THE INFLUENCE OF SOME FUNGI ASSOCIATED WITH FLOUR AND HUMIDITY ON THE SURVIVAL AND DEVELOPMENT OF *CRYPTOLESTES TURCICUS* (GROUVELLE) (COLEOPTERA:CUCUJIDAE)

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Shen-Sin Chang

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ABSTRACT

by

Shen-Sin Chang

THE INFLUENCE OF SOME FUNGI ASSOCIATED WITH FLOUR AND HUMIDITY ON THE SURVIVAL AND DEVELOPMENT OF *Cryptolestes turcicus* (GROUVELLE) (COLEOPTERA:CUCUJIDAE)

The stored-product insect, *Cryptolestes turcicus* (Grouvelle) was reared on seven different diets at $28\pm1^{\circ}C$ and at two relative humidities of 90% and 60%. These diets were: flour from the part of a mill at Medicine Hat, Alberta that had previously been infested with *C. turcicus*; commercial flour sterilized with propylene oxide; unenriched commercial flour; and four laboratory-prepared diets each containing a different concentration of fungi isolated from the Medicine Hat flour. The four latter diets were prepared by mixing the freeze-dried mixture of fungi to unenriched flour to provide mixtures containing 1%, 0.001%, 0.0001%, and 0.00001% by weight of fungi.

At 90% R.H., larvae developed most rapidly on prepared flour-fungi diets and in flour from the mill and slowest in sterilized flour. The highest survival of larvae occurred on the flour-fungi diets and the highest mortality on the flour from the mill. Rate of

pupal development was uniform on all the diets tested. Survival of pupae was about 20% higher on the flour-fungi diets than on the sterilized flour. At 60% R.H., larvae survived and completed their development on the flour-fungi diet containing 1% by weight of fungi and on the flour from the mill but not on any of the other diets.

In general, fungi present in flour encouraged the growth and development of C. turcicus, and the flour-fungi diet containing 1% of fungi was the most favourable food for these insects.

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CHAPTER I

INTRODUCTION

It is well-known that stored products deteriorate when attacked by insects or fungi. The interrelationship between insects and microflora in the stored products environment had been neglected until recently when Agrawal *et al.* (1957), Van Wyk *et al.* (1959), Misra *et al.* (1961), Sikorowski (1964), Abdel-Rahman (1969) showed a close and constant association between certain grain-infesting insects or mites, and the microflora of the grain. These studies opened a number of fascinating problems of practical and fundamental interest in the ecology of both the insects and the fungi.

Even though the association of insects and fungi in stored products was reported by the above workers and frequently by some others in North America and abroad, little is known of the factors which influence the survival and development of stored products insects.

The Problem

It has often been observed in flour mills in Canada that the flat grain beetle, *Cryptolestes turcicus* (Grouvelle) thrives in the presence of moulds associated with flour at high moisture level. During the milling operation some stocks become warm and moist as a result of passing through the break rolls and consequently provide ideal conditions for the growth of microorganisms. The musty odour that is detectable on these stocks indicates that microflora are present. Stocks of similar composition but without the musty odour are unlikely to

become infested by C. turcicus. The presence of this insect in mill-stocks containing a high proportion of microorganisms strongly suggests an insect-fungus relationship. Are fungi essential for the survival and development of C. turcicus? This study was launched to attempt to answer this question by determining the effect of some fungi associated with flour on the growth and development of C. turcicus reared at two different humidities and one temperature.

Organization of the Thesis

The review of literature covers the history and biology of the flat grain beetle, C. turcicus, many aspects of storage fungi and the association of microorganisms with stored products insects. This review is contained in Chapter II. Chapter III explains the materials and methods of the experiments. Chapter IV contains the results and discussions of the studies involving the survival and development of C. turcicus reared on flour mixed with different quantities of a mixture of six species of fungi isolated from flour taken from a flour mill at Medicine Hat. A summary of the principal findings appears in Chapter V.

CHAPTER II

REVIEW OF THE LITERATURE

Historical Review of the Biology of *Cryptolestes turcicus*

Distribution and Status

The flat grain beetle, *Cryptolestes turcicus* was originally described by Grouvelle in 1876 and 1877 (Lefkovitch, 1962a). It was recorded in dried fruit imported into France from Turkey. Howe and Lefkovitch (1957) gave an account of the geographical distribution of this and other species of *Cryptolestes* of economic importance but their data were limited to a great extent to specimens collected on ships at British ports and in Britain and to a smaller extent to specimens collected elsewhere. Since then, specimens have been collected in Japan and South Africa and the species is now known to be established in the temperate holarctic, neotropical and ethiopean regions but not from Australasia (Lefkovitch, 1962a).

At the present time, *C. turcicus* is found commonly in the machinery of flour and provender mills. It is very difficult to assess the damage caused by such a small insect in this habitat. It would seem that the worst damage due to it is likely to be the miller's reputation (Lefkovitch, 1962a). Dyte (1961) reported on the numbers of *C. turcicus* which occurred in the covers of centrifugals in a London flour mill. Bishop (1958) gave records of *C. turcicus* from flour mills farms and grain bins in the western U.S.A. In Canada,

C. turcicus is usually found in flour mills and warehouses (Smith, 1965). A related species, C. ferrugineus (Stephens), has often been found in stored grain where it feeds on, and damages the germ (Rilett, 1949, Liscombe, 1964a). These two species are found throughout the grain-producing areas of Canada and often at points of embarkation (Sinha, 1965a). Hurlock (1963, 1964) indicated that both species have been found in produce originating in Canada and the United States of America, and subsequently unloaded at United Kingdom ports.

Description of Life History

The life history of C. turcicus is similar to that of C. ferrugineus (Rilett, 1949), C. minutus (Davies, 1949) and C. ugandae Steel and Howe (Lefkovitch, 1957). Lefkovitch (1962a) and Bishop (1959) referred to the biology of C. turcicus but neither author gave much detail of the various stages.

Eggs are deposited loosely in the food material and the incubation period depends on the temperature and relative humidity of the environment. Segmentation of the larva can be seen just prior to emergence from the egg. By a series of stretching and undulating movements the chorion is broken and the larva emerges. The caudal hoods or "egg bursters" are used to assist the larva to escape from the egg shell (Liscombe, 1964b).

There are four larval instars. During the late third or early fourth instar, a pair of silk glands begins to develop on the ventral surface of the first thoracic segment (Bishop, 1960). The silk glands develop rapidly, and shortly before the onset of the

prepupal stage in the latter part of the fourth instar, a cocoon is spun. The cocoon may take several forms. In a fine food medium such as flour, the cocoon may be formed entirely of silk, while in coarse food medium the cocoon usually consists of particles of food held together and lined with the silken material. The larva will occasionally pupate without forming a cocoon. The pupa emerges anteriorly through the split larval skin of the fourth instar larva. The duration of the larval period varies greatly, depending on the temperature and relative humidity at which the insect is reared (Lefkovitch, 1962a). The adult remains within the pupal case for about two days and then chews its way out of the cocoon. It soon commences to feed and search for a mate.

Description of Stages

Bishop (1960) published a taxonomic description of the larvae of C. turcicus, C. ferrugineus and C. minutus and outlined a key for their separation.

Egg

The egg of C. turcicus is sausage-shaped and glistening white when laid, but becomes yellowish and opaque just before hatching. It is slightly more than three times longer than wide with one end more tapered than the other (Figure 1).

Larva

The newly hatched larva is whitish and slightly longer than

the egg. There are four instars, the last of which is partly a prepupa. In this condition the larva is shortened, thickened and completely immobile (Figure 2).

Pupa

After passing through the prepupal phase, the larva of the last instar becomes a pupa. During the last larval stage, the insect secretes a tough silken cocoon in which it pupates (Figure 3).

Adult

The beetle is light amber in colour when formed. It remains in the cocoon until the exoskeleton has hardened and the body has attained its normal red-brown colour. The species shows sexual dimorphism with the male antennae being as long as the body and the female antennae half the body length (Figure 4).

Cannibalism

Lucas and Oxley (1947) reported adults but not larvae of *Cryptolestes* spp. to be cannibalistic. Lefkovitch (1957) observed cannibalism in cultures of *C. ugandae* when larval density was high. Ashby (1961) and Rilett (1949) showed that *C. ferrugineus* was cannibalistic and Lefkovitch (1962a, b) reported the same characteristic for *C. turcicus*.

Biology

Lefkovitch (1962a) studied the rate of development of *C. turcicus* reared on wheatfeed (bran). Development was most rapid

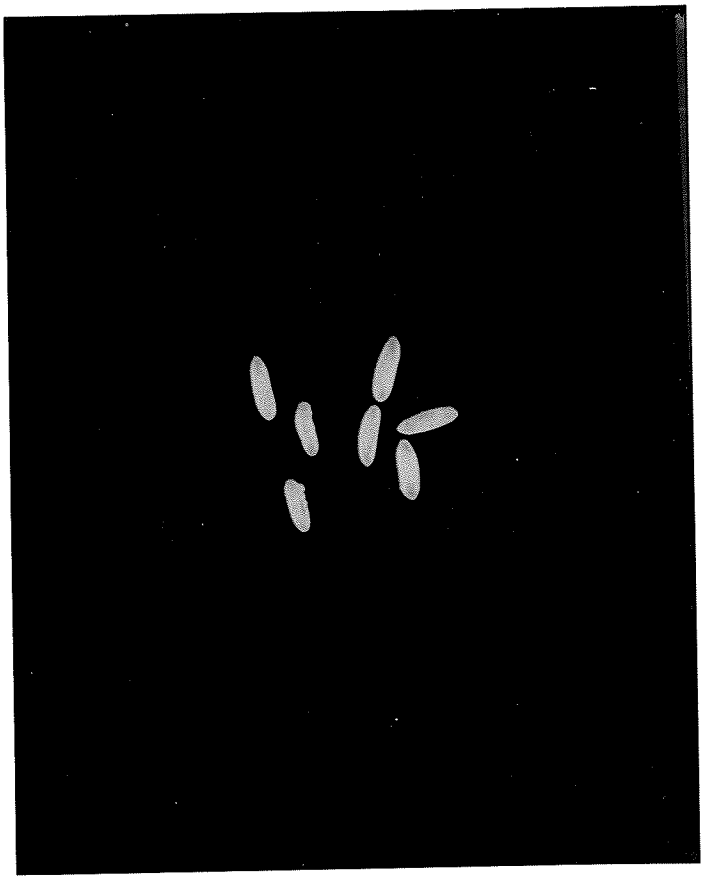


Figure 1. Eggs of C. turcicus

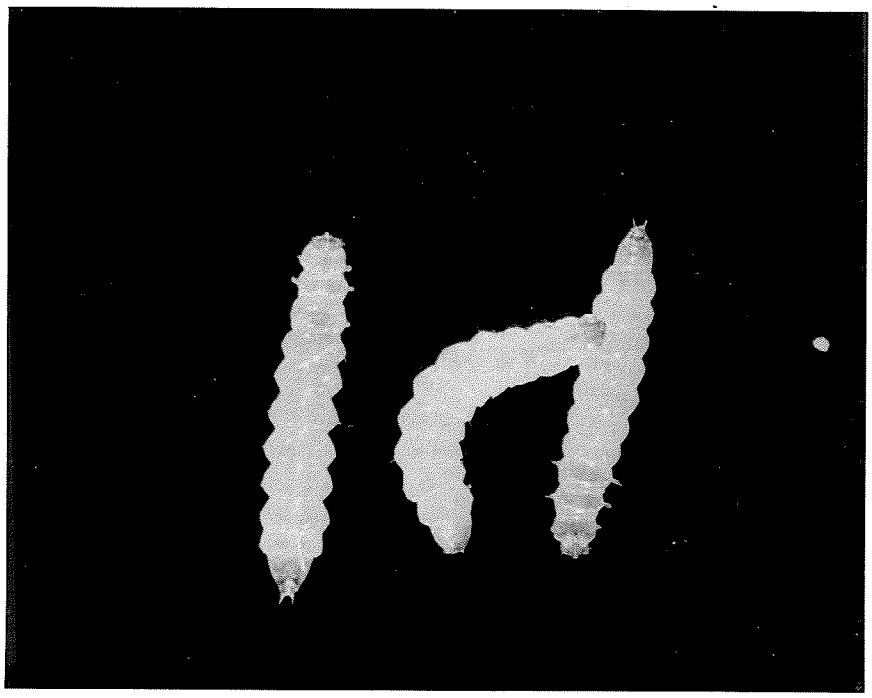


Figure 2. Larvae of C. turcicus

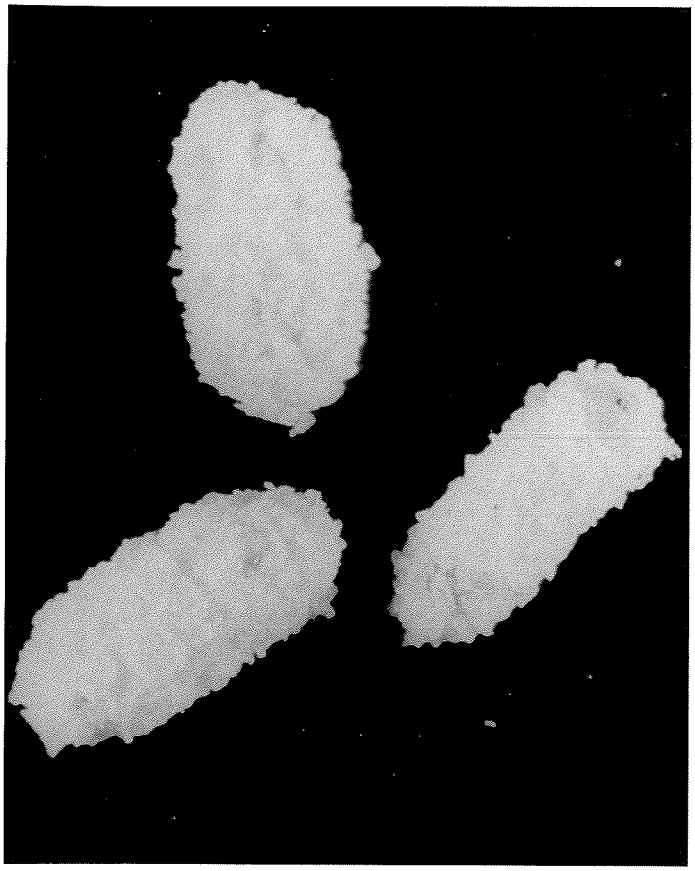


Figure 3. Cocoons of C. turcicus

Figure 4. Adults of C. turcicus show distinct sexual dimorphism, male (left) possessing antennae almost as long as the body whereas those of the female (right) are only half the body length.

at 35°C. and 90% R.H., the life cycle minus the incubation period of the egg requiring 25.8 days. Survival however, was only 20 per cent. At the same relative humidity the greatest survival was 73.3 per cent at 22.5°C. and the least was 16.7 per cent at 17.5°C., the duration of development being 44.3 days at 22.5°C. and 141.3 days at 17.5°C. The range of temperature at which the insect could complete development when the relative humidity was reduced to 70 per cent was narrowed to 22.5°C. to 32.5°C. At 70% R.H. the rate of development at each temperature was slower than that observed at the higher humidity, the shortest time being 34.5 days at 32.5°C. and the longest being 60.7 days at 22.5°C. Survival at 70% R.H. ranged from 23.3 per cent at 30°C. to 76.6 per cent at 22.5°C. At 50% R.H. all larvae died at 22.5°C. and 32.5°C., but there was 66.7 per cent survival at 27.5°C.

Bishop (1959) studied rate of development of C. turcicus on food consisting of one-half a wheat kernel containing germ. At 32.5°C. and 90% R.H., the average time required to complete development (first instar larva to adult) was 25.2 days, while at the same temperature but at 70 and 50% R.H. all first instar larvae died.

For oviposition studies, Bishop (1959) used flakes of wheat germ as food and Lefkovitch (1962a) used wheatfeed (bran). At 32.5°C., Bishop (1959) reported a lifetime average of 55.6, 6.6 and 0.5 eggs per female at 90, 70 and 50% R.H., respectively. At 21°C., he reported a lifetime average of 131.0, 21.8 and 5.6 eggs per female, respectively, at 90, 70 and 50% R.H. on food consisting of equal parts of wholemeal flour and wheat germ. Lefkovitch (1962a) reported a 12 week total of

102.5 eggs per female at 32.5°C. and 90% R.H., 112.9 at 27.5°C. and 90% R.H. and 90.4 eggs at 27.5°C. and 70% R.H.

Lefkovitch (1962a) claimed the optimum conditions for C. turcicus to be near 28°C. and 90% R.H., while Bishop (1959) stated the optimum may be near 21°C. and 90% R.H.

Storage Fungi

Fungi belonging to all three classes (Table I) infect stored products, the majority belonging to the Fungi Imperfecti (or Deuteromycetes as they are called occasionally) (Clarke, 1968).

The genus Aspergillus, of which 132 species are recognized in the latest monograph (Raper & Fennell, 1965), contains more species attacking stored products (such as A. glaucus, A. candidus, A. flavus), than any other single genus, and often dominates the mycoflora of tropical products. The genus Penicillium, consisting of 137 species according to Raper & Thom (1949), also contains a large number of species, such as P. citrinum and P. cyclopium, which infect stored products. They are more prevalent in temperate than in tropical conditions, some species even growing in moist barley stored at 3.8°C. (Burrell et al., 1966). Species of Penicillium, on account of their predominantly blue-green colour and their few 'good' morphological characters, are more difficult to identify than those of Aspergillus, since the majority of species in these two genera have no sexual stage, they are both placed among the Fungi Imperfecti.

TABLE I

SYSTEMATIC POSITION OF THE STORAGE FUNGI (AFTER CLARKE, 1968)

CLASSES		
		Fungi
Phycomycetes	Ascomycetes	Imperfecti
Absidia	Byssochlamys	Alternaria
Mucor	Hansenula	Aspergillus
Rhizopus	Monascus	Aureobasidium
		Candida
		Epicoccum
		Cladosporium
		Fusarium
		Helminthosporium
		Penicillium
		Sporobolomyces
		Verticillium

Christensen (1957) and Christensen & Kaufman (1965) reviewed the deterioration of stored grain due to the activities of fungi. Christensen and Cohen (1950) reported that the mould counts of approximately five hundred samples of flour, collected principally in commercial mills, ranged from several hundred to more than 5,000 per gm. Three samples of washed wheat from one mill, collected as the wheat went to the first break rolls, contained only a few hundred moulds per g., while the flours milled from these wheat contained up to several thousand moulds per g. The chief source of mould contamination of flour appears to be moulds growing and sporulating within the milling system itself. The predominant moulds in most of the flours were Aspergillus glaucus and A. candidus. Unidentified species of Penicillium made up a major portion of the mould flora in only a few samples of commercial flour. Several other genera were found in most samples, but only in small numbers. The factors that influence the numbers and kinds of moulds cultured from a given sample were: the composition of the medium on or in which the flour was cultured, the techniques of making the dilutions, and the method of counting the number of colonies in the cultured dishes (Christensen, 1946). The fungi already mentioned above undoubtedly exist and grow during storage.

Since, like insect pests, fungi growing in stored products are using them as food substrates, they cause chemical breakdown with consequent loss in nutritive value and marketing quality. Coursey (1966) showed that a number of fungi, in particular Aspergillus tamarii and A. niger, accelerated the liberation of free fatty acids from palm

oil. Butt (1966) showed that there was a significant correlation between fungal spore load and 'mustiness' of Ugandan coffee.

Heavy growth of fungi in stored products may make them difficult to handle. For instance, Clarke et al. (1967) reported that excessive growth of fungi, particularly yeasts, in moist barley stored in 'sealed' silos in England interrupted unloading of the grain by auger.

Association of Storage Insects with Fungi

The storage insects associated with fungi are able to thrive if conditions are favourable, and consequently to cause deterioration of the stored products. Sikorowski (1964) stated that reproduction of stored products insects and of fungi is greatly influenced by moisture and temperature. Either insects or fungi alone, or both can cause heating in stored products. Usually, storage fungi accompany or follow insect infestations. Some experiments have shown that the granary weevil's activity increased moisture content of wheat and favoured development of storage fungi (Agrawal et al., 1957; Christensen et al., 1960).

Practically no fungi will grow at a relative humidity below 70 per cent (Ayerst, 1966., Panasenko, 1967), whereas insects in stored products can still survive and breed at much lower relative humidities (Howe, 1965).

Areas within stored grain where either insect or mite or fungal activity is high are referred to as "hot spots" (Sinha, 1961).

According to Sinha, the centre of the hot spot has a temperature of 95°F and a moisture content exceeding 17%. This centre is surrounded by drier wheat of 14.5% of moisture or less at a temperature of 86°F. Under suitable conditions, the hot spot temperature may reach 175°F. Hot spots may develop quickly in farm-stored grain in Western Canada in winter. Heavy infestations of mites, insects and fungi may accompany such hot spots. Once the heating process is initiated, whatever its cause, it brings about a rapid deterioration of grain, through charring of kernels, and reduction of their germinability, and by providing optimum conditions for the growth and reproduction of stored insects, fungi and mites. The most common beetles observed by Sinha were Cryptolestes spp. Other insects less often present included 14 species of various genera. Wallace and Sinha (1962) reported that the grain in hot spots was predominantly infected by storage fungi, primarily Penicillium spp. Other fungi commonly found were Aspergillus flavus and A. versicolor.

Direct feeding by insects is not serious in itself, but their presence is associated with an increase of moisture and temperature in the grain. This condition favours mould development which in turn leads to down-grading of the grain. Lindgren (1935) pointed out that the metabolic water and heat given off by insects hasten deterioration of grain in storage. Sikorowski (1964) showed that the saw-toothed grain beetle, Oryzaephilus surinamensis (L.), the red flour beetle, Tribolium castaneum (Herbst) and the flat grain beetle, Cryptolestes minutus (Olivier) were all attracted to artificially-induced hot spots.

This result suggested that the beetle-fungus association is not a coincidence, but that environmental factors are responsible for this relationship. Rilett (1949) reported that the enzyme diastase, produced by moulds, acts on raw starch and splits it through the dextrin stage into maltose. The maltose in turn is usually hydrolized to glucose by the maltase in the enzyme mixture. When larvae of C. ferrugineus were fed on mouldy wheat and on diastase-treated wheat, approximately equal numbers reached maturity.

Misra et al. (1961) demonstrated that storage fungi, principally Aspergillus amstelodami, A. repens, and A. ruber in the Aspergillus glaucus group, were consistently isolated from nonsurface-disinfected and surface-disinfected larvae, pupae and adults of the Angoumois grain moth, Sitotroga cereallella (Olivier), and from the alimentary tract of larvae; excreta of the insect contained up to almost a billion spores of those fungi per gram. Adult moths were attracted to air passed through mouldy grain in preference to air passed through clean grain or to air alone, and preferred grain invaded by members of the A. glaucus group to clean grain.

During the last 12 years Agrawal et al. (1957), Van Wyk et al. (1959) and Griffiths et al. (1959) showed that a close and constant association exists between some of the grain-infesting insects and mites, and the microflora of infested grain. It seemed worth while to determine whether a similar relationship might exist between the Angoumois grain moth, Sitotroga cereallella, and the microflora,

especially storage fungi. In general, the relationship between the Angoumois grain moth and storage fungi is similar to that found by Agrawal et al. (1957) between the granary weevil, Sitophilus granarius and storage fungi. As populations of the moth developed in wheat, the moisture content of the grain increased. This increase was not so great as was found with the granary weevil, but still amounted to between 5 and 7% within 3 to 4 months. Spores of a number of the common storage fungi were carried in abundance on the outside of the adult moths, when the moths emerged from the mouldy wheat, and some inoculum was carried even within the bodies of the adults, in spite of the fact that the adults do not feed. These fungi developed in the grain and their spores were consumed by the larvae along with the interior portion of the wheat grains. The alimentary canals of nearly all larvae cultured carried large numbers of spores of Aspergillus spp. amounting to nearly a billion per gm. of grain. When clean and mouldy samples of wheat were infested with equal numbers of adult moths and stored under identical conditions, more larvae developed in the mouldy than in the clean grain. The major fungal flora associated with the Angoumois grain moth were from the Aspergillus glaucus group, principally A. repens. A. amstelodami was abundant in grain infested by the granary weevil (Agrawal et al., 1957).

Flour harbors a moderate to heavy load of various species of fungi, yeast and bacteria (Christensen and Cohen, 1950). It was thought possible that some of these microflora might influence or be influenced by developing populations of Tribolium confusum. Van Wyk et al. (1959)

isolated large numbers of bacteria and lesser numbers of storage fungi from larvae and adults of T. confusum, and found that the bacteria were more numerous in the insects than in the food from which the insects were taken. These bacteria, when added to autoclaved whole wheat flour, promoted growth and reproduction of T. confusum. The authors considered that the bacteria appeared to supply the B vitamins necessary to normal development of the insects about as effectively as did brewer's yeast.

Fungi as a Source of Food for Stored Product Insects

Sinha (1965b) studied two stored-product insects, C. ferrugineus (Stephens) and Oryzaephilus mercator (Fauvel.) reared on 23 species of seed-borne fungi and showed that C. ferrugineus completed development on 10 and C. mercator on 18 species. Further work (Sinha, 1966) showed that adults of T. castaneum and T. confusum reared at $31\pm1^{\circ}C$ and $70\pm3\%$ R.H. fed voraciously on Alternaria spp. and Mucor sphaerosporus. Adult T. castaneum fed well also on Mormodendrum spp. and Nigrospora spp. and T. confusum on 10 other species of fungi. These studies apparently indicated that some stored products insects can utilize fungi at least partially, as a source of food.

The use of fungi and some other microorganisms as sources of nutrients for insects has also been reported by Van Wyk et al. (1959). They added Aspergillus flavus, A. repens, A. candidus, and/or yeast to a basic medium consisting of casein, starch cholesterol, and salts. The larvae of T. confusum fed readily on the basic diet

plus Aspergillus spp. and Penicillium spp., but did not increase in size. Insects fed on the basic diets plus yeast developed normally. Addition of bacteria to a vitamin-free diet allowed normal growth of the larvae. Olfactometer and "free-choice" tests showed that the beetles were attracted more to flour containing fungus spores than to the flour assumed to be fungus-free. The percentage of survival of the adults was higher in mouldy wheat than in mould-free wheat at 60 and 70% R.H.

Rilett (1949) indicated that the presence of mould in stored wheat may greatly increase the total amount of food suitable for the developing larvae by making the starch portion of wheat more readily available as larval food. Woodroffe (1962) reported that the foreign grain beetle, Ahasverus advena (Waltl) occurred in large numbers of mouldy produce and it has always been regarded as a mould feeder rather than a direct destroyer of stored food stuffs. Recently, Sikorowski (1964) reported that the flat grain beetle, Cryptolestes minutus, the red flour beetle, Tribolium castaneum and the saw-toothed grain beetle, Oryzaephilus surinamensis could complete the developmental stages from egg to adult on a diet of Aspergillus versicolor only. A. repens and A. parasiticus have detrimental effects on both mature and immature stages of the insects tested. He concluded that some species of fungi might have toxic effects. Therefore, whether a given fungus-insect association is beneficial or detrimental to the insect depends upon the species of insect and of fungus.

Ample evidence is provided in the literature review to show that fungi play a role in the development of storage insects. As mentioned above, many workers have reported associations of storage insects with fungi.

CHAPTER III

MATERIALS AND METHODS

Rearing and Handling of Insects

Initially, two strains of Cryptolestes turcicus were used and their rates of development determined. The Keewatin strain was collected from a flour mill in Canada, and the Slough strain from the Pest Infestation Laboratory at Slough, England.

Adults were kept in one gallon glass jars containing a mixture of commercial unenriched flour and brewer's yeast (95:5 w/w). The jar was fitted with filter paper held in place with melted paraffin. The filter paper allowed air to penetrate the jar but prevented the entry of mites. The stock cultures were stored in cabinets maintained at 28$\pm$1°C and 90% R.H.

Comparison of Survival and Rate of Development of Two Strains of C. turcicus

Experiments were carried out in desiccators containing a solution of potassium hydroxide maintaining a constant known relative humidity (Solomon, 1951).

Eggs of uniform age were obtained by allowing adults to oviposit in food medium for 24 hours. About 6,000 adults of each strain handled separately were placed on 145 gm of a mixture of culture medium that had been passed through a 100-mesh screen. This culture was maintained at 28$\pm$1°C and 90% R.H. After 24 hours, the adults were

separated and removed by sifting the medium over a 40-mesh screen. The medium was re-sifted with an 80-mesh screen to retain the eggs. One egg was then transferred with a fine camel hair brush into each of 200 small vials (1 cm in diameter and 4.5 cm deep) containing approximately 55 mg of flour previously conditioned at the temperature and relative humidity of the experiment for at least six days.

Daily observation commenced two days before the eggs were expected to hatch. The newly-hatched larvae were left undisturbed until just before cocoon construction when daily observations were continued. After larval development was completed the insects were not examined until just before adult emergence. The duration of the egg, larval and pupal periods was recorded for each strain. The results are shown in Table II. No significant difference was found in the duration of the developmental stages between the two strains. Therefore, in subsequent experiments insects from the Keewatin strain were used exclusively.

The Isolation of Fungi

The sample of second and fourth break flour from which fungi were isolated was obtained from a flour mill in Medicine Hat, Alberta. The source of the sample was a "boot" which had periodically been infested with C. turcicus. A small portion (0.2 gm) of the sample was placed in a flask containing 100 ml of sterile water, well shaken and allowed to settle for 15 seconds. One ml was removed with a pipette and poured on the surface of acidified potato sucrose agar (PSA) on

TABLE II

NUMBER OF SURVIVORS, AND DURATION OF EGG, LARVAL AND PUPAL DEVELOPMENT PERIODS OF TWO STRAINS OF *C. TURCICUS* AT 28±1°C, 90% R.H.

Strain	Stage	Initial Number	Number of Survivors	Duration (days) Range	Mode	Mean	S.D.
	Egg	100	90	3-4	3	3.2	0.4
Slough	Larva	90	80	14-23	16	16.4	2.1
	Pupa	80	77	7-14	13	12.1	1.3
	Egg	100	86	3-4	3	3.4	0.5
Keewatin	Larva	86	79	12-23	15	16.3	3.1
	Pupa	79	78	5-16	13	11.5	2.2

petri plates. The plates were then incubated at room temperature for about 5-6 days during which a number of colonies of different species of fungi, bacteria, and yeasts grew on the surface on the PSA medium. An inoculum of each species of fungus was transferred to PSA slants at about p^H 6 in 8 mm x 150 mm test tubes by making two or three cuts deep into the surface of the medium with a No. 22 inoculating needle. The culture tubes were incubated at room temperature until a thick matrix of mycelia and spores had grown over the surface of the agar slants (Figure 5). This usually required 1-2 weeks. Large numbers of replicates were prepared for each fungus. Those that showed signs of contamination were discarded.

The species of fungi isolated from the flour were identified as follows:-

Alternaria triticina Prasada & Prablu

Cladosporium herbarum (Pers.) Link ex Fr.

Epicoccum purpurascens Ehrenb. ex Schlecht.

Aspergillus amstelodami (Mangin) Thom & Church

Penicillium cyclopium Westling

Penicillium decumbens Thom.

The bacteria and yeasts that occurred in the flour were not considered in this study.

Bulk quantities of each fungus were obtained by adding inoculum to potato sugar water in 1000 ml Erlenmeyer flasks plugged with sterile cotton. Before addition of the inoculum the flasks and contents were autoclaved for about 30 min. at 121°C and 15 psi (pounds

per square inch). The inoculated flasks were incubated in a well-lighted room at room conditions until sporulation was well defined, usually about 3 weeks for most species (Figures 6-8).

At this stage of growth, the mycelial mat and spores of each species were removed from each flask, placed on a vacuum funnel, rinsed briefly with distilled sterile water and freeze-dried. The freeze-dried fungi were pulverized in a blender, passed through a 20-mesh sieve, and stored in a refrigerator until ready for use.

Rearing C. turcicus on Mixtures of Fungi and Flour at Two Different Relative Humidities

The freeze-dried fungi were mixed with the commercial unenriched flour to obtain a stock culture containing 1% by weight of fungi in flour, 0.1 gm of dry fungi was weighed out on an electric balance and added to a sterilized mortar containing 9.9 gm of flour. The mixture was stirred and pounded with a pestle until the flour particles were mixed uniformly with the fungi. It was then transferred into a glass jar and shaken violently in order to make the mixture as homogeneous as possible. From this stock, dilutions were made in the following proportions:-

A 1:100,000 fungi:flour mixture was prepared by adding 0.1 gm of the stock to 99.9 gm of flour. Further dilutions were prepared to provide 1:1,000,000, and 1:10,000,000 fungi-flour mixture.

Besides the fungus-flour mixed media, the following diets were tested: (1) unenriched flour, (2) flour from the Medicine Hat

mill, (3) sterilized flour. The procedure used to sterilize the flour was described by Hansen and Snyder (1947), Barlow and House (1956). Flour to be sterilized was placed in a jar to which was added the sterilant, liquid propylene oxide, at the rate of 1 ml per 100 gm of flour. The operation was first conducted in a cold room ($35^{\circ}F$) with thoroughly chilled materials. After two hours in the cold room, the jar was allowed to reach room temperature in a well-ventilated area. It was then shaken to remove any remaining traces of the sterilant, sealed and left for at least 24 hours at room temperature. Then the contents were aerated.

Newly-hatched larvae were surface-sterilized by immersing them in 1% sodium hypochlorite for 2-3 min. followed by rinsing in sterile distilled water 3 or 4 times (Sinha, 1964). The surface-sterilized larvae were introduced singly into each of 30 small vials (1 cm in diameter and 4.5 cm deep) containing approximately 0.5 gm of different diets described above. The vials were held in separate desiccators that contained aqueous solution of KOH formulated according to Solomon (1951) to provide a relative humidity of 60 per cent in one and 90 per cent in the other. The desiccators were placed in temperature-controlled cabinets and kept at $28\pm1^{\circ}C$ during the course of the experiment.

Daily observations were started just before the formation of cocoons and the emergence of adults. Survival and rate of development were recorded. Ranges, modes, means and standard deviations were calculated for each set of data.

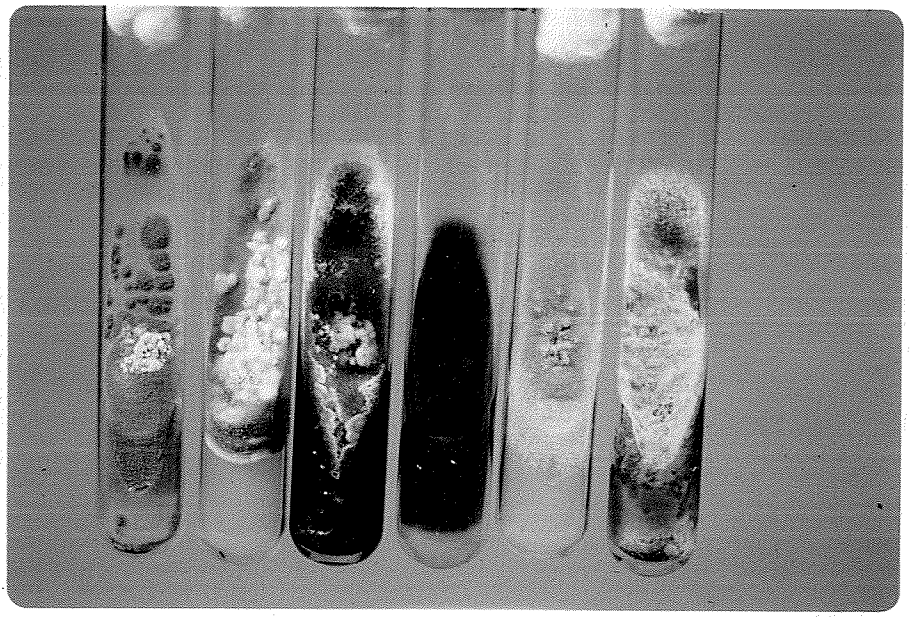
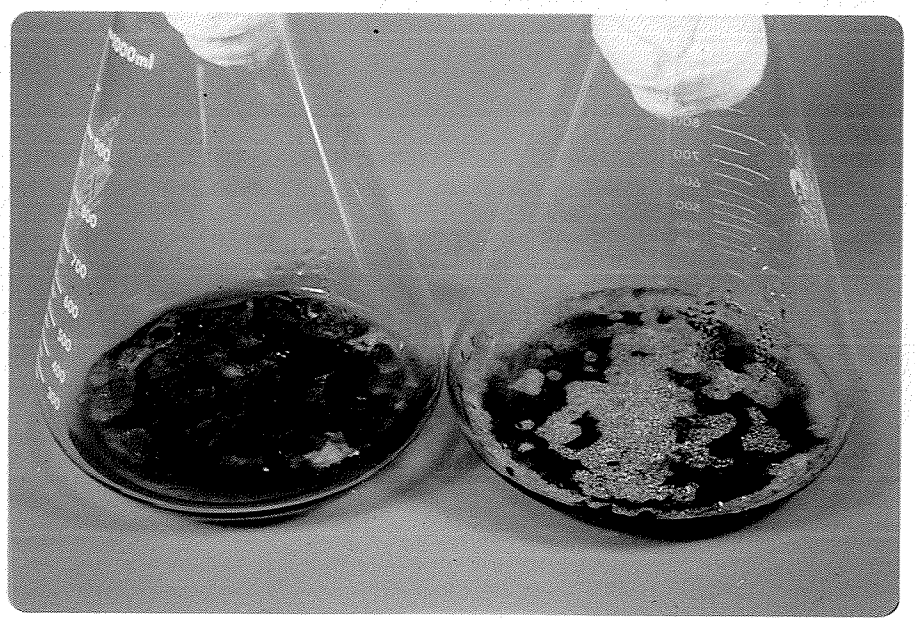
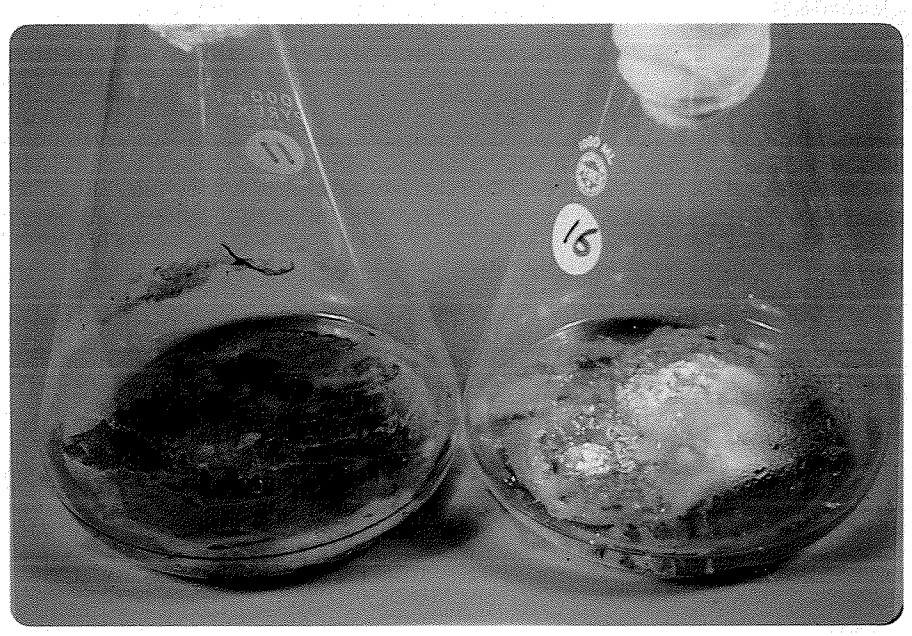


Figure 5. Various species of fungi grown on the PSA slant in test tubes. From left to right: Penicillium cyclopium, P. decumbens, Alternaria triticina, Cladosporium herbarum, Aspergillus amstelodami, and Epicoccum purpurascens.



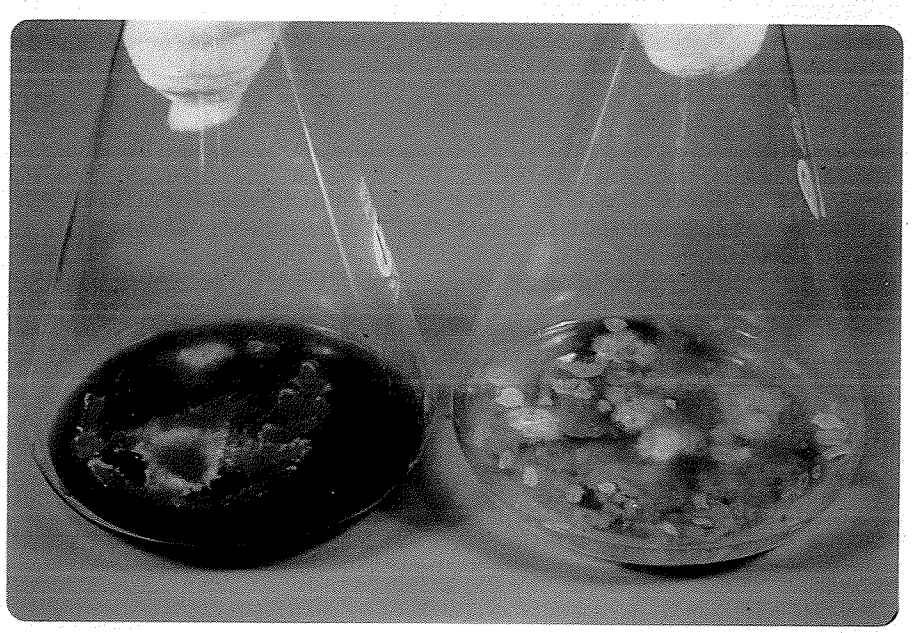
(a) (b)

Figure 6. Fungi grown on surface of potato sugar water in 1000 ml Erlenmeyer flasks. (a) Alternaria triticina and (b) Cladosporium herbarum.



(a) (b)

Figure 7. Fungi grown on surface of potato sugar water in 1000 ml Erlenmeyer flasks. (a) Penicillium cyclopium and (b) P. decumbens.



(a) (b)

Figure 8. Fungi grown on surface of potato sugar water in 1000 ml Erlenmeyer flasks. (a) Epicoccum purpurascens and (b) Aspergillus amstelodami.

CHAPTER IV

RESULTS AND DISCUSSION

Section 1.

The Rate of Development and Survival of Larvae and Pupae of C. turcicus on Various Diets at 28°±1°C and 90% R.H.

Results

The rate of development and survival of larvae of C. turcicus reared on various diets at 28°±1°C and 90% R.H. are given in Table III. Larvae developed faster on the prepared flour-fungi diets and on the flour from the Medicine Hat mill than on the sterilized flour. The difference was highly significant at the one per cent level.

Of the prepared fungal-flour diets, the one containing 1% by weight of a mixture of fungi was the best for larval development. There was an inverse relationship between the amount of fungi present in the diets and rate of development. As the proportion of fungi decreased the larval developmental period increased. Larval development on the unenriched flour was the slowest compared to those of the prepared fungal-flour diets. (Although no fungi were added to the unenriched flour, it is not unreasonable to assume that some fungi are present, because stored products such as grain and flour are never free from fungi). On the sterilized flour, which is presumably free of fungi, larval development was slowest. The most rapid rate of larval development occurred on the flour from the Medicine Hat mill.

The highest survival of larvae of C. turcicus occurred on the flour-fungi diets and the highest mortality on the flour from the mill at Medicine Hat. Larval mortality on the sterilized flour was about 23%.

Table IV shows the rate of development and survival of pupae of C. turcicus reared on the same conditions as those of the larvae. The rates of pupal development on the prepared flour-fungi diets containing 1% or 0.001% by weight of fungi, and on the unenriched flour were somewhat faster than on sterilized flour. As the proportion of fungi to flour increased in the flour-fungi mixtures, the duration of the pupal stage decreased only slightly, and the time required by the beetles to complete pupal development was essentially the same on all the diets studied.

Survival of pupae was affected by the type of food materials at 28±1°C and 90% R.H. (Table IV). Per cent survival was lowest on the sterilized flour. Survival was uniformly high on the remaining diets.

Discussion

The results of this experiment suggested that the more mouldy the flour, the more favourable it became for larval development of the beetles within the range tested. The effects of fungi are more marked when one compares the larval survival and rate of development on the flour-fungi diets and on the sterilized flour. Larvae survived in greater numbers and developed more rapidly in the flour-fungi diets

than in the sterilized flour. It might be questioned that the sterilization process of unenriched flour with propylene oxide may have produced deleterious effects on nutrients. However, since Barlow and House (1956) showed that ethylene oxide did not have a deleterious effect on a diet used to rear the larvae of a dipterous parasite, it was assumed that the sterilization of flour had little or no effect on the nutrients.

It is possible that the poor growth and high mortality observed on the sterilized flour is due to the absence of fungi. These fungi may produce some nutrients which are essential to the growth of the beetles. Rapid larval development on the flour-fungi diets and on the flour from the Medicine Hat mill, from which the fungi were originally isolated support the hypothesis that the fungus-insect association is beneficial to the insects. The high mortality of larvae on flour from the Medicine Hat mill may be due to the presence of some other microorganisms which might be unfavourable or toxic to the insects. The low larval survival rate on the sterilized flour may be due to the absence of fungi and suggests that their presence in the diet is beneficial to the beetles.

Pupal development did not appear to be appreciably affected by the presence of fungi in the diets. On the other hand, low pupal survival on the sterilized flour suggests that fungi in the larval diet may affect a later stage of development.

The results in Table III and Table IV appear to agree with the findings of several workers. Rilett (1949) reported that the

TABLE III

RATE OF DEVELOPMENT AND SURVIVAL OF 30 NEWLY-HATCHED LARVAE OF CRYPTOLESTES TURCICUS ON VARIOUS DIETS AT 28±1°C AND 90% R.H.

	Survival to pupal stage		Developmental period (days)			
Diet	No.	%	range	mode	mean	s.d.
Flour containing 1% fungi	27	90.0	19-25	19	20.1**	1.7
Flour containing 0.001% fungi	25	83.4	21-28	22	23.2**	2.3
Flour containing 0.0001% fungi	28	93.4	22-38	28	23.5**	3.9
Flour containing 0.00001% fungi	25	83.4	20-33	20,23	23.8**	2.2
Unenriched flour	27	90.0	21-28	23	24.0**	2.1
Sterilized flour	23	76.7	23-50	26	31.4	8.5
Flour from Medicine Hat mill	17	57.0	13-16	13	13.8**	1.0

**Significant difference at the one per cent level, compared with the sterilized flour.

TABLE IV

RATE OF DEVELOPMENT AND SURVIVAL OF PUPAE OF

CRYPTOLESTES TURCICUS ON VARIOUS

DIETS AT 28±1° AND 90% R.H.

Diet	Survival to adult stage		Developmental period (days)			
	No.	%	range	mode	mean	s.d.
Flour containing 1% fungi	26	96.3	4-12	10	8.3*	2.0
Flour containing 0.001% fungi	25	100.0	5-11	9	8.6*	1.3
Flour containing 0.0001% fungi	27	96.5	7-12	9	9.1	1.3
Flour containing 0.00001% fungi	24	96.0	6-15	10	10.7	2.1
Unenriched flour	26	96.3	6-11	9	8.7*	1.0
Sterilized flour	18	78.3	5-12	11	9.7	1.8
Flour from Medicine Hat mill	17	100.0	8-13	10	9.8	1.1

*Significant difference at the 5 per cent level compared with the sterilized flour.

enzyme diastase produced by moulds favoured the growth of larvae of C. ferrugineus (Stephens) on mouldy wheat. He concluded that this enzyme greatly increased the total amount of food for the development of larvae by making the starch portion of the wheat more readily available as food. Van Wyk et al. (1959) considered that the confused flour beetle, Tribolium confusum (Duval) was attracted to, and developed somewhat better in flour or wheat containing storage fungi than on flour or wheat free of them. Griffiths et al. (1959) found that the grain mite, Tyrophagus castellanii required more time to reach maturity on sterilized than on unsterilized wheat germ, whereas Misra et al. (1961) working on the Angoumois grain moth claimed that mouldy grain was much preferred by these insects for oviposition and development than was clean grain. Woodroffe (1962) reported that the foreign grain beetle, Ahasverus advena (Waltl) thrived in mouldy stored food stuffs.

Some stored products are deficient in certain nutrients which may be supplied by fungi or other microorganisms. The results of the present experiment suggest that a diet consisting of flour and fungi encouraged the rate of development of C. turcicus.

Section 2.

Rate of Development and Survival of Larvae and Pupae of C. turcicus on Various diets at 28$\pm$1°C and 60% R.H.

Results

The rate of development and per cent survival of larvae of

C. turcicus on various diets at 28±1°C and 60% R.H. are given in Table V. Of all the diets tested, only the flour-fungi diet containing 1% by weight of fungi and the flour from the Medicine Hat mill supported development to the pupal stage. The larval developmental period was about 34 days on the former diet and about 21 days on the latter. Larvae failed to develop or survive on the remaining diets and died mostly in the second or third stadium.

Survival rate of larvae on the two diets upon which larvae completed development was low. About 50 per cent larval mortality occurred on the flour-fungi and 40 per cent on the flour from the Medicine Hat mill.

Table VI shows the rate of development and per cent survival of pupae of C. turcicus on two diets at 28±1°C and 60% R.H. The duration of pupal period was about 8 days on the flour-fungi diet containing 1% by weight of fungi, and 10 days on the flour from the Medicine Hat mill. Survival to the adult stage was 50 per cent on the former diet and about 83 per cent on the latter diet. As larvae did not complete development on the other five diets, pupal development on these diets could not be measured.

Discussion

The low survival and long developmental periods on two diets, and complete mortality on five others indicates the inability of C. turcicus to survive at 60% R.H. (According to Lefkovitch, 1962a the optimum conditions for these insects are about 28°C and 90% R.H.)

TABLE V

RATE OF DEVELOPMENT AND SURVIVAL OF 30 NEWLY-HATCHED LARVAE OF *CRYPTOLESTES TURCICUS* ON VARIOUS DIETS AT 28±1°C AND 60% R.H.

Diet	Survival to pupal stage		Developmental period (days)			
	No.	%	range	mode	mean	s.d.
Flour containing 1% fungi	16	53.4	27-46	27,46	34.2	7.5
Flour containing 0.001% fungi	[a]	0	———	———	———	———
Flour containing 0.0001% fungi	[a]	0	———	———	———	———
Flour containing 0.00001% fungi	[a]	0	———	———	———	———
Unenriched flour	[a]	0	———	———	———	———
Sterilized flour	[a]	0	———	———	———	———
Flour from Medicine Hat mill	18	60	20-25	20	21.3	1.7

[a] Died during early larval development.

TABLE VI

RATE OF DEVELOPMENT AND SURVIVAL OF PUPAE OF

CRYPTOLESTES TURCICUS ON VARIOUS DIETS

AT 28±1°C AND 60% R.H.

Diet	Survival to adult stage		Developmental period (days)			
	No.	%	range	mode	mean	s.d.
Flour containing 1% fungi	8	50	6-9	9	7.8	1.4
Flour containing 0.001% fungi	[a]	0	———	———	———	———
Flour containing 0.0001% fungi	[a]	0	———	———	———	———
Flour containing 0.00001% fungi	[a]	0	———	———	———	———
Unenriched flour	[a]	0	———	———	———	———
Sterilized flour	[a]	0	———	———	———	———
Flour from Medicine Hat mill	15	83.4	8-11	10	9.4	0.9

[a]Died during early larval development.

However, even under these adverse conditions the two diets containing the greater amounts of fungi, namely the flour-fungi diet containing 1% by weight of fungi and the flour from the Medicine Hat mill promoted some survival and development. These results suggest that this species requires certain fungi in their diet to promote survival and development.

It is interesting that larval development was fastest on flour from the Medicine Hat mill at both 90% and 60% R.H.

General Discussion

Figures 9-12 summarize the results of these experiments on larval and pupal survival and rate of development of C. turcicus on various diets at 28±1°C, 60% and 90% R.H. It is clear from Figures 9 and 10 that higher survival of larvae and pupae occurred on the flour-fungi diets at 90% R.H. Figure 11 indicates that faster larval development occurred at 90% than at 60% R.H. Figure 12 shows that on the two diets on which pupae survived and developed, the duration of the pupal stage was essentially the same at both humidities.

Humidity and temperature are two primary physical conditions which greatly influence survival, rate of development, and fecundity of stored-product insects. Some workers feel that moisture is the most important factor that influences the build-up of large populations of C. turcicus in milling machinery and that nutritive value of food material has little consequence in this regard (Dyte, 1961). However, since high humidity usually encourages the growth of fungi, it is questionable that the successful growth of C. turcicus

can be attributed to moisture alone. It is more likely that moisture induces the growth of fungi which may provide nutrients that favour insect development.

In the present experiments the conditions of 28±1°C and 90% R.H. were considered optimal (Lefkovitch, 1962a). At these conditions survival and development may be affected by the kind of food. If significant differences are found in survival and development, then it is reasonable to assume that the food is the factor causing the change. Therefore, the rapid development and high per cent survival of C. turcicus on the flour-fungi diets at 90% R.H. were attributed to the influence of the fungi present in the diets.

During the course of these experiments the mycelia and spores of fungi were observed on the surface of the prepared flour-fungi diets and the flour from the Medicine Hat mill at 90% but not at 60% R.H. These observations indicated that high humidity encourages the growth of fungi. They agree with the findings of Ayerst (1966) and of Panasenko (1967) who found that practically no fungi will grow at relative humidity below 70%. Therefore, the dependence of fungi on humidity probably accounts for the great difference in survival and rate of development of C. turcicus on various diets at the two humidities of 90% and 60%. At 90% R.H. there is a high probability that this species ingests fungi as food since fungi grow profusely at this high moisture level. As a result, the insects can fully utilize the fungi and thrive. On the other hand, the probability of C. turcicus taking fungi as food at 60% R.H. is extremely low simply because no

fungi will grow at a relative humidity below 70%. However, if mycelia or spores of fungi are present in very large quantities in the diets, the probability of ingesting fungi would increase. As these experiments showed, more than 50 per cent of larvae survived and developed on the flour-fungi diet containing 1% of fungi and on the flour from the Medicine Hat mill despite the unfavourably low humidity of 60%. This result suggests that the fungi present in the flour were utilized by the insects to aid their survival and development. Therefore, it is reasonable to state that even under adverse humidity conditions the presence of fungi in the flour is beneficial to the growth of C. turcicus.

It has been reported that certain species of storage fungi are suitable as food for a particular species of insect or mite (Agrawal et al., 1957; Van Wyk et al., 1959; Sikorowski, 1964; Sinha, 1965; Loschiavo and Sinha, 1965). There is general agreement that fungi as a group have an important role in the successful growth of stored-products insects.

Some workers have found that some species of fungi may produce toxins which are harmful to insects (Evlakhova, 1953; Kodaira, 1961). However, toxicity or pathogenicity can be affected by many factors, for example, kind of substrate, temperature, and period of incubation (Armolik et al., 1956). Consequently, it is sometimes difficult to determine which factor or combination of factors is responsible for a given effect on the host organism. In viewing the results of the present experiment, it seems most unlikely that the fungi used in the diets have a toxic effect on C. turcicus. However,

other microorganisms such as yeasts and bacteria that naturally occur in flour may have toxic effects or may deter growth.

Since no attempt was made to show the response of C. turcicus to single species of fungi isolated from the flour, it is not known, which of the six species of fungi was the most suitable for the growth and development of C. turcicus. This aspect of the insect-fungus relationship is worthy of further investigation.

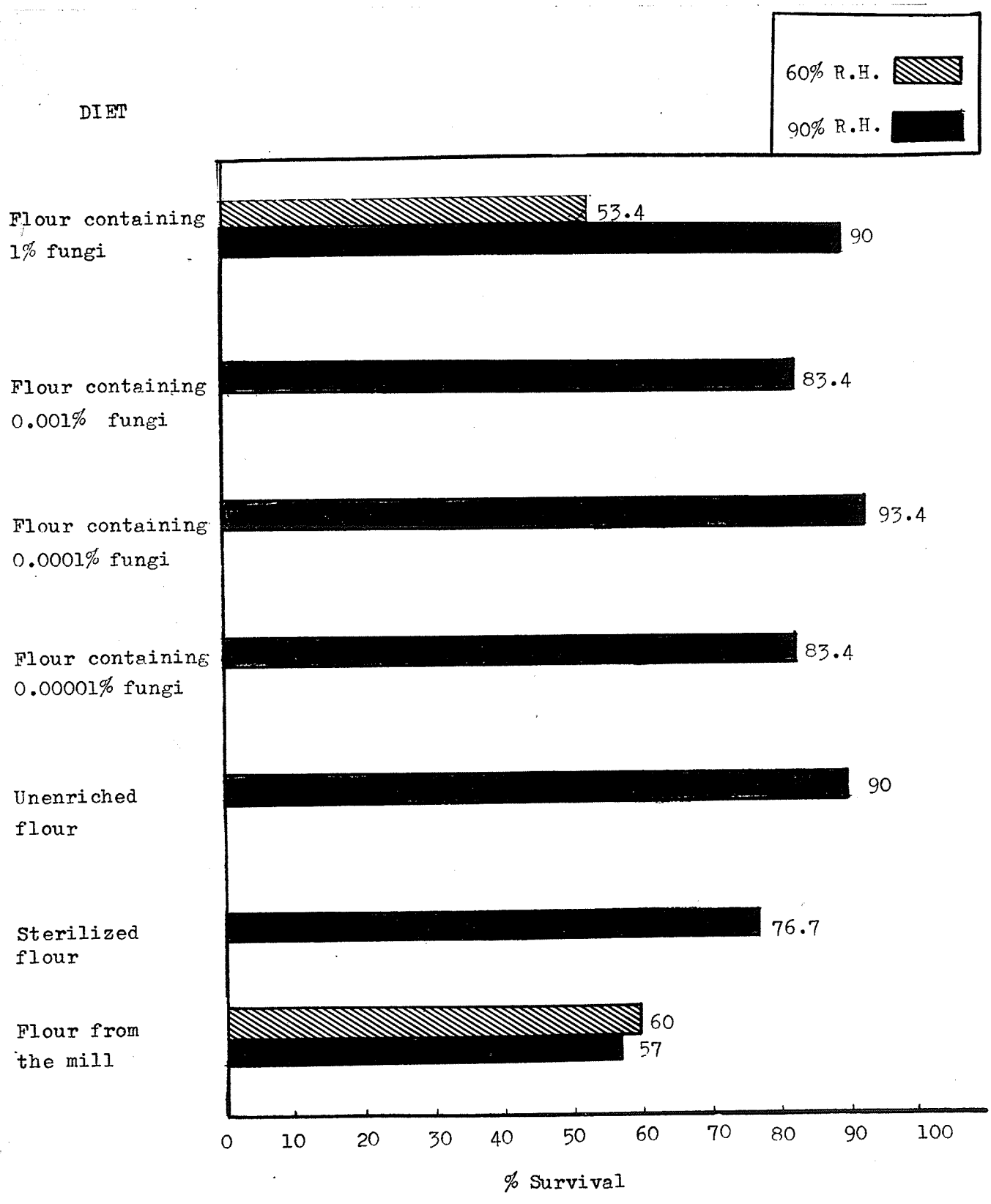


FIGURE 9. PER CENT SURVIVAL OF LARVAE OF Cryptolestes turcicus ON VARIOUS DIETS AT 28±1°C, 90% and 60% R.H.

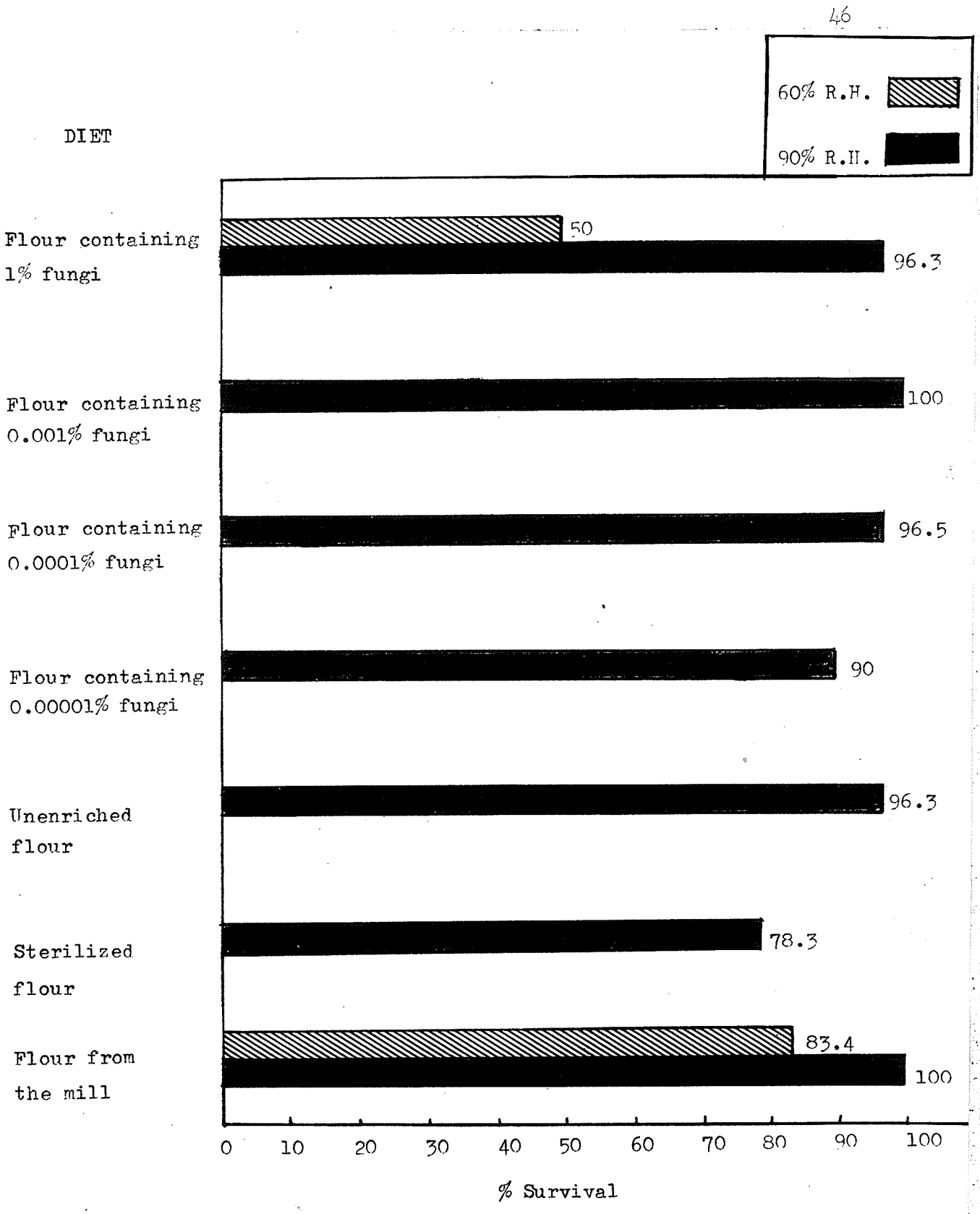


FIGURE 10. PER CENT SURVIVAL OF PUPAE OF Cryptolestes turcicus ON VARIOUS DIETS AT 28±1°C, 90% and 60% R.H.

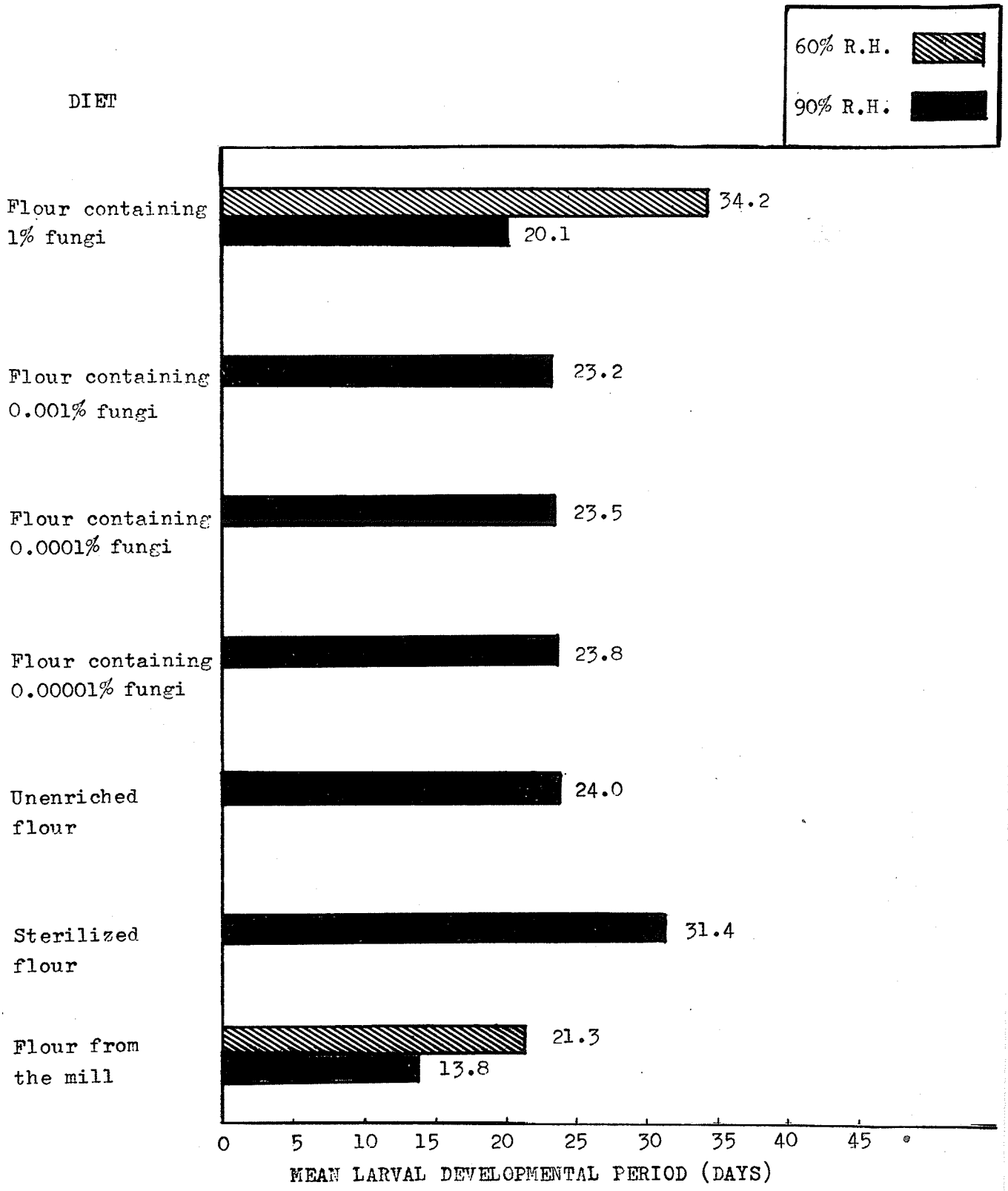


FIGURE 11. RATE OF LARVAL DEVELOPMENT OF Cryptolestes turcicus ON VARIOUS DIETS AT 28±1°C, 90% and 60% R.H.

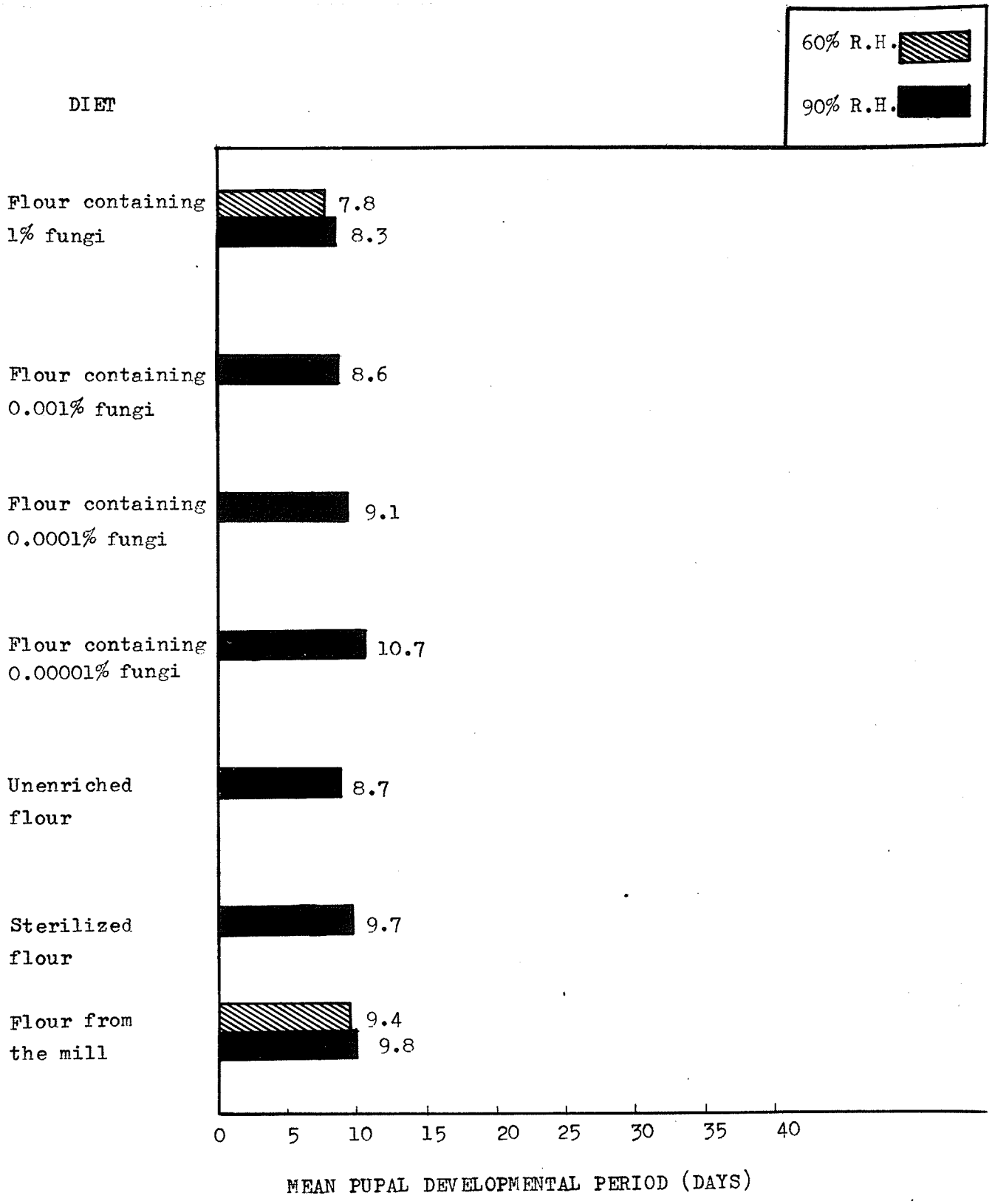


FIGURE 12. RATE OF PUPAL DEVELOPMENT OF Cryptolestes turcicus ON VARIOUS DIETS AT 28±1°C, 90% and 60% R.H.

CHAPTER V

SUMMARY AND CONCLUSIONS

1. The flat grain beetle, Cryptolestes turcicus (Grouvelle) is an important pest of flour mills in Canada and elsewhere in the world.
2. Direct feeding on stored products by C. turcicus is not so serious in itself but under conditions that favour the growth of certain fungi, this insect thrives and may cause deterioration of the infested stored products.
3. It has been commonly observed in flour mills in Canada that C. turcicus thrives in the presence of fungi associated with flour of high moisture content.
4. It was found that under the conditions of 28±1°C and 90% R.H., larvae of C. turcicus developed faster on prepared flour-fungi diets and on flour from a flour mill than on sterilized flour. The difference was highly significant at the one per cent level. The most rapid rate of larval development occurred on the flour from a flour mill and the slowest rate on sterilized flour. Larval survival was highest on the flour-fungi diets. The highest mortality occurred on the flour from the flour mill. The pupal period was about 9 days on all the diets studied. Thus, rate of pupal development was not affected by the presence of fungi in the flour. Survival of pupae was higher on the flour-fungi diets than

on the sterilized flour. Approximately 98% of the pupae survived on the former and about 78% on the latter diet.

5. At 60% R.H. and 28$\pm$1°C, some larvae of C. turcicus survived and completed their development only on the flour-fungi diet containing 1% by weight of fungi and on the flour from the flour mill.

6. Generally, the flour-fungi diet containing 1% of fungi was the most favourable diet for the survival and development of C. turcicus.

In conclusion, the presence of fungi associated with flour accelerated the rate of development and increased survival of C. turcicus. Humidity was also important since the insects were unable to grow at 60% R.H. Humidity may be important not only to satisfy the moisture requirements of C. turcicus but also to induce the growth of certain fungi which in turn may contribute some essential nutrients that favour rapid growth and development of this insect species.

LITERATURE CITED

Abdel-Rahman, H. A., C. M. Christensen, and A. C. Hodson. 1969. The relationship between Plodia interpunctella (Hb.) (Lepidoptera, Phycitidae) and stored-grain fungi. J. Stored Prod. Res. 4: 331-337.

Agrawal, N. S., C. M. Christensen, and A. C. Hodson. 1957. Grain storage fungi associated with the granary weevil. J. Econ. Ent. 50: 659-663.

Armolik, N., J. G. Dickson, and A. D. Dickson. 1956. Deterioration of barley in storage by microorganisms. Phytopath. 46: 457-461.

Ashby, K. R. 1961. The population dynamica of C. ferrugineus (Steph.) (Col., Cucujidae) in flour and on Manitoba wheat. Bull. Ent. Res. 52: 365-379.

Ayerst, G. 1966. The influence of physical factors on deterioration by moulds, in Microbiological Deterioration in the Tropics. Soc. Chem. Ind. Monogr. 23: 14-20.

Barlow, J. S., and H. L. House. 1956. Ethylene oxide for sterilizing diets. Science 123: 229.

Bishop, G. W. 1958. The taxonomy and bionomics of western Laemophloeus with special reference to the stored product species. Ph.D. thesis. State College of Washington University.

_____ 1959. The comparative bionomics of the American grain Cryptolestes (Col., Cucujidae) that infest stored grain. Ann. Ent. Soc. Amer. 52: 657-665.

_____ 1960. Taxonomic observations on the larvae of the three American Cryptolestes (Col., Cucujidae) that infest stored grain. Ann. Ent. Soc. Amer. 53: 8-11.

Burrell, N. J., J. H. J. Laundon, S. T. Hill, J. Emerson, and L. Hollingworth. 1965. Refrigerated damp grain storage. Laboratory tests. Pest. Infest Res. 1965. 17-19.

Butt, D. J. 1966. The microbiological deterioration of Uganda dry processed Robusta coffee, in Microbiological Deterioration in the Tropics. Soc. Chem. Ind. Monogr. No. 23, pp. 80-97.

Christensen, C. M. 1946. The quantitative determination of moulds in flour. Cereal Chem. 23: 322-329.

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

Christensen, C. M., and M. Cohen. 1950. Numbers, kinds and source of moulds in flour. Cereal Chem. 27: 178-185.

Christensen, C. M., and A. C. Hodson. 1960. Development of granary weevils and storage fungi in columns of wheat-II. J. Econ. Ent. 53: 375-380.

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

Clarke, J. H. 1968. Fungi in stored products. Trop. Stored Prod. Inf. 15: 3-14.

Clarke, J. H., E. V. Niles, and S. T. Hill. 1967. Ecology of the microflora of moist barley. Barley in 'sealed' silos on farms. Pest Infest. Res. 1966. pp. 15-16.

Coursey, D. G. 1966. Biodeteriorative processess in palm oil stored in West Africa, in Microbiological Deterioration in the Tropics. Soc. Chem. Ind. Monogr. 23: 44-56.

Davies, R. G. 1949. The biology of Laemophloeus minutus Oliv. (Col., Cucujidae). Bull. Ent. Res. 40: 63-82.

Dyte, C. E. 1961. A study of the development of beetle infestation in flour-milling machinery. Ann. Appl. Biol. 49: 378.

Evlakhova, A. A. 1953. The application of the microbiological method in the control of the noxious little tortoise. (In Russian). Dokl. vseoyuz. Akad. sel.-khoz. Nauk Lenina 18(3): 36-39. Moscow. (From author's abstract in Rev. Appl. Ent. A. 41(A): 428-428, 1953).

Griffiths, D. A., A. C. Hodson, and C. M. Christensen. 1959. Grain storage fungi associated with mites. J. Econ. Ent. 52: 514-518.

Hansen, H. N., and W. C. Snyder. 1947. Gaseous sterilization of biological materials for use as culture media. Phytopath. 37: 369-371.

Howe, R. W. 1965. A summary of estimates of optimal and minimum conditions for population increase of some stored products insects. J. Stored Prod. Res. 1(2): 177-184.

Howe, R. W., and L. P. Lefkovitch. 1957. The distribution of the storage species of Cryptolestes (Col., Cucujidae). Bull. Ent. Res. 48: 795-809.

Hurlock, E. T. 1963. The infestation of Canadian produce inspected in United Kingdom ports between 1953 and 1959. Can. Ent. 95: 1263-84.

_____ 1964. Infestation of foodstuffs from the United States of America inspected in the U.K. between 1953 and 1961. Bull. Ent. Res. 53: 173-192.

Kodaira, Y. 1961. Toxic substances to insects, produced by Aspergillus ochraceus and Copsra destructor. Agr. Biol. Chem. 25: 261-262.

Lefkovitch, L. P. 1957. The biology of Cryptolestes ugandae Steel and Howe (Col., Cucujidae). A pest of stored products in Africa. Proc. Zool. Soc. Lond. 128: 419-429.

_____ 1962a. The biology of Cryptolestes turcicus (Grouvelle) (Col., Cucujidae). A pest of stored and processed cereals. Proc. Zool. Soc. Lond. 138: 23-35.

_____ 1962b. Food quantity and density effects in pre-adult Cryptolestes turcicus (Grouvelle) (Col. Cucujidae). Proc. Zool. Soc. Lond. 138: 37-47.

Lindgren, D. L. 1935. The respiration of insects in relation to the heating and fumigation of grain. Univ. Minnesota Agr. Sta. Tech. Bull. 109. 32pp.

Liscombe, E. A. R. 1964a. Stored-product insect surveys in Canada. Proc. Ent. Soc. Manitoba. 20: 12-18.

_____ 1964b. An investigation of the effects of milling fraction of bread wheat on the biology of two Cucujids Cryptolestes turcicus (Gr.) and C. ferrugineus (Steph.) Ph.D. thesis, University of Manitoba.

Loschiavo, S. R., and R. N. Sinha. 1966. Feeding, oviposition, and aggregation by the rusty grain beetle, Cryptolestes ferrugineus (Col., Cucujidae) on seed-borne fungi. Ann. Ent. Soc. Amer. 59: 578-585.

Lucas, C. E., and T. A. Oxley. 1945. A study of an infestation by Laemophloeus spp. (Col., Cucujidae) in bulk wheat. Ann. Appl. Biol. 33: 289-293.

Misra, C. P., C. M. Christensen, and A. C. Hodson. 1961. Angoumois grain moth, Sitotroza cereallella, and storage fungi. J. Econ. Ent. 54: 1032-33.

Panasenko, V. T. 1967. Ecology of microfungi. Bot. Rev. 33(3): 189-215.

Raper, K. B., and D. I. Fennell. 1965. The genus Aspergillus. Baltimore: Williams & Wilkins. 686pp.

Raper, K. B., and C. Thom. 1949. Manual of the Penicillia. Baltimore: Williams & Wilkins. 875pp.

Rilett, R. O. 1949. The biology of Laemophloeus ferrugineus (Steph.) Can. J. Res. D. 27: 112-48.

Sikorowski, P. P. 1964. Interrelation of fungi and insects to deterioration of stored grains. Wash. State Univ. Agr. Exp. Sta. Tech. Bull. 42, 35pp.

Sinha, R. N. 1961. Insects and mites associated with hot spots in farm stored grain. Can. Ent. 93: 609-621.

_____ 1964. Ecological relationships of stored-products mites and seed-borne fungi. Acarologia 6: 372-89.

_____ 1965a. Insects associated with stored products in Canada. Can. Insect Pest Rev. Supplement 2, Can. Dep. Agr. Res. Branch.

_____ 1965b. Development of Cryptolestes ferrugineus and Oryzaephilus mercator on seed-borne fungi. Ent. Exp. Appl. 8: 309-13.

_____ 1966. Development and mortality of Tribolium castaneum and T. confusum on seed-borne fungi. Ann. Ent. Soc. Am. 59: 192-201.

Smith, L. B. 1965. The intrinsic rate of natural increase of Cryptolestes ferrugineus (Stephens) (Col., Cucujidae). J. Stored Res. 1: 35-49.

Solomon, M. E. 1951. Control of humidity with potassium hydroxide, sulphuric acid, or other solutions. Bull. Ent. Res. 42: 543-54.

Van Wyk, J. H., A. C. Hodson, and C. M. Christensen. 1959. Microflora associated with the confused flour beetle, Tribolium confusum. Ann. Ent. Soc. Am. 52: 452-463.

Wallace, H. A. H., and R. N. Sinha. 1962. Fungi associated with hot spots in farm stored grain. Can. J. Plant Sci. 42: 130-141.

Woodroffe, G. E. 1962. The status of the foreign grain beetle, Ahasverus advara (Waltl) (Col., Silvanidae), as a pest of stored products. Bull. Ent. Res. 53: 537-540.