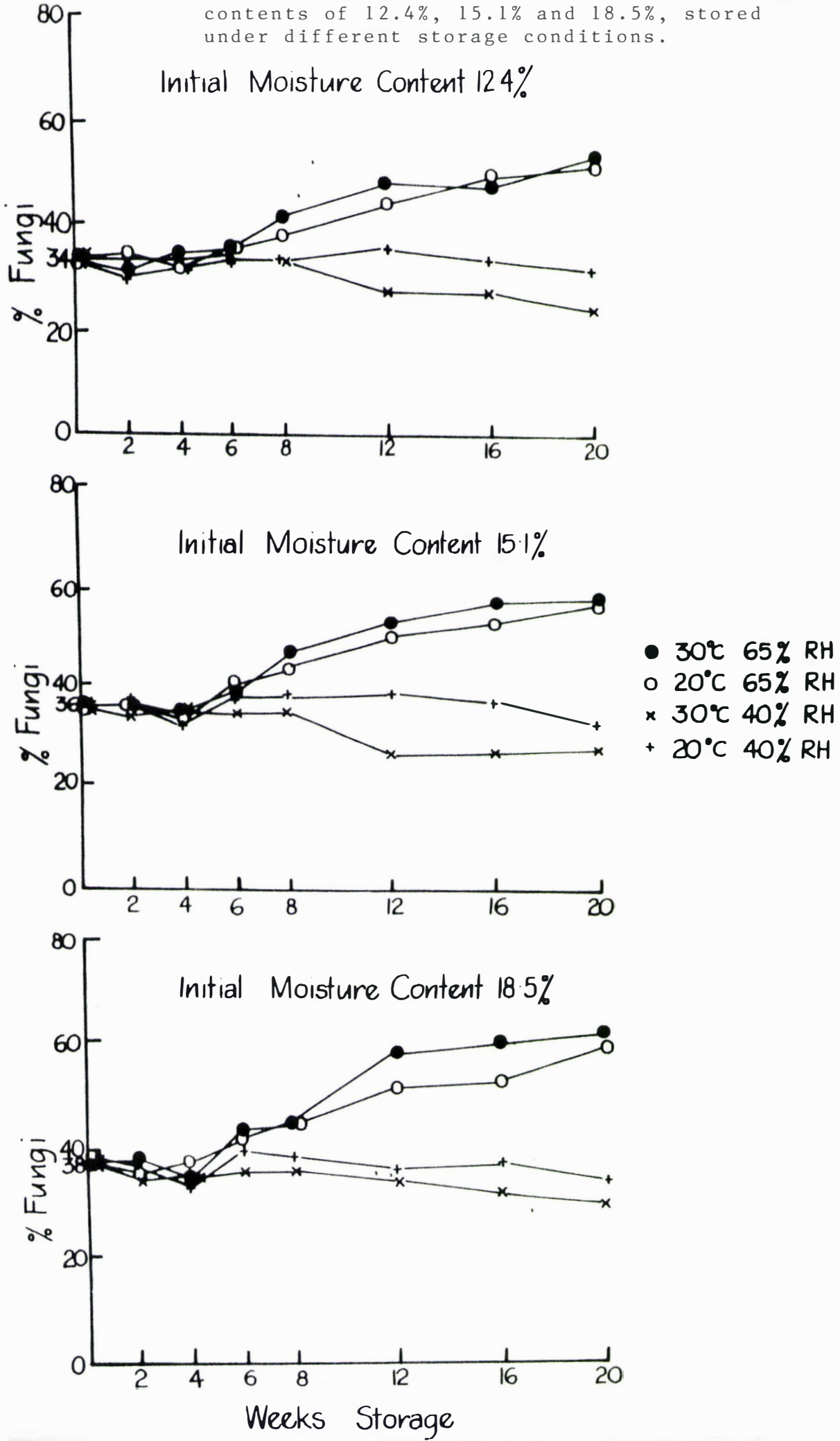
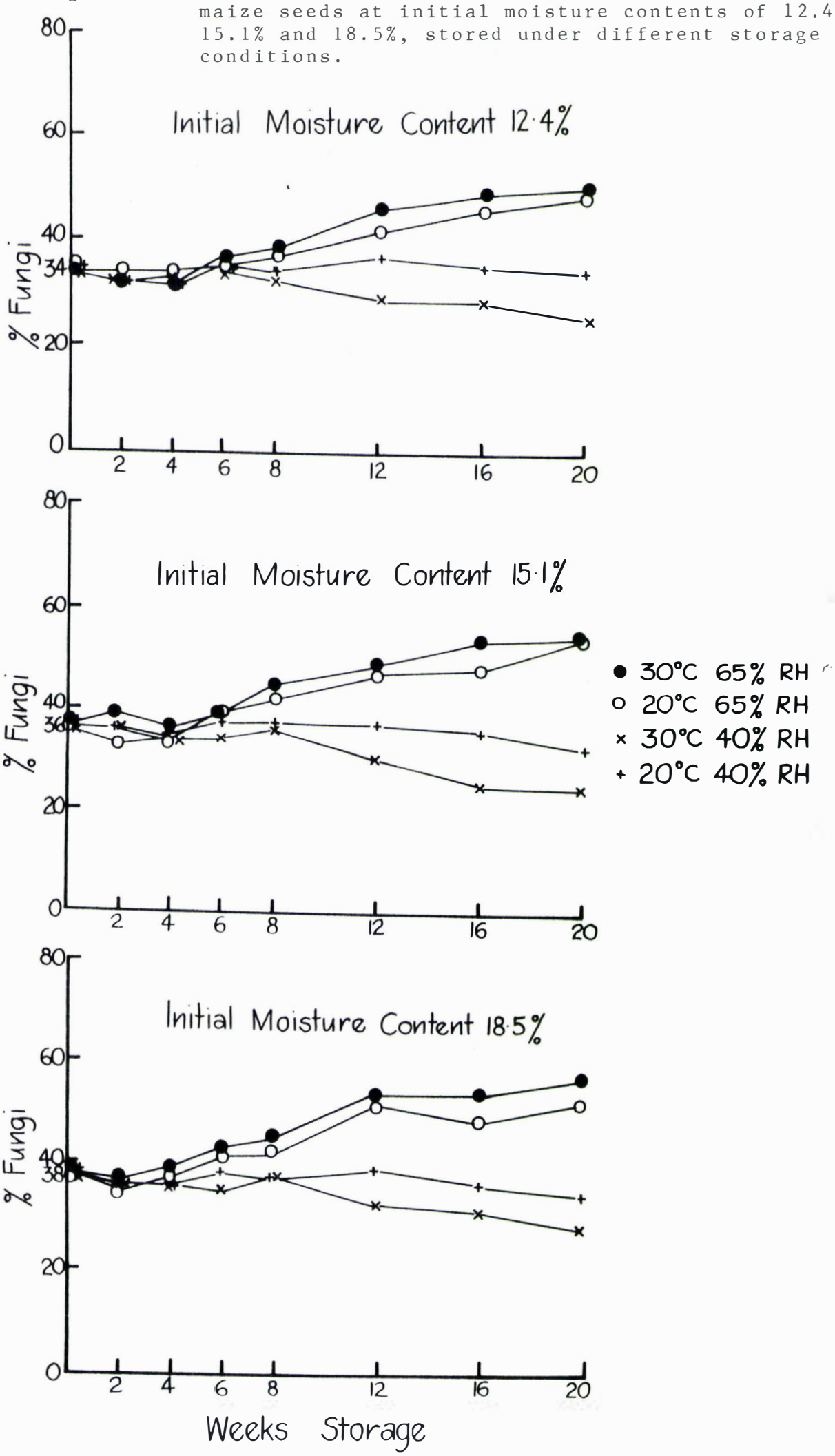


Figure 6: Changes in percentage of fungal development in maize seeds at initial moisture contents of 12.4%, 15.1% and 18.5%, stored under different storage conditions.



weeks storage, an occasional isolate of *Fusarium* was obtained on culture medium. Subsequently no field fungi were detected in samples of maize seed tested during storage.

In all three initial moisture content treatments a similar pattern of storage fungus development was observed. The results in Figure 6 show that after an initial 4 weeks storage period a slow increase in the percentage of storage fungi occurred through to 8 weeks storage. This was followed by a rapid increase in the incidence of storage fungi in 65% relative humidity storage environments with 30°C storage samples developing more extensively than 20°C stored samples. In seed stored at 40% relative humditiy, however, a decline in storage fungus percentage occurred generally after 8 weeks storage. This decline was greater in seed stored at 30°C - 40% RH than in seed stored at 20°C - 40% RH. This was a direct reflection of the lower equilibrium moisture content (7.4-7.6%) of seed at 30°C - 40% RH compared with 9.1-9.4% in seed stored at the same relative humidity but at 20°C. Presumably, the lower seed moisture level was more deleterious to the development of storage fungi.

At low relative humidities the presence of insects did not seem to cause any consistent difference in the percentage of storage fungi recovered from the seed during storage. Any differences which did occur were extremely small.

At the higher storage relative humidity level (65% RH) however, there was generally a small increase in the percentage of storage fungi recovered from seed samples containing insects. This effect occurred at both 20°C and 30°C storage temperatures. The stimulation of storage fungi in seed samples containing insects was presumably a reflection of the higher seed moisture contents occurring in insect infested samples as a result of increased populations during the last 8 weeks of the 20 week storage period (Figure 5).

The highest percentage of storage fungi detected was 60% in the most extreme treatments (18.5% initial seed moisture content, 30°C -65% RH) storage environment containing insects. This represented only an increase to 58% from the initial 38% infection in this treatment, suggesting that even this extreme combination of moisture, temperature and humidity was not entirely favourable for storage fungus development (Appenix 5a and b).

The main species of storage fungi detected in all samples were *Aspergillus glaucus* (Plate 30), *A. ochraceus*, *A. candidus*, *A. flavus* and *A. niger* (Plate 31). *Penicillium* species were also commonly isolated from maize kernels in seed samples tested before storage. *Penicillium* also remained dominant throughout the storage period (Plates 32 and 33). The relative rate of isolation of *Penicillium* and *Aspergillus* species in 12.4, 15.1 and 18.5% initial moisture seedlots after 16 and 20 weeks storage were in the approximate ratios of 3:2, 3:1 and 2:1 respectively.

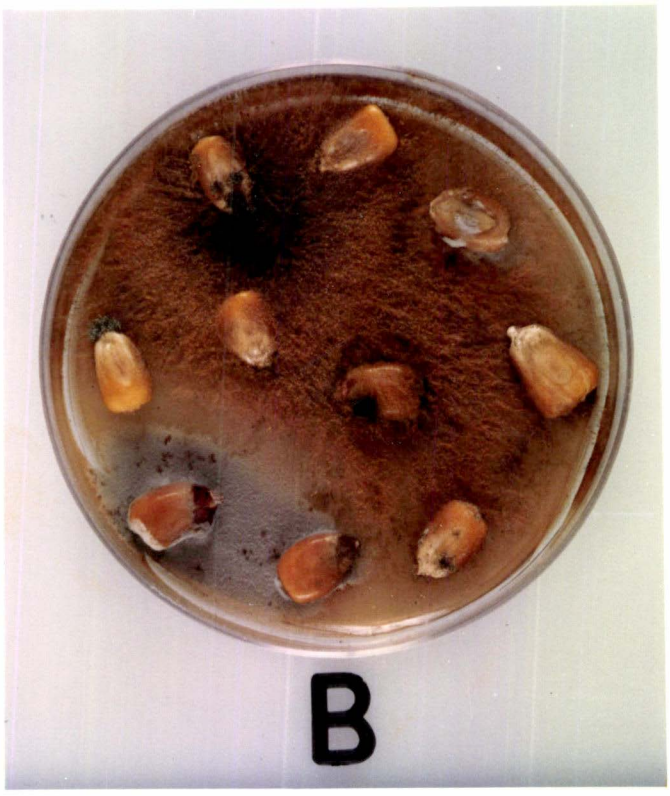


Plate 30: *Aspergillus Glaucus*

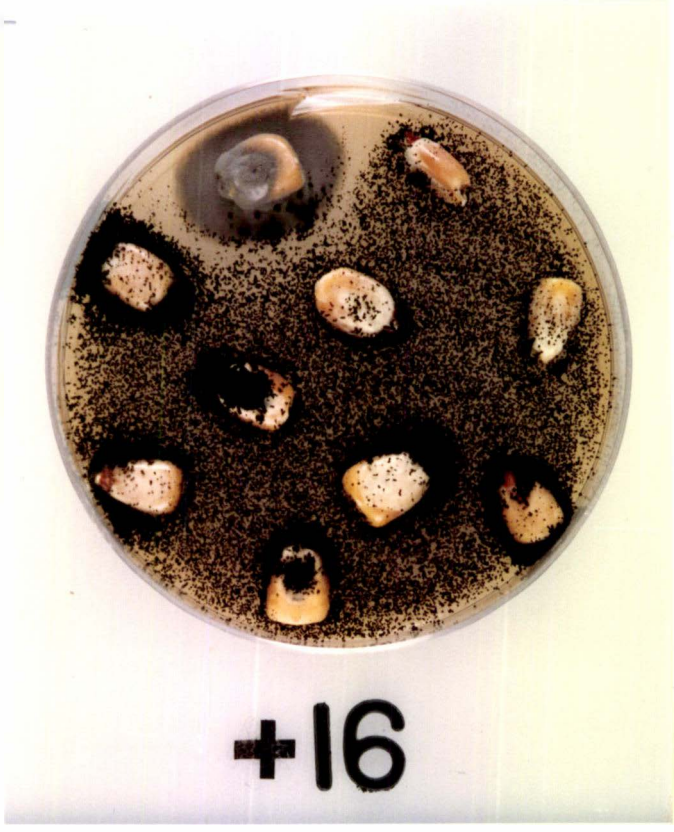


Plate 31: *Aspergillus Niger*

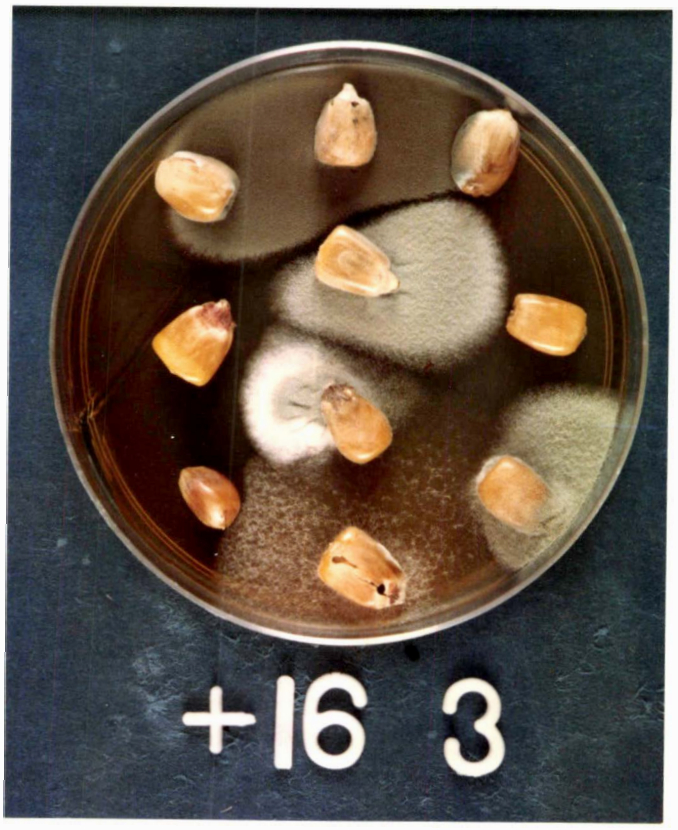


Plate 32: *Penicillium*

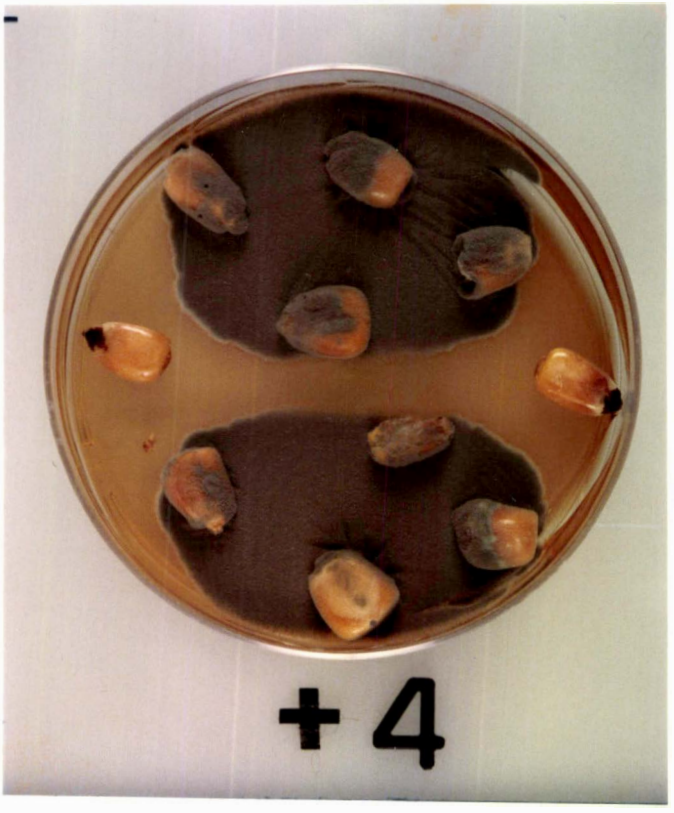


Plate 33: *Penicillium*

CHAPTER IV

GENERAL DISCUSSION

Relative humidity and temperature of the storage environment are the most important factors affecting the maintenance of seed quality. Of these two factors, relative humidity has the greater effect due to its influence on seed moisture content, and also on the infestation, growth and reproduction of both storage insects and fungi.

The hygroscopic nature of maize seed allowed a rapid (2 weeks) change in the initial level of seed moisture to reach equilibrium moisture content. Even storage conditions involving a humidity level of 65%, as used in this study, resulted in a equilibrium moisture content below the 15% generally considered to be safe for the short term storage of maize. It appeared, however, that the storage of maize at the highest initial moisture content (18.5%), may have been sufficient to increase the subsequent deterioration of seed germination towards the end of the storage period.

Storage temperature appeared to play only a minor role in affecting the rate or extent of moisture content change in environments involving a 65% relative humidity. However at 40% relative humidity seed stored at 20^{o}C showed a slower change in moisture content and a higher equilibrium value than seed stored at 30^{o}C. The difference in E.M.C. was generally about 3%.

Within the range of storage treatments used, no appreciable loss of viability, or increase in the number of abnormal seedlings was apparent in most treatments during the first 10-12 weeks. However, during the last 8-10 weeks storage some loss of germination and increase in seedling abnormality was apparent in the most unfavourable treatment combinations. Heydecker (1972) has discussed

what he considers to be the probable order of events in the process of seed deterioration. The sequence, which is a modification of an earlier suggestion by Delouche (1969), is as follows:

- membrane damage
- impaired biosynthesis
- slower germination
- slower and more uneven growth and development
- greater susceptibility to environmental stress
- poor emergence potential
- morphological aberrations

and finally - inability to germinate

This sequence suggests the possibility that the increase in abnormal seedlings and the categories such as unbalanced development, missing and damaged structures and decay may indicate that at least some of the seedlings classed as viable were in fact exhibiting symptoms of advanced deterioration likely to result eventually in seed death.

A temperature of 20°C is considered to be the minimum value for significant development of rice weevil infestations (Cottan and Wilbur, 1960) so that below this temperature serious damage to seed is unlikely to occur. However, temperatures above 45°C are also unfavourable as oviposition ceases and adults are short lived. The storage temperatures of 20°C and 30°C used in this study are therefore close to the minimum and optimum levels respectively for likely damage to maize seed from this species.

Grain moisture content and relative humidity of the storage environment are also important factors with respect to the ability of stored grain pests to survive and reproduce, as most species depend entirely on the moisture content of seed for the water that is essential to sustain their life processes. As shown in the results of the present study,

adult rice weevils were unable to survive at 40% RH and larval populations did not develop. Furthermore, the seed stored at 40% RH rapidly reached an equilibrium moisture content of only 7-9%, irrespective of temperature and initial moisture content. The death of the population under these conditions confirms the finding of Cotton and Wilbur (1960) that adult rice weevils can survive for only a week in 8% moisture content seed and that in 9% moisture seed 70% mortality occurred after three weeks. They considered the critical minimum moisture content for rice weevil development in cereals to be between 11-12%. The results of the present study are in general agreement with their values, because, although initial moisture contents used were above 12%, they declined to below 10% after 2-4 weeks for seed stored at 40% RH. The short period of suitable moisture content was evidently insufficient to permit establishment and survival of the insect population.

The necessity for both temperature and seed moisture content (and hence humidity of the storage environment) to be above certain critical levels for development of rice weevil populations is clearly shown from the results of the present study. Populations increased only in seed stored at 65% RH (which maintained seed moistures above 12.5%) and the increase was much greater at 30°C (close to optimum) than at 20°C (only slightly above minimum threshold temperature).

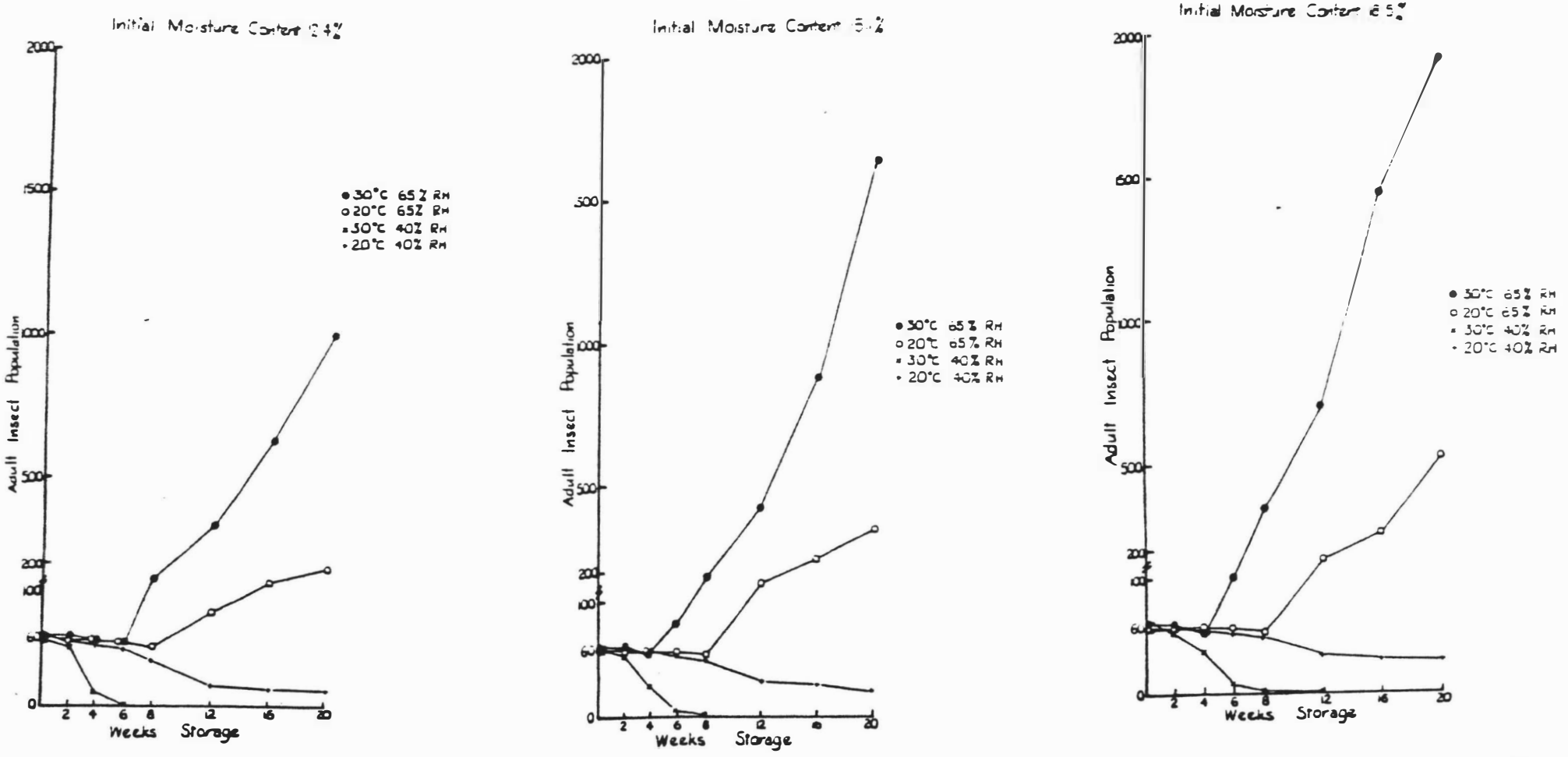
Cotton and Wilbur (1960) have stated that under optimum conditions rice weevils may complete development from egg to adult in about four weeks. Each adult female is capable of laying several hundred eggs so that an enormous rate of increase is possible. The maximum increase in the present study (at 30°C, 65% RH and 18.5% IMC) was from 60 introduced insects to 1908 after 20 weeks, a factor of x32. Although impressive, this is still clearly well below the optimum for the species. Grain species and variety (not examined in the present study) may well be an additional complicating factor.

Most of our knowledge of damage to seed by insect pests, including that caused by *Sitophilus* species, is based on studies of stored food grains. This is pertinent in drawing attention to storage practices that should be avoided with seed grains, but provides little information about actual effects on seed, as losses have usually been assessed in terms of loss of seed weight, increase in dust fraction, development of off-flavours and the presence of insect fragments. This tells us little or nothing about seed viability and seedling vigour when such seed is planted. It appears that very few researchers have critically examined the influence of insect oviposition or feeding by adults or larvae on germination or other determinants of seed quality.

Results of the present study suggest that in the case of rice weevil infesting maize, germination is not greatly affected by adult feeding or by oviposition or even by feeding of young larvae during the first few weeks of storage. However, germination does become more seriously affected when the larvae grow large enough to eat sufficient of the endosperm to prevent adequate food reserves being available for the seedling. *S. oryzae* also clearly damages areas of the seed which influence the growth of the seedling as shown by the effects on seedling abnormalities. There is thus a real danger that even though percentage germination may not be seriously affected, many seeds which germinate will subsequently produce abnormal seedlings which are incapable of development into a productive plant. In this respect the present study has shown that damage to seed viability by *S. oryzae* is clearly a function of the size of the insect population and the time over which it persists (Figure 7).

X-ray photographs of insect-attacked seeds showed that internal injury from larval feeding may occur in many instances without external visible evidence being apparent. However, in most cases where seed was significantly affected this was apparent from external visual examination.

Figure 7: Effect of different storage conditions on the population of rice weevils in maize seeds.



X-ray examination of infected seeds did not strongly suggest that larvae of *S. oryzae* feed preferentially on the embryo. However, even if the embryo is not directly eaten, so much of the endosperm may be consumed that insufficient remains to support the seedling. Howe (1973) has suggested that *Sitophilus* deliberately lay their eggs in the scutellum area around the embryo. If this is so, it seems unlikely that larvae continue to preferentially feed on that part of the seed. In general the effect of rice weevil infestation on grain seed was both to reduce percentage germination and to increase the incidence of abnormal seedling development (Figures 8 and 9).

Aspergillus species have been reported to invade and destroy stored seeds, including cereal grains (Christensen and Kaufmann, 1965). Certainly the invasion of seeds by storage fungi has been shown to be very rapid under conditions of relatively high ambient temperature and relative humidity (Christensen and Kaufmann, 1969). In the present study, the seedlot of maize showed a prestorage level of invasion by storage fungi of 35-38%. Storage of this seed at a range of initial moisture contents under a range of conditions including high temperature ($30^{o}C$) and moderate humidity (65%) for 5 months did not cause total loss of germinability despite some increase in the level of *Aspergillus* and *Penicillium* storage fungi. This was surprising since these species have generally been found to cause rapid and adverse effects on seed quality. The present results are supported by findings by Kulik (1973) who showed that some vegetable seeds may not be susceptible to rapid invasion by *A. amstelodami* and *A. flavus* even when they were stored under conditions that are favourable for fungal development. He suggested this may be due to the relatively low moisture content attained by seeds even when they are stored at humidity levels up to 85%. This possibly explains the only slow increase in fungi in maize seed stored at 40% and 65% RH in the present study, an effect which occurred similarly in the presence or absence

Figure 8: Effect of different storage conditions and rice weevils on the germination of maize seeds.

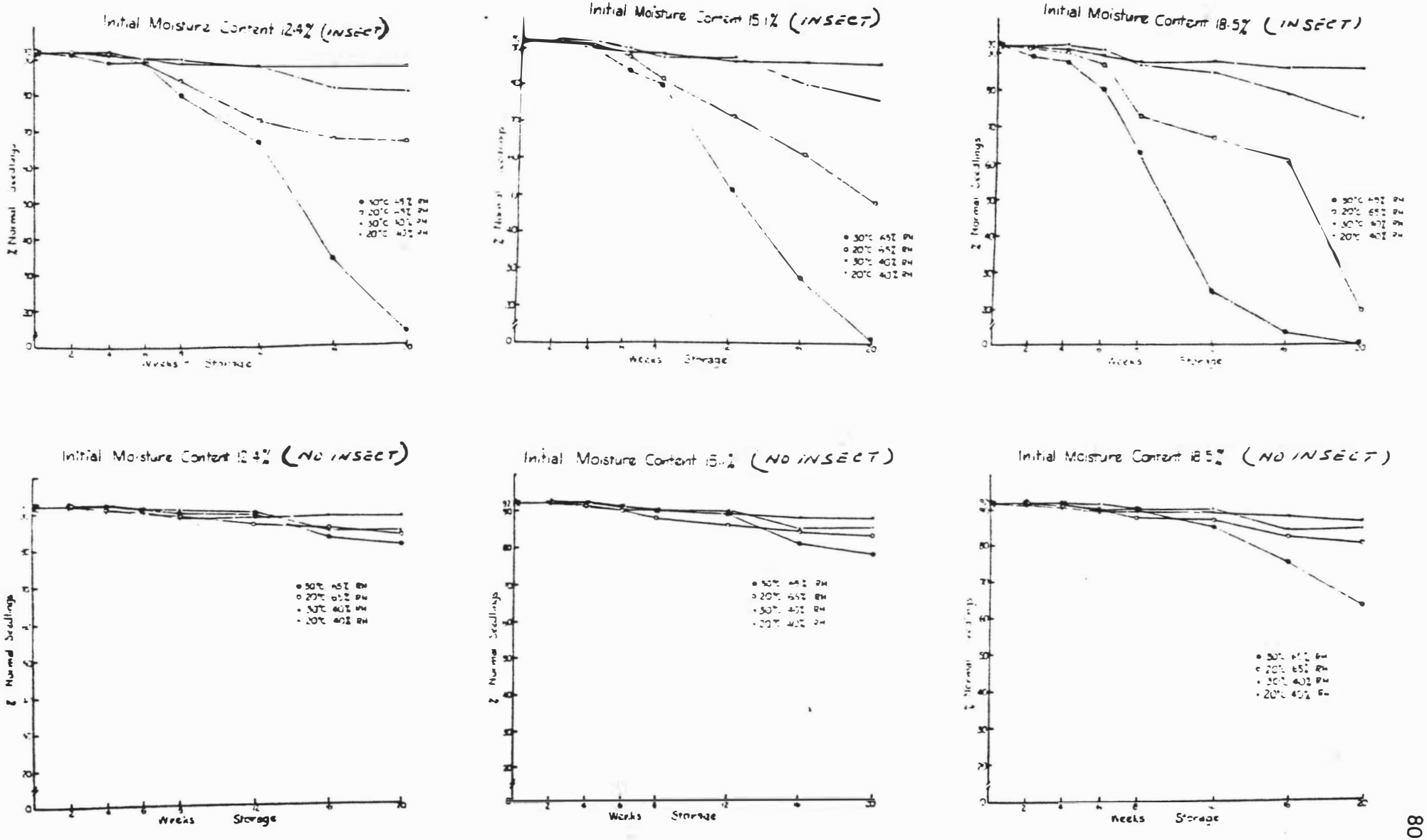
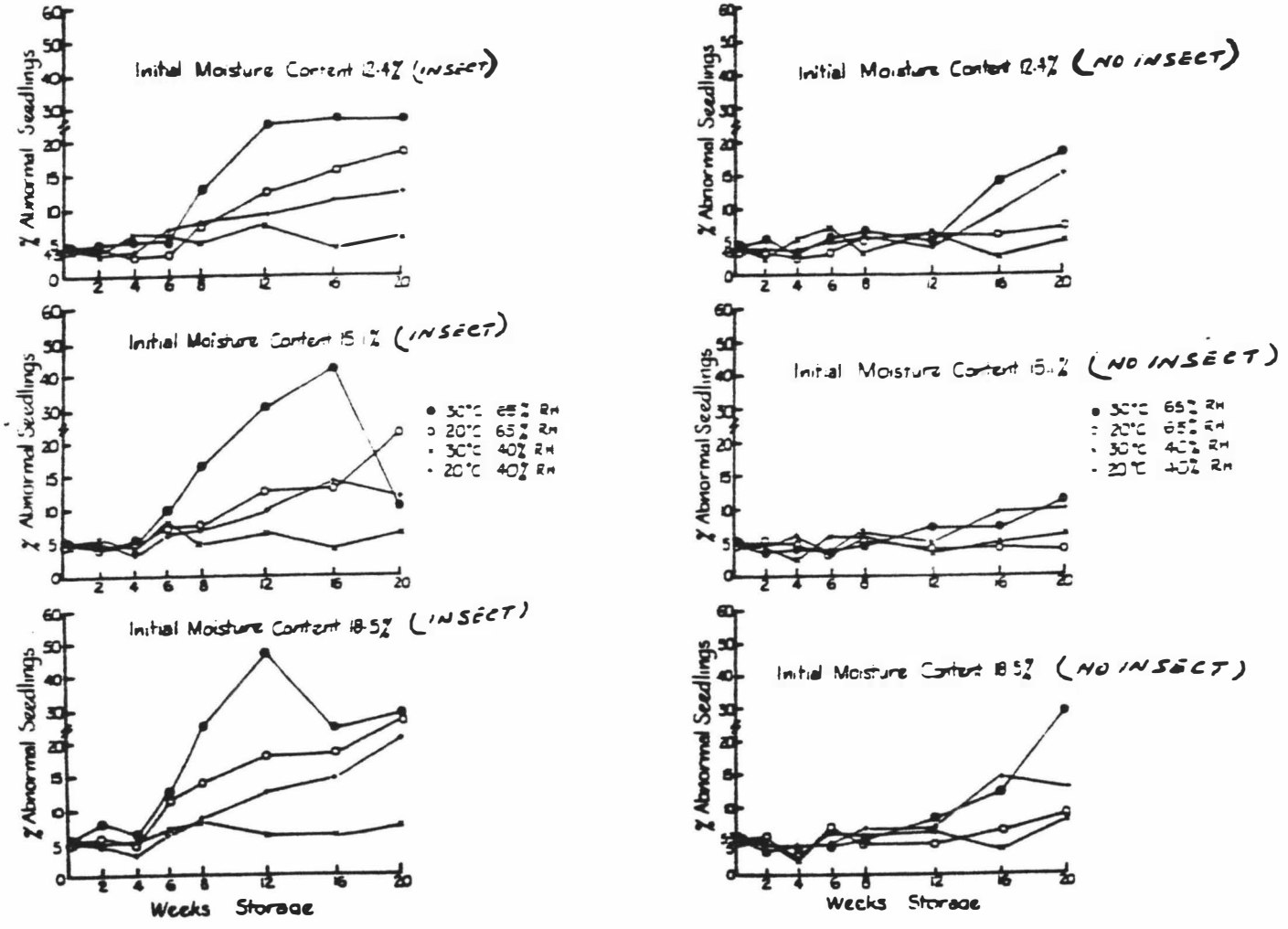


Figure 9: Effect of different storage conditions and rice weevils on abnormal seedling development of maize.



of insects. The rapid change in seed moisture content to equilibrium during the first 2 weeks of storage suggests that the level of initial seed moisture content, even though it may have been high, was of only transient importance and apparently did not provide suitable conditions for long enough to greatly aid the development of storage fungi. This is also supported by the findings of Christensen and Kaufmann (1969) that storage fungi cannot grow and reproduce significantly on seed in equilibrium with a relative humidity less than 65-70%. It appears therefore that the levels of humidity used in this study were relatively "safe" in terms of microfloral activity.

BIBLIOGRAPHY

BAILEY, C.H. 1918. Respiration of stored wheat. Jour. of Agric. Res., 12: 685-711.

BAKER, J.E. and MABIE, J.M. 1973b. Growth responses of larvae of *Sitophilus granarius* (Coleoptera, curculionidae) on a meridic diet. Ann. Entomol. Soc. Am., 66: 723-726.

BARTON, L.V. 1961. Seed preservation and longevity. 216 pp., Illus., New York. (Cited by Justice and Boss).

BIRCH, L.C. 1944. The effect of temperature and dryness on the survival of the eggs of *Calandra oryzae L.* (small strain) and *Rhizopertha dominica Fab.* (Coleoptera). Aust. J. Exp. Biol. Med. Sci., 22: 265-269.

BIRCH, L.C. 1945a. The influence of temperature on the development of the different stages of *Calandra oryzae L.* and *Rhizopertha dominica Fab.* (Coleoptera). Aust. J. Exp. Biol. Med. Sci., 23: 29-35.

BIRCH, L.C. 1945d. The influence of temperature, humidity and density on the oviposition of the small strain of *Calandra oryzae L.* and *Rhizopertha dominica Fab.* Aust. J. Exp. Biol. Med. Sci., 23: 197-203.

BIRCH, L.C. 1946b. The movements of *Calandra oryzae L.* (small strain) in experimental bulks of wheat. J. Aust. Inst. Agric. Sci., 12: 21-26.

BIRCH, L.C. 1953a. Experimental background to the study of the distribution and abundance of insects. I. The influence of temperature, moisture and food on the innate capacity for increase of three grain beetles. Ecology, 34: 698-711.

BIRCH, L.C. 1953b. Experimental background to the study of the distribution and abundance of insects. II. The relation between innate capacity for increase in numbers and the abundance of three grain beetles in experimental populations. Ecology, 34: 712-726.

BIRCH, L.C. 1954. Experiments on the relative abundance of two sibling species of grain weevils. Aust. J. Zool., 2: 66-74.

BOSWELL, V.R., TOOLE, E.H., TOOLE, V.K. and FISHER, D.F. 1940. A study of rapid deterioration of vegetable seeds and methods for its preservation. U.S. Dept. Agr. Tech. Bull., 708. 40 pp.

BOTTOMLEY, R.A., CHRISTENSEN, C.M. and GEDDES, W.F. 1950. Grain Storage Studies IX. The influence of various temperatures, humidities and oxygen concentrations on mold growth and biochemical changes in stored yellow corn. Cereal Chem., 27: 271-296.

BOTTOMLEY, R.A., CHRISTENSEN, C.M. and GEDDES, W.F. 1952. Grain Storage Studies X. The influence of aeration, time and moisture content on fat acidity non reducing sugars, and mould flora of stored yellow corn. Cereal Chem., 29: 53-64.

CHAMP, B.R. and CYTE, C.E. 1976. Report of the FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Production and Protection Ser. No.5. Rome.

CHRISTENSEN, C.M. 1957. Deterioration of stored grains by fungi. Bot. Rev., 23: 108-134.

CHRISTENSEN, C.M. and GORDON, D.R. 1948. The mold flora of stored wheat and corn and its relation to heating of moist grain. Cereal Chem., 24: 40-51.

CHRISTENSEN, C.M. and KAUFMANN, H.H. 1965. Deterioration of stored grains by fungi. Ann. Rev. Phytopath., 3: 69-84.

CHRISTENSEN, C.M. and KAUFMANN, H.H. 1969. Grain storage: The role of fungi in quality loss. 153 pp., Illus. Minneapolis, Minn.

COTTON, R.T. 1963. Pests of stored grain and grain products (rev. ed.). 318 pp. Burgess Publ. Co., Minneapolis, Minn.

COTTON, R.T., WALKDEN, H.H.,, WHITE, G.D. and WILBUR, D.A. 1960. Causes of outbreaks of stored grain insects. 35pp. Kans. Agr. Exp. Sta. Bull. 416.

DAVIDSON, J. 1940. Wheat storage problems in South Australia. Jour. Dept. Agric. S. Australia., 44 (3, 5, 7-8): 124-36.

DELOUCHE, H.C. 1963. Seed deterioration. Seed Wld., 92 (4): 14-15. (Cited by Delouche, 1973).

DELOUCHE, J.C. 1964. Observation on seed deterioration. Proceedings, 1964 Mississippi Short Course for Seedsmen, 102-108. Seed Technology Laboratory, Mississippi State University. State College, Mississippi. (Cited by Delouche, 1973).

DELOUCHE, J.C. 1965. Deterioration of crimson clover seed in storage. Proc. Asso. of Seed Analysts N. Am., 55: 65-75. (Cited by Delouche, 1973).

DELOUCHE, J.C. 1968b. Precepts for seed storage. Proc., 1968 Mississippi Short Course for Seedsmen, 85-119. Seed Technology Laboratory, Mississippi State University, State College, Mississippi. (Cited by Delouche, 1973).

DELOUCHE, J.C. 1969. Planting seed quality. Proceedings, 1969 Beltwide Cotton Production Mechanization Conference, 16-18. National Cotton Council, Memphis, Tenn. (Cited by Delouche, 1973).

DELOUCHE, J.C. 1971. Determinants of seed quality. Proceedings, 1971 Mississippi Short Course for Seedsmen, 14, 53-68. Seed Technology Laboratory, Mississippi State University, State College, Mississippi. (Cited by Delouche, 1973).

DELOUCHE, J.C. and BASKIN, C.E. 1970. Seed vigour sets performance. Int. Coll. Congr., 37: 65-69. (Cited by Delouche, 1973).

DELOUCHE, J.C. and BASKIN, C.E. 1971. Accelerating ageing techniques for predicting the realtive storability of seed lots. Seed Sci. & Technol., 1: 427-452.

DELOUCHE, J.C., MATTHES, R.K., DAUGHERTY, G.M. and BOYD, A.H. 1973. Storage of seed in sub-tropical and tropical regions. Seed Sci. & Technol., 1: 671-700.

DEXTER, S.T. 1957. Moisture equilibrium values in relation to mold formation of seeds of several grass and small seeded legumes. Agron. Jour., 49: 485-488.

DOUGLAS, J.E. 1975. Seed storage and packaging. In Cereal Seed Technology. (Ed. Feistritzer, W.P.) pp. 87-107. Illus. FAO Rome.

FAO Monthly Bulletin of Statistics. 1982. Vol 5, No. 12 December 1982.

GILL, N.C. and DELOUCHE, J.C. 1973. Deterioration of seed corn during storage. Proc. AOSA, 53: 33-50.

GOLEBIOWSKA, Z., PRADZYNSKA, A. and NAWROT, J. Investigation upon the seeding of some species of storage beetles. 71-88. Institute for Plant Protection, Poznon, Poland.

HARDMAN, J.M. 1977. Environmental changes associated with the growth of populations of *Sitophilus oryzae (L.)* confined in small cells of wheat. J. Stored Prod. Res., 13: 45-52.

HARRINGTON, J.F. 1960. Drying, storing and packaging seed to maintain germination and vigor. Proc. 1959 Mississippi Short Course for Seedsmen. 89-107.

HARRINGTON, J.F. 1963. The value of moisture resistance containers in vegetable seed packaging. Bulletin 792. California Agricultural Experiment Station, Davis, California.

HARRINGTON, J.F. 1973. Packaging seed for storage and shipment. Seed Sci. & Technol., 1: 701-709.

HARINGTON, J.F. and DOUGLAS, J.F. 1970. Seed storage and packaging application for India. New Delhi, India. pp 222.

HARRIS, W. VICTOR. 1943. Maize storage. East Afr. Agr. Jour., April 1943: 190-196.

HELMER, J.D., DELOUCHE, J.C. and LIENHARD, M. 1962. Some indices of vigour and deterioration in seed of crimson clover. Proc. Asso. of Seed Analysts N. Am., 52: 154-161. (Cited by Delouche, 1973).

HEYDECKER, W. 1972. In seed ecology. (Edited by W. Heydecker). London. pp. 433-463.

HILL, DENNIS S. 1975. Agricultural insect pests of the tropics and their control. pp. 338-389.

HOWE, R.W. 1943. An investigation of the changes in a bin of stored wheat infested by insects. Bull. Entomol. Res., 34: 145-158.

HOWE, R.W. 1952. The biology of the rice weevil, *Calandra oryzae L.* Ann. Appl. Biol., 39: 168-180.

HOWE, R.W. 1973. Loss of viability of seed in storage attributable to infestation of insects and mites. Seed Sci. & Technol., 1: 563-586.

HUBBARD, J.E., EARLE, F.R. and SENTI, F.R. 1957. Moisture relation in wheat and corn. Cereal Chem., 34: 422-433.

JAMES, E. 1967. Preservation of seed stocks. Adv. Agron., 19: 87-106.

JUSTICE, O.L. and BASS, L.N. 1978. In principles and practices of seed storage. Agriculture Handbook No. 506. Washington, D.C. pp. 29 and 49.

KAMEL, A.H. and ZEWAR, M.M. 1973. Loss in weight in stored corn and millet due to *Sitophilus oryzae* and *Rhizopertha dominica* infestations. Agric. Res. Rev. 1973, 51 (1): 29-31. (EN) Plant Protection Research Institute. Ministry of Agriculture, Cairo, Egypt.

KHAN, M.Q. 1948. A contribution to a further knowledge of the structure of biology of the weevils, *Sitophilus oryzae (L.)* and *S. Granarius (L.)* with special reference to the effects of temperature and humidity on their rate of development. Ind. J. Entomol., 11: 143-201.

KOURA, A. and EL-HALFAWY, M.A. 1972. Weight loss in stored grains caused by insect infestation in Egypt. Bulletin de La Societe Eutomologique d, Egypte. 56: 413-417. Plant Protection Department. Ministry of Agriculture, Arab Republic of Egypt.

KULIK, M.M. 1973. Susceptibility of stored vegetable seeds to rapid invasion by *Aspergillus amstelodami* and *Aspergillus flavus* and effect of germinability. Seed Sci. & Technol., 1: 799-803.

LITYNSKI, M. 1957. Effect of environmental moisture on the viability of certain species of vegetables. Roczn. Naukro In., 76: 217-298., (English translation from Office of Technical Services, U.S. Department of Commerce, Washington, D.C. 1964).

LONGSTAFF, BARRY C. 1981. Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: curculionidae): A critical review. Protection Ecology, 2: 83-130.

MATHLEEN, R. 1938. Undersokningar rorande forradsskadedjur. I. Kornviveln, *Calandra granaria L.* och risviveln, *C. oryzae L.* Derasbiologi och bekampning. Nat. Swed. Inst. Plant Prot., 23.

MATTHES, R.K., WELCH, G.B., DELOUCHE, J.C. and DAUGHERTY, G. 1969. Drying, processing and storage of corn seed in tropical and subtropical regions. Paper No. 69, 577 American Society of Agriculture Engineers, St. Joseph, Michigan. (Cited by Delouche, 1973).

MCROSTIE, G.P. 1939. The thermal death point of corn from low temperatures. Sci. Agric., 19: 687-699.

MILNER, M., CHRISTENSEN, C.M. and GEDDES, W.F. 1947. Grain Storage Studies. VII. Influence of certain mold inhibitors on respiration of moist wheat. Cereal Chem., 24: 507-517.

MOORE, R.P. 1963. Previous history of seed lots and differential maintenance. Proc. Int. Seed Test Asso., 28: 691-699. (Cited by Delouche, 1973).

MOUTIA, L.A. 1942. La teneur en humidité du mais en grain en relation avec divers etats hygrometriques de l'air. Rev. Agr. Maurice, 21 (3): 122-126.

MUNFORD, R.S. 1965. Controlled temperature and humidity storage. Proceedings, 1965 Short Course for Seedsmen, 145-156. Seed Technology Laboratory, Mississippi State University, State College, Mississippi. (Cited by Delouche, 1973).

OLAFSON, JOHN H., CHRISTENSEN, C.M. and GEDDES, W.F. 1954. Grain Storage Studies. XV. Influence of moisture content, commercial grade and maturity on the respiration and chemical deterioration of corn. Cereal Chem., 31: 333-340.

OWEN, E.B. 1956. The storage of seeds for maintenance of viability. Bulletin 43. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.

OXLEY, T.A. 1948. Scientific principles of grain storage. 103 pp. North Pub. Co., Liverpool.

OXLEY, T.A. and HOWE, R.W. 1944. Factors influencing the course of an insect infestation in bulk wheat. Ann. Appl. Biol., 31: 76-80.

PAPAVIZAS, G.C. and CHRISTENSEN, C.M. 1957. Grain Storage Studies. XXV. Effect of invasion by storage fungi upon germination of wheat seed and upon development of sick wheat. Cereal Chem., 34: 350-359.

PIXTON, S.W. and WARBURTON, S. 1968. The time required for conditioning grain to equilibrium with specific relative humidity. Stored Prod. Res. Jour., 4: 261-265.

PIXTON, S.W. and WARBURTON, S. 1971. Moisture content/ relative humidity equilibrium of some cereal grains at different temperatures. J. Stored Prod. Res., 6 (4): 283-295.

REDDY, D.P. 1950a. Ecological studies of the rice weevil. J. Econ. Entomol., 43: 203-206.

RENGIFO, G., PFOST, H.B. 1976. High temperature and high humidity grain storage. Research report, Food and Feed Grain Institute, Kansas State University. No. 10, 44 pp. Food and Feed Grain Inst., Kansas State University, Manhattan, Kansas, U.S.A.

RICHARDS, O.W. 1944. The two strains of the rice weevil, *Calandra oryzae L.* Trans. R. Entomol. Soc. London, 94: 187-200.

RICHARDS, O.W. 1947. Observations of grain weevils, *Calandra* (Col., curculionidae) 1. General biology and oviposition, Proc. Zool. Soc. London, 117: 1-43.

ROBERTS, E.H. and ROBERTS, D.L. 1972a. Viability nomographs in viability of seeds (ed. E.H. Roberts). pp. 417-423. Chapman and Hall, London.

SAUER, D.B. and CHRISTENSEN, C.M. 1968. Germination percentage, storage fungi isolated from, and fat acidity values of export corn. Phytopathology, 58: 1356-1359.

SIJBRING, P.H. 1963. Results of some storage experiments under controlled conditions (agriculture seed). Proc. Int. Seed Test Asso., 28: 845-851. (Cited by Delouche, 1973).

SINHA, R.N. and WALLACE, H.A.H. 1965. Ecology of a fungus induced hot spot in stored grain. Canad. Jour. Plant Sci., 45: 48-59.

SINGH, N.B., SINHA, R.N. and WALLACE, H.A. 1977. Changes in O_2, CO_2 and microflora of stored wheat induced by weevils. Environmental Entomology, 6 (1): 111-117. Research Station, Agriculture Canada, Winnipeg, Manitoba R3T2M9, Canada.

STOYANOVIC, T. 1966. Uticaj pocetne gustine populacije *Sitophilus granarius L.* and *S. oryzae L.* (Coleoptera, curculionidae) na gubitak tezine napadnute psenice. The effect of the initial population density of *Sitophilus granarius* and *S. oryzae* on the loss of weight in infested wheat. Let. Nauc. Rad. 1966 No. 10. pp. 63-72.

THEIMER, O. 1978. Conditions for safe storage of cereals. Bedingungen fur sichene Getreidelagerung. Muhle and Mischfutter Technik. 115 (6): 71-72 (De).

TOOLE, E.H. 1950. Relation of seed processing and conditions during storage on seed germination. Proc. Int. Seed Test Asso., 16: 214-225.

TOOLE, E.H. 1957. Storage of vegetable seeds. U.S. Dept. Agr. Leafl. 220.

TSAI, PANG-HWA and CHANG, YEN NIEN. 1935. Experimental studies regarding the influence of temperature and relative humidity on the oviposition of the rice weevil, Calandra oryzae. Agri, Siwica, 1 (6): 175-188.

WELCH, G.B. and DELOUCHE, J.C. 1967. Seed processing and storage facilities in tropical areas. Paper No. 62, 318. American Society of Agricultural Engineers. St. Joseph Michigan. (Cited by Delouche, 1973).

KEY TO APPENDICES

Treatment 1 =	IMC 12.4%	Temperature	20°C,	RH 40%
Treatment 2 =	IMC 12.4%	Temperature	20°C,	RH 65%
Treatment 3 =	IMC 12.4%	Temperature	30°C,	RH 40%
Treatment 4 =	IMC 12.4%	Temperature	30°C,	RH 65%
Treatment 5 =	IMC 15.1%	Temperature	20°C,	RH 40%
Treatment 6 =	IMC 15.1%	Temperature	20°C,	RH 65%
Treatment 7 =	IMC 15.1%	Temperature	30°C,	RH 40%
Treatment 8 =	IMC 15.1%	Temperature	30°C,	RH 65%
Treatment 9 =	IMC 18.5%	Temperature	20°C,	RH 40%
Treatment 10 =	IMC 18.5%	Temperature	20°C,	RH 65%
Treatment 11 =	IMC 18.5%	Temperature	30°C,	RH 40%
Treatment 12 =	IMC 18.5%	Temperature	30°C,	RH 40%

APPENDIX 1: Analysis of variance of the experimental data on the percentage of initial seed moisture content after 2, 4, 6, 8, 12, 16 and 20 weeks.

A. Seed without insect.

Week 2.

1

***** ANALYSIS OF VARIANCE *****

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	152.20901	99.71	13.83718	737.547
RESIDUAL	24	0.45027	0.29	0.01876	
TOTAL	35	152.65927	100.00	4.36169	
GRAND TOTAL	35	152.65927	100.00		

GRAND MEAN 12.326
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE SEDM

GRAND MEAN 12.326

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	12.317	12.500	8.950	12.413	12.400	13.717	9.117	13.483	13.600	15.133	9.300

TREATMNT	12
	14.983

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.1118

APPENDIX 1: CONTINUED

Week 4.

***** ANALYSIS OF VARIANCE *****

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	1476.8	34.99	134.3	1.174
RESIDUAL	24	2743.6	65.01	114.3	
TOTAL	35	4220.4	100.00	120.6	
GRAND TOTAL	35	4220.4	100.00		

GRAND MEAN 14.4
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 14.4

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
REP	11.0	13.1	8.6	12.6	25.0	30.4	8.5	13.3	12.0	14.5	8.7
	3	4	3	3	3	3	3	2	3	3	3

TREATMNT	12
REP	14.7
	3

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	UNEQUAL
SED	10.69X MIN REP
	9.26 MAX-MIN
	7.56X MAX REP

APPENDIX 1: CONTINUED

Week 6.

1

***** ANALYSIS OF VARIANCE *****

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	166.74139	99.74	15.15831	852.656
RESIDUAL	24	0.42667	0.26	0.01778	
TOTAL	35	167.16803	100.00	4.77623	
GRAND TOTAL	35	167.16803	100.00		

GRAND MEAN 11.586
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 11.586

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	10.700	13.083	8.500	12.517	11.067	13.733	8.450	13.217	10.883	14.100	8.400

TREATMNT	12
	14.383

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.1089

APPENDIX 1: CONTINUED

Week 8.

***** ANALYSIS OF VARIANCE *****

VARIATE SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	155.09686	99.85	14.09971	1409.966
RESIDUAL	24	0.24000	0.15	0.01000	
TOTAL	35	155.33685	100.00	4.43820	
GRAND TOTAL	35	155.33685	100.00		

GRAND MEAN 11.312
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE SEDM

GRAND MEAN 11.312

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	10.583	13.000	8.283	12.550	10.483	12.800	8.350	13.000	10.500	13.950	8.350

TREATMNT	12
	13.900

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.0816

APPENDIX 1: CONTINUED

Week 12.

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	165.87741	99.85	15.07976	1457.378
RESIDUAL	24	0.24833	0.15	0.01035	
TOTAL	35	166.12573	100.00	4.74645	
GRAND TOTAL	35	166.12573	100.00		

GRAND MEAN 11.207
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 11.207

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	10.083	12.883	8.200	12.617	10.133	12.883	8.300	12.983	10.217	14.100	8.283

TREATMNT	12
	13.800

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.0831

APPENDIX 1: CONTINUED

Week 16.

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	213.14075	99.74	19.37643	827.953
RESIDUAL	24	0.56167	0.26	0.02340	
TOTAL	35	213.70239	100.00	6.10578	
GRAND TOTAL	35	213.70239	100.00		

GRAND MEAN 10.899
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 10.899

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	9.733	12.950	7.650	13.033	9.583	13.167	7.433	13.000	9.550	13.517	7.567

TREATMNT	12
	13.600

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.1249

APPENDIX 1: CONTINUED

Week 20.

***** ANALYSIS OF VARIANCE ***

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	263.28406	99.85	23.93491	1485.616
RESIDUAL	24	0.38667	0.15	0.01611	
TOTAL	35	263.67072	100.00	7.53345	
GRAND TOTAL	35	263.67072	100.00		

GRAND MEAN 11.043
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 11.043

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	9.117	13.950	7.633	13.233	9.367	13.933	7.517	13.150	9.400	14.200	7.517

TREATMNT	12
	13.500

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.1036

APPENDIX 1: CONTINUED

B. Seed with insect.
Week 2.

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	155.83026	99.82	14.16639	1220.362
RESIDUAL	24	0.27860	0.18	0.01161	
TOTAL	35	156.10886	100.00	4.46025	
GRAND TOTAL	35	156.10886	100.00		
GRAND MEAN		12.374			

TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 12.374

TREATMNT	1	3	4	5	6	7	8	9	10	11
	12.250	12.600	8.900	12.407	12.467	13.750	9.300	13.467	13.783	15.233	9.250

TREATMNT	12
	15.083

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.0880

APPENDIX 1: CONTINUED

Week 4.

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	165.95740	99.70	15.08704	721.774
RESIDUAL	24	0.50166	0.30	0.02090	
TOTAL	35	166.45905	100.00	4.75597	
GRAND TOTAL	35	166.45905	100.00		

GRAND MEAN 11.915
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 11.915

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	11.250	12.950	8.600	12.500	11.617	13.767	8.617	13.350	12.267	14.567	8.683

TREATMNT	12
	14.817

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.1180

APPENDIX 1: CONTINUED

Week 6.

VARIATE SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS* STRATUM					
TREATMNT	11	172.45306	99.38	15.67755	351.099
RESIDUAL	24	1.07167	0.62	0.04465	
TOTAL	35	173.52472	100.00	4.95785	
GRAND TOTAL	35	173.52472	100.00		
GRAND MEAN		11.653			

TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE SEDM

GRAND MEAN 11.653

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	10.783	13.083	8.500	12.600	11.267	13.967	8.500	13.283	10.700	14.233	8.450

TREATMNT	12
	14.467

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.1725

APPENDIX 1: CONTINUED

Week 8.

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS* STRATUM					
TREATMNT	11	167.41138	99.56	15.21922	491.383
RESIDUAL	24	0.74333	0.44	0.03097	
TOTAL	35	168.15469	100.00	4.80442	
GRAND TOTAL	35	168.15469	100.00		
GRAND MEAN		11.497			

TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 11.497

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	10.667	13.033	8.300	12.650	10.833	13.300	8.400	13.167	10.750	14.167	8.400

TREATMNT	12
	14.300

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.1437

APPENDIX 1: CONTINUED

Week 12.

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	170.20721	99.85	15.47338	1456.321
RESIDUAL	24	0.25500	0.15	0.01062	
TOTAL	35	170.46219	100.00	4.87035	
GRAND TOTAL	35	170.46219	100.00		
GRAND MEAN		11.378			

TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 11.378

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	10.233	13.100	8.300	12.667	10.517	13.183	8.367	13.133	10.483	14.150	8.317

TREATMNT	12
	14.083

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.0842

APPENDIX 1: CONTINUED

Week 16.

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	271.97449	99.62	24.72495	573.370
RESIDUAL	24	1.03493	0.38	0.04312	
TOTAL	35	273.00940	100.00	7.80027	
GRAND TOTAL	35	273.00940	100.00		
GRAND MEAN		11.312			

TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 11.312

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	9.933	13.383	7.550	13.750	9.867	13.633	7.450	13.767	9.967	14.200	7.533

TREATMNT	12
	14.707

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.1696

APPENDIX 1: CONTINUED

Week 20.

VARIATE: SEDM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	350.61383	99.83	31.87398	1264.423
RESIDUAL	24	0.60500	0.17	0.02521	
TOTAL	35	351.21881	100.00	10.03482	
GRAND TOTAL	35	351.21881	100.00		
GRAND MEAN		11.544			

TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: SEDM

GRAND MEAN 11.544

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	9.250	14.383	7.583	13.800	9.617	15.217	7.433	13.983	9.717	15.117	7.583

TREATMNT	12
	14.850

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.1296

Appendix 2: The Effect of Storage Conditions on Insect Population (Number of Adults of *Sitophilus oryzae* Per 300 Seeds) After 20 Weeks of Storage. Figures in the Body of the Appendix are Number of Insects.

Storage Periods (weeks)	Initial Moisture Content 12.4%				Initial Moisture Content 15.1%				Initial Moisture Content 18.5%			
	20°C		30°C		20°C		30°C		20°C		30°C	
	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH
0	60	60	60	60	60	60	60	60	60	60	60	60
2	59	59	55	60	59	59	55	60	59	59	55	60
4	57	59	14	59	59	59	27	57	57	59	37	58
6	50	57	0	57	53	59	5	89	54	59	9	106
8	40	54	0	149	49	55	0	193	50	56	2	343
12	18	82	0	335	31	162	0	433	34	172	0	709
16	16	138	0	633	29	255	0	892	31	265	0	1443
20	14	181	0	1000	22	351	0	1659	28	529	0	1908

APPENDIX 3: Analysis of variance of the experimental data on normal germination percentages of corn seed after 2, 4, 6, 8, 12, 16 and 20 weeks.

A. Seed without insect.

Week 2.

***** ANALYSIS OF VARIANCE *****

VARIATE: GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS* STRATUM					
TREATMNT	11	2.222	4.48	0.202	0.102
RESIDUAL	24	47.333	75.52	1.772	
TOTAL	35	49.556	100.00	1.416	
GRAND TOTAL	35	49.556	100.00		

GRAND MEAN 92.11
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 92.11

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	92.33	92.00	92.00	92.00	92.33	92.33	91.67	92.33	92.33	92.33	91.67

TREATMNT	12
	92.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.147

APPENDIX 3: CONTINUED

Week 4.

***** ANALYSIS OF VARIANCE *****

VARIATE: GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	18.472	38.57	1.679	1.370
RESIDUAL	24	29.417	61.43	1.226	
TOTAL	35	47.889	100.00	1.368	
GRAND TOTAL	35	47.889	100.00		

GRAND MEAN 91.94
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 91.94

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	92.00	91.75	91.33	92.00	93.33	93.33	91.00	92.00	92.00	91.67	91.00
REP	3	4	3	3	3	3	3	2	3	3	

TREATMNT	12
	92.00
REP	3

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	UNEQUAL
SED	1.107X MIN REP
	0.959 MAX-MIN
	0.783X MAX REP

APPENDIX 3: CONTINUED

Week 6.

***** ANALYSIS OF VARIANCE *****

VARIATE: GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	17.417	37.25	1.583	1.295
RESIDUAL	24	29.333	62.75	1.222	
TOTAL	35	46.750	100.00	1.336	
GRAND TOTAL	35	46.750	100.00		

GRAND MEAN 90.58
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 90.58

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	91.33	89.67	90.67	91.33	91.33	90.00	90.33	91.00	91.67	90.00	89.67

TREATMNT	12
	90.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.903

APPENDIX 3: CONTINUED

Week 8.

1

***** ANALYSIS OF VARIANCE *****

VARIATE: GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	27.556	40.39	2.505	1.478
RESIDUAL	24	40.667	59.61	1.694	
TOTAL	35	68.222	100.00	1.949	
GRAND TOTAL	35	68.222	100.00		

GRAND MEAN 89.78
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 89.78

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	90.67	89.67	89.67	90.00	90.33	88.33	90.00	90.33	90.33	87.67	89.67

TREATMNT	12
	90.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.063

APPENDIX 3: CONTINUED

Week 12.

***** ANALYSIS OF VARIANCE *****

VARIATE: GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	94.333	75.47	8.576	6.711
RESIDUAL	24	30.667	24.53	1.278	
TOTAL	35	125.000	100.00	3.571	
GRAND TOTAL	35	125.000	100.00		

GRAND MEAN 88.50
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 88.50

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	90.00	87.33	88.67	89.67	90.00	86.33	89.33	89.33	90.33	87.00	89.00

TREATMNT	12
	85.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.923

APPENDIX 3: CONTINUED

Week 16.

VARIATE: GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	481.889	91.06	43.808	22.213
RESIDUAL	24	47.333	8.94	1.972	
TOTAL	35	529.222	100.00	15.121	
GRAND TOTAL	35	529.222	100.00		

GRAND MEAN 84.28
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 84.28

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	84.67	85.67	89.33	83.00	84.33	85.33	88.00	81.33	82.00	84.33	88.33

TREATMNT	12
	75.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.147

APPENDIX 3: CONTINUED

Week 20.

VARIATE: GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	1452.000	98.37	132.000	132.000
RESIDUAL	24	24.000	1.63	1.000	
TOTAL	35	1476.000	100.00	42.171	
GRAND TOTAL	35	1476.000	100.00		

GRAND MEAN 82.33
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN	82.33										
TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	84.00	84.67	89.33	81.33	83.33	85.33	87.67	78.33	80.00	84.33	86.00

TREATMNT	12
	63.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.816

APPENDIX 3: CONTINUED

B. Seed with insect.
Week 2.

VARIATE GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	25.639	50.97	2.331	2.268
RESIDUAL	24	24.667	49.03	1.028	
TOTAL	35	50.306	100.00	1.437	
GRAND TOTAL	35	50.306	100.00		

GRAND MEAN 91.64
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE GERM

GRAND MEAN 91.64

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	92.00	91.67	92.00	91.67	92.33	91.67	92.33	92.00	92.00	91.67	91.33

TREATMNT	12
	89.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.828

APPENDIX 3: CONTINUED

Week 4.

VARIATE. GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	58.222	62.23	5.293	3.595
RESIDUAL	24	35.333	37.77	1.472	
TOTAL	35	93.556	100.00	2.673	
GRAND TOTAL	35	93.556	100.00		

GRAND MEAN 90.89
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 90.89

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	92.33	92.00	91.00	92.00	92.00	90.67	90.33	90.00	92.00	90.00	90.67

TREATMNT	12
	87.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.991

APPENDIX 3: CONTINUED
Week 6.

VARIATE GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	300.2222	92.79	27.2929	28.073
RESIDUAL	24	23.3333	7.21	0.9722	
TOTAL	35	323.5555	100.00	9.2444	
GRAND TOTAL	35	323.5555	100.00		

GRAND MEAN 88.11
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE. GERM

GRAND MEAN 88.11

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	90.00	89.33	90.00	89.00	90.33	88.33	89.33	84.33	90.67	86.67	89.00

TREATMNT	12
	80.33

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	0.805

APPENDIX 3: CONTINUED

Week 8.

VARIATE GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	2047.639	94.06	186.149	34.543
RESIDUAL	24	129.333	5.94	5.389	
TOTAL	35	2176.972	100.00	62.199	
GRAND TOTAL	35	2176.972	100.00		

GRAND MEAN 82.97
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 82.97

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	90.00	94.00	88.67	81.33	88.33	82.33	89.00	80.67	87.33	73.00	87.67

TREATMNT	12
	63.33

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.895

APPENDIX 3: CONTINUED

Week 12.

VARIATE: GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	12263.416	98.45	1114.856	138.396
RESIDUAL	24	193.333	1.55	8.056	
TOTAL	35	12456.748	100.00	355.907	
GRAND TOTAL	35	12456.748	100.00		

GRAND MEAN 73.42
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 73.42

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	88.67	73.00	88.67	67.00	86.67	72.00	87.67	52.00	85.00	67.00	88.33

TREATMNT	12
	25.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	2.317

APPENDIX 3: CONTINUED

Week 16.

VARIATE: GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	23638.219	99.51	2148.929	444.606
RESIDUAL	24	116.000	0.49	4.833	
TOTAL	35	23754.219	100.00	678.692	
GRAND TOTAL	35	23754.219	100.00		

GRAND MEAN 63.78
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 63.78

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	82.33	68.00	88.33	34.67	81.00	62.33	87.33	28.33	79.00	61.00	86.00

TREATMNT	12
	7.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.795

APPENDIX 3: CONTINUED

Week 20.

VARIATE GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	42069.633	99.86	3824.512	1546.994
RESIDUAL	24	59.333	0.14	2.472	
TOTAL	35	42128.961	100.00	1203.685	
GRAND TOTAL	35	42128.961	100.00		

GRAND MEAN 53.03
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 53.03

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	81.33	67.00	88.33	8.00	77.00	49.00	86.67	2.00	71.67	19.00	86.33

TREATMNT	12
	0.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.284

APPENDIX 4: Analysis of variance of the experimental data on the percentage of abnormal seedlings after 2, 4, 6, 8, 12, 16 and 20 weeks.

A. Seed without insect.

Week 2.

***** ANALYSIS OF VARIANCE *****

VARIATE: ABN

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	28.083	37.57	2.553	1.313
RESIDUAL	24	46.667	62.43	1.944	
TOTAL	35	74.750	100.00	2.136	
GRAND TOTAL	35	74.750	100.00		

GRAND MEAN 4.42
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: ABN

GRAND MEAN 4.42

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	3.67	3.33	3.00	5.67	4.67	5.00	4.67	3.67	4.67	5.67	5.33

TREATMNT	12
	3.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.139

APPENDIX 4: CONTINUED

Week 4.

***** ANALYSIS OF VARIANCE *****

VARIATE: ABN

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	49.139	52.48	4.467	2.409
RESIDUAL	24	44.500	47.52	1.854	
TOTAL	35	93.639	100.00	2.675	
GRAND TOTAL	35	93.639	100.00		

GRAND MEAN 3.69
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: ABN

GRAND MEAN 3.69

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	3.67	3.00	5.33	3.33	6.00	4.33	2.33	4.50	4.00	2.33	2.00
REP	3	4	3	3	3	3	3	2	3	3	3

TREATMNT	12
	4.00
REP	3

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	UNEQUAL
SED	1.362X MIN REP
	1.179 MAX-MIN
	0.963X MAX REP

APPENDIX 4: CONTINUED

Week 6.

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	65.556	59.84	5.960	3.251
RESIDUAL	24	44.000	40.16	1.833	
TOTAL	35	109.556	100.00	3.130	
GRAND TOTAL	35	109.556	100.00		

GRAND MEAN 5.11
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: ABN

GRAND MEAN 5.11

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	5.00	3.33	7.33	5.67	3.67	3.67	6.00	3.67	4.67	7.00	6.67

TREATMNT	12
	4.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.106

APPENDIX 4: CONTINUED

Week 8.

***** ANALYSIS OF VARIANCE *****

VARIATE: ABN

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	28.972	23.56	2.634	0.672
RESIDUAL	24	94.000	76.44	3.917	
TOTAL	35	122.972	100.00	3.513	
GRAND TOTAL	35	122.972	100.00		

GRAND MEAN 5.47
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: ABN

GRAND MEAN 5.47

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	5.67	5.67	3.33	6.67	6.33	5.00	6.00	4.67	6.67	5.00	5.33

TREATMNT	12
	5.33

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.616

APPENDIX 4: CONTINUED

Week 12.

1

***** ANALYSIS OF VARIANCE *****

VARIATE: ABN

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	82.750	53.47	7.523	2.508
RESIDUAL	24	72.000	46.53	3.000	
TOTAL	35	154.750	100.00	4.421	
GRAND TOTAL	35	154.750	100.00		

GRAND MEAN 5.58
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE. ABN

GRAND MEAN 5.58

TREATMNT	1	2	3	4	5	6	7	8	9	10	11	12
	4.00	6.00	6.00	5.00	5.00	3.67	3.33	7.67	7.00	4.67	6.33	8.33

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.414

APPENDIX 4: CONTINUED

Week 16.

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	537.889	91.18	48.899	22.569
RESIDUAL	24	52.000	8.82	2.167	
TOTAL	35	589.889	100.00	16.854	
GRAND TOTAL	35	589.889	100.00		

GRAND MEAN 7.94
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: ABN

GRAND MEAN 7.94

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	9.33	6.00	2.67	14.00	9.33	4.33	5.00	7.67	14.67	6.33	3.67

TREATMNT	12
	12.33

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.202

APPENDIX 4: CONTINUED

Week 20.

SOURCE OF VARIATION	DF	SS	SSX	MS	VR
UNITS STRATUM					
TREATMNT	11	1534.972	93.05	139.543	29.207
RESIDUAL	24	114.667	6.95	4.778	
TOTAL	35	1649.638	100.00	47.133	
GRAND TOTAL	35	1649.638	100.00		

GRAND MEAN 11.31
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: ABN

GRAND MEAN 11.31.

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	15.00	7.00	5.00	18.00	10.33	4.33	6.33	11.00	13.00	9.00	8.0

TREATMNT	12
	28.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.785

APPENDIX 4: CONTINUED
B. Seed with insect.
Week 2.

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	44.972	50.55	4.088	2.230
RESIDUAL	24	44.000	49.45	1.833	
TOTAL	35	88.972	100.00	2.542	
GRAND TOTAL	35	88.972	100.00		

GRAND MEAN 4.97
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE ABN

GRAND MEAN 4.97

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	3.33	4.33	4.00	4.67	5.33	4.33	5.67	4.67	4.67	5.67	5.00

TREATMNT	12
	3.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.106

APPENDIX 4: CONTINUED

Week 4.

VARIATE ABN

SOURCE OF VARIATION	DF	SS	%SS	MS	VR
UNITS STRATUM					
TREATMNT	11	41.667	38.94	3.788	1.391
RESIDUAL	24	65.333	61.06	2.722	
TOTAL	35	107.000	100.00	3.057	
GRAND TOTAL	35	107.000	100.00		

GRAND MEAN 4.50
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: ABN

GRAND MEAN 4.50

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	3.67	3.00	6.33	5.33	3.33	5.00	4.00	5.00	3.00	4.33	5.00

TREATMNT	12
	6.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.347

APPENDIX 4: CONTINUED

Week 6.

VARIATE: ABN

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS* STRATUM					
TREATMNT	11	212.972	75.98	19.361	6.901
RESIDUAL	24	67.333	24.02	2.806	
TOTAL	35	280.305	100.00	8.009	
GRAND TOTAL	35	280.305	100.00		

GRAND MEAN 7.64
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE ABN

GRAND MEAN 7.64

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	7.00	3.67	6.00	6.00	6.33	7.67	8.00	10.00	6.00	11.33	7.00

TREATMNT	12
	12.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.368

APPENDIX 4: CONTINUED

Week 8.

VARIATE ABN

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS. STRATUM					
TREATMNT	11	1134.333	89.25	103.121	18.109
RESIDUAL	24	136.667	10.75	5.694	
TOTAL	35	1271.000	100.00	36.314	
GRAND TOTAL	35	1271.000	100.00		

GRAND MEAN 10.50
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE ABN

GRAND MEAN 10.50

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	8.00	7.67	5.00	13.67	6.67	7.33	5.00	16.67	8.67	14.00	8.33

TREATMNT	12
	25.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.948

APPENDIX 4: CONTINUED

Week 12.

VARIATE: ABN

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	5129.22	94.75	466.29	39.405
RESIDUAL	24	284.00	5.25	11.83	
TOTAL	35	5413.22	100.00	154.66	
GRAND TOTAL	35	5413.22	100.00		

GRAND MEAN 16.72
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: ABN

GRAND MEAN 16.72

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	9.33	12.67	7.67	25.67	10.00	12.67	6.67	31.67	12.67	18.00	6.00

TREATMNT	12
	47.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	2.809

APPENDIX 4: CONTINUED

Week 16.

***** ANALYSIS OF VARIANCE *****

VARIATE: ABN

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	4100.08	93.81	372.73	33.050
RESIDUAL	24	270.67	6.19	11.28	
TOTAL	35	4370.75	100.00	124.88	
GRAND TOTAL	35	4370.75	100.00		

GRAND MEAN 16.25
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: ABN

GRAND MEAN 16.25

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	11.67	16.00	3.67	26.33	14.33	13.33	3.67	43.33	14.33	18.67	5.67

TREATMNT	12
	24.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	2.742

APPENDIX 4: CONTINUED

Week 20.

VARIATE: ABN

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREATMNT	11	2282.305	75.46	207.482	45.824
RESIDUAL	24	108.667	4.54	4.528	
TOTAL	35	2390.972	100.00	68.313	
GRAND TOTAL	35	2390.972	100.00		

GRAND MEAN 14.53
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: ABN

GRAND MEAN 14.53

TREATMNT	1	2	3	4	5	6	7	8	9	10	11
	12.67	18.67	5.67	26.33	11.33	23.00	6.67	10.33	21.67	26.67	7.33

TREATMNT	12
	4.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREATMNT
REP	3
SED	1.737

Appendix 5a: Percentage of Storage Fungi Infected Seeds at Different Storage Conditions After 2, 4, 6, 8, 12, 16 and 20 Weeks Storage (Without Insects). Figures in the Body of the Appendix are Percentage Fungi.

Storage Periods (weeks)	Initial Moisture Content 12.4%				Initial Moisture Content 15.1%				Initial Moisture Content 18.5%			
	20°C		30°C		20°C		30°C		20°C		30°C	
	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH
0	34	34	34	34	36	36	36	36	38	38	38	38
2	33	34	33	33	36	33	36	39	36	35	36	37
4	32	34	33	32	35	34	34	36	36	37	36	39
6	35	35	34	37	37	39	34	39	38	42	35	43
8	34	37	33	39	37	42	36	45	37	42	37	45
12	37	42	29	46	37	47	30	49	39	52	32	54
16	35	46	29	49	36	48	25	54	36	49	31	54
20	32	51	25	53	32	57	28	58	35	60	30	62

Appendix 5b: Percentage of Storage Fungi Infected Seeds at Different Storage Conditions After 2, 4, 6, 8, 12, 16 and 20 Weeks Storage (With Insects). Figures in the Body of the Appendix are Percentage Fungi.

Storage Periods (weeks)	Initial Moisture Content 12.4%				Initial Moisture Content 15.1%				Initial Moisture Content 18.5%			
	20°C		30°C		20°C		30°C		20°C		30°C	
	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH	40% RH	65% RH
0	34	34	34	34	36	36	36	36	38	38	38	38
2	31	35	34	32	36	36	34	36	37	36	35	38
4	33	33	34	35	33	34	35	35	33	38	35	35
6	34	36	35	36	38	40	35	39	40	43	36	44
8	34	39	34	42	38	44	35	47	39	45	36	45
12	36	44	28	48	39	50	28	53	37	52	34	58
16	34	49	28	48	37	53	27	57	38	53	32	60
20	34	49	26	50	32	55	25	55	35	53	28	57