March 9, 1966

To the Graduate Council:

I am submitting herewith a dissertation written by Turuvekere Somaiah Thontadarya entitled "Influence of Temperature and Humidity on the Longevity of the Spores of the Entomogenous Fungus, Beauveria bassiana (Bals.) Vuill., (Fungi Imperfecti), and the Potentialities of Utilizing This Fungus for the Control of Some Coleopterous Stored Grain Pests." I recommend that it be accepted in partial fulfillment of the requirements for the degree Doctor of Philosophy, with a major in Entomology.

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INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE SPORES OF THE ENTOMOGENOUS FUNGUS, BEAUVERIA BASSIANA (BALS.) VUILL., (FUNGI IMPERFECTI), AND THE POTENTIALITIES OF UTILIZING THIS FUNGUS FOR THE CONTROL OF SOME COLEOPTEROUS STORED GRAIN PESTS

A Dissertation
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fullfillment
of the Requirements for the Degree
Doctor of Philosophy

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by

Turuvekere Somiah Thontadarya

March 1966

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SECTION I

INTRODUCTION

Of the three principal biological agents that are employed in the control of insects pests; namely, parasites, predators, and pathogens, use of pathogenic microorganisms has made very rapid progress within recent years. However, microbial control is not new. Considerable natural control is microbial. It is only an increasing appreciation of the role played by insect pathogens in nature, with some of its advantages over other methods of control, including chemical control, that have given an impetus to this method. As Tanada (1959) observes,

> Microbial control is of special value when other methods have been found inadequate, for example, when insecticidal residues are toxic to man, insect parasites, and predators.

Some of the spectacular successes achieved by the use of pathogens to control forest tree and field crop insect pests by Canada and the United States, in addition to other countries, have contributed to the use of microbial control. Polyhedrosis virus has been used very successfully to control the European spruce sawfly, Diprion hercyniae (Htg.), and the European pine sawfly, Neodiprion sertifer, in both Canada and the United States. As expressed by Bergold (1958),

> As an aftermath of these successes, the virus has gained wide usage in Canada and the United States for control of the European pine sawfly, and replacing many other methods of control.

Another successful attempt made to obtain microbial control of an insect on a field crop was the use of polyhedrosis virus for the control of the alfalfa caterpillar in California (Hall, 1964).

Bacteria have given spectacular results in the control of some of the lepidopterous pests of field crops in the United States and in other countries. As Hall (1964) states,

> The discovery of Bacillus popilliae Dutky and B. lentimorbus Dutky, the causative agents of types A and B milky diseases in the Japanese beetle, Popillia japanica Newn., and the development of techniques for their use beginning in about 1940, gave added encouragement for the utilization of bacteria in the control of insects. Successful utilization of another spore-forming, crystal-bearing bacterium, Bacillus thuringiensis Berl. var. thuringiensis was achieved a few years back and at present the bacterium is produced on a commercial scale in the United States and supplied to the farmers under trade names. In fact commercial firms which are producing the insecticidal products containing the bacillus, are distributing the product throughout the world for wide-scale testing against susceptible insects.

With regard to entomogenous fungi, the greatest success has been achieved by introduction or colonization of effective pathogens in new areas where susceptible host populations occur. Among the pathogenic fungi that have proved effective and have been introduced to new areas are some species of Entomophthoreceae, and Beauveria bassiana in the group Fungi Imperfecti. However, other pathogens, including those that are in use, have not been extensively utilized, as noted by Tanada (1959), ". . . Mainly because of difficulties inherent in their use or because of their extreme dependence on environment."

The part played by environmental factors, particularly

temperature and humidity, varies with the kind of insect pathogens that are used, their mode of entry into the body of the host, and their mode of action on the host. For example, temperature appears to have a serious effect on the survival of some of the pathogens when they are outside the host body. Viruses have been known to infect better in warmer climates than cold climates, with retardation of activity under low temperatures (Hall 1963). Moreover, fungi and nematodes are known to be influenced by the humidity of the environment. As Steinhaus (1964) opines, "Humidity is the most frequently cited physical factor affecting the initiation and development of eipzootics among insect populations." This has been demonstrated by Hart and MacLeod (1955), among other workers, in the case of the white muscardine fungus, B. bassiana where they noticed optimum germination of spores occuring at relative humidities above ninety-four per cent and at a temperature of 28° C., but with negligible germination at 10°, 38°, and 44° C. Schaefer (1936) did observe the infection of the red locust, Nomadacris septemfasciata (Serv.), with the fungus Entomophthora grylli Fres., at relative humidities below sixty per cent. In the case of bacteria and viruses, humidity seems to have very little effect, which may be due to their method of entry, i.e., infection is through ingestion. On the other hand, in 1940, White observed the lessened effectiveness of type A milky disease organism, Bacillus popilliae Dutky, on the Japanese beetle, when weather conditions were neither extremely wet nor completely dry. Humidity

has been found to have no effect in cases of polyhedrosis viruses, and nematodes are found to be dependent on high humidity and moisture at moderate temperatures (Steinhaus 1964).

After considerable research on some of the entomogenous microorganisms, a few have shown promising results, and have been used for control of either forest insect pests or field crop pests in different parts of the world. Very little attempt has been made to apply microbial control to suppress the insects that attack stored grains and legumes, except for the works of Steinhaus and Bell (1953) and Dunn and Mechalas (1963). Very few additional reports are found in the literature on the infection of the stored grain insects.

Control of stored grain pests is still a problem in spite of modern methods of storage. Grains are frequently either exposed to hot sun or dried to bring the level of moisture below the critical point (eight per cent) or they are subjected to severe cold. Fumigant treatment is also used. All of these methods will kill or suppress the insect pests. Approximately five to six per cent of the stored food grains and legumes in the whole world may be completely spoiled by insects alone. To cite an example of an annual loss due to insects and other pests, the United States has incurred an annual loss of $1,042,063,000 during the years 1951-1960, according to a report published by the United States Department of Agriculture in August 1965 (Agricultural Handbook No. 291). The other part of the story is the effect of consuming

grains treated with insecticides directly, which needs serious consideration in view of the health hazards to both man and animals. Under these circumstances there seems to be a need to find a safe and effective way of controlling stored grain pests.

Work of Dunn and Mechalas (1963) with *B. bassiana* and some of the stored grain insects has given an indication concerning the potentialities of infectivity of stored grain insects by *Beauveria bassiana*.

In this investigation, the effect of different temperatures and humidities on the longevity of the spores of two strains of the fungus was studied. The longevity of two host insects, namely *Sitophilus oryzae* (Linn.), and *Callosobruchus maculatus* (Fabr.), was also studied, with a view to estimate the mortality rate under different conditions and to select suitable temperature and humidity conditions, under which the insects would survive for a long period. Both of these investigations are presented in Section II.

During the course of these investigations, an attempt was also made to determine the potentialities of utilizing the spores of this fungus to control the two species of coleopterous stored grain pests, by treating food grains with different concentrations of the pathogen. As a prerequisite, studies were made to find out the pathogenicity of the fungus at different concentrations under suitable conditions of temperature and humidity. Both of these investigations are presented in Section III.

SECTION II

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE SPORES OF THE ENTOMOGENOUS FUNGUS, *BEAUVERIA BASSIANA* (BALS.) VUILL., (FUNGI IMPERFECTI)

I. REVIEW OF LITERATURE

B. bassiana belongs to the group Hyphomycetes (Deuteromycetes) of the Fungi Imperfecti, and is responsible for causing the so called muscardine disease in the silkworm, *Bombyx mori* (Linn.). Originally, its generic name was *Botrytis*, named by Balsamo who gave the specific name as *bassiana*, in honor of Bassi, who first identified this fungus on silkworm as the causative organism of muscardine disease. Subsequently, in 1911, Beauverie studied the organism in comparison with another closely related species, *Botrytis effusa*, which was also found on silkworms and expressed the importance of treating it as a separate genus. Vuillemin (1912) implemented his wish by creating a new genus and named it *Beauveria* in honor of Beauverie and treated the species *bassiana* as the type. Thereafter, the name *Beauveria bassiana* (Bals.) Vuill., was employed instead of *Botrytis bassiana* (Steinhaus 1949).

Prior to 1954, there were fourteen species listed under the genus *Beauveria*. MacLeod then revised and reduced the number

of species to two, namely B. tenella and B. bassiana. He treated the species B. stephanoderis, B. globulifera, B. effusa, B. vexans, B. doryphorae, and B. acridiorum as synonyms of B. bassiana.

Influence of Different Temperatures and Humidities on the Germination and Infectivity of the Spores of B. bassiana

Spore germination involves change from relative inactivity to an active growth condition. This is accompanied in most spore-forming fungi by the formation of a germ tube which continues to elongate and ultimately leads to the development of a vegetative body and a fruiting body. The fruiting body frequently develops after the death of the host in the case of pathogenic forms. The influence of environment on ther germination and infectivity of the pathogen is complex. Further, the environmental factors which control the percentage and speed of germination and the rate of vegetative growth of the pathogen also differ. Optimum temperature and humidity are very important factors as are adequate oxygen and a suitable pH. Different species of fungi have different requirements for germination and infection of spores. Moreover, not all spores respond with exact uniformity to a given factor, but preserve a certain individuality in relation to it.

Regarding the influence of temperature and humidity on the germination and infection of the spores of B. bassiana, the literature seems to be scanty. However, the importance of climate, particularly the humidity of the microclimate for the infection of

entomogenous fungi was recognized in 1896 when Snow studied the control of chinch bug, Blissus leucopterus, by B. bassiana. Metalnikov and Toumanoff (1928) observed the infection of the larvae of Ostrinia nubilalis (Hübner) (= Pyrausta nubilalis Hübner) by B. bassiana and B. globulifera at a relative humidity of about forty-six per cent.

Rozypal (1930) noticed infestation of the sugar beet weevil, Bothynoderes punctiventris, by various microorganisms of which B. bassiana was considered the most important. The author opines that during periods of inaction of the intestinal tract which occurs commonly on chilly days, the fungal spores germinate and thus infection occurs.

Steyaert (1934 and 1935), who worked on the natural parasitism of B. stephanoderis and B. bassiana on the coffee berry borer, Stephanoderes hampi, in a coffee plantation, reports that humidity in excess of eighty per cent increases the effectiveness of B. stephanoderis, and a relative humidity of seventy-nine per cent seems to be approximately optimum for parasitism by B. bassiana. In 1938, Gösswald demonstrated the infection of ants by B. bassiana at lower humidities, when the spores were ingested by the insects.

Müller-Kögler (1942) observed the rate of increase in germination with increase in humidity, while studying the effect of humidity on the germination of spores of B. bassiana. According to his report, infested larvae of the pine moth, Bupalus piniarius,

were found dying more rapidly at very high and at very low air humidities and more slowly at intermediate humidities. He also observed an increase in the rate of germination of spores at high humidity, and a reduction in the resistance of the insect at low humidity. Furthermore, the mortality of infected and control larvae seemed to increase with increase in temperature. Successful infection of the codling moth, Carpocapsa pomonella (Linn.) by B. bassiana was observed under low humidity, by Ferreira (1943), when the insects and their plants were dusted with spores.

Schaerffenberg (1947) observed germination of spores of B. bassiana, when ingested by Colorado potato beetles. York (1958) and Ferreira (1943) found similar results with the European corn borer, Ostrinia nubilalis, when infected by B. bassiana, in the open dry air. Apparently the spores were ingested by the caterpillars along with the food. This indicated that the spores of B. bassiana can also infect hosts by ingestion, as is the case with bacteria and viruses. It also indicated the suitability of the digestive tract for the germination of spores.

Germination and development of the spores of B. bassiana, within the temperature ranges of 0° to 40° C., has been reported by Schaerffenberg (1957). Therefore, within limits, Beauveria spp., may be somewhat independent of temperature. Schaerffenberg noticed a decline in the infectivity of the larvae of Leptinotarsa decemlineata by B. bassiana and B. densa at temperatures below 6° and 10° C. respectively. Müller-Kögler (1942) made similar

observations in the case of Bupalus piniarius larvae, where B. bassiana failed to infect at 5° C. but did so slowly at 8° C.

Dresner (1949) who worked on mass production of spores of B. bassiana and on its host range and practicability of field control, has reported a low rate of spore production at high relative humidity, which favored quick germination and rapid vegetative growth of the fungus. According to him, the ideal conditions for spore production were found to be a very moist but not wet medium with low humidity maintained after germination. In laboratory tests, most of the insects which he tried, including some stored product insects, were found to be susceptible when they were dusted or sprayed with the spores of the fungus. With respect to stored product insects, heavy spore concentrations at high humidities and normal temperatures were found effective. In consideration of results obtained from both laboratory and field tests, he drew the following conclusions:

> The main limiting ecological factor in the use of artificially spread fungus spores was atmospheric humidity. The best form of moisture was rainfall, an optimum period of about one-half hour being sufficient to cause infection of insects. This optimum humidity was present at the time of the observations reported here and is common each summer in the lower New York area. However, humidity alone is not sufficient to insure natural control of insects. It is believed that entomogenous fungi can be depended upon to control insect pest infestations in the frequent rainfall and humidity areas of the Atlantic Coast of the United States. Areas outside of this weather pattern should not depend on entomogenous fungi for the control of the pest populations. If the exacting requirements for entomogenous fungi are met, an inexpensive, long-lasting control is likely to result.

Some additional workers who have studied these ecological factors are Schneider (1953) and Hsu et al. (1959). Schneider reported that a relative humidity of ninety-two per cent was a limiting factor in the germination of the spores of B. bassiana and B. septori. Hsu et al. reported germination and infection of B. bassiana at temperatures of 21° and 28° C. and at a relative humidity of ninety-five per cent.

Hart and MacLeod (1955) also studied the effects of temperature and humidity on the germination of the spores of B. bassiana. They devised an apparatus for such testing, and their observations showed that optimum germination of the spores occurred at relative humidities above ninety-four per cent and at a temperature of 28° C. It was slightly less at 25° C. and there was negligible germination at 10°, 38° and 44° C. with relative humidity above ninety-four per cent. At ninty-four per cent relative humidity germination was very low. But, as the humidity increased above this value, there was a corresponding increase in germination at 25° and 28° C.

Dunn and Mechalas (1963) got results similar to those observed by Dresner (1949) regarding the infection of B. bassiana, on some of the stored grain insects with reference to humidity and temperature. These workers also studied the infection of both Tribolium confusum and Sitophilus granarius at 26° C. and sixty-five to seventy per cent relative humidity. In addition to these insects, Dunn and Mechalas observed a one hundred per cent

kill in the case of lygus bugs, Lygus hesperus Knight, when they were held at forty and seventy per cent relative humidity following treatment with 0.5 per cent B. bassiana spore dust at 75° to 85° F. (about 24° to 29.4° C).

Influence of Different Temperatures and Humidities on the Longevity of the Spores of B. bassiana

The epidemic potential of a pathogen is dependent not only on its infective capacity, but also on dispersal, speed of germination, speed of sporulation or multiplication, and the biological fitness of infective stages. Unfavorable temperatures and humidities may interfere with the metabolic activity of the spores and result in shortening of life. Hence, studies made on the influence of temperature and humidity on the longevity, or viability of spores of the entomogenous fungus, B. bassiana are reviewed in the following paragraphs.

Humidity plays a more important role in the longevity of fungal spores than does temperature. High humidity is favorable to the germination of spores, but not to the biological life span of spores. Therefore, conservation of spores depends upon a very dry environment. Except when exposed to high temperature for a long period, temperature seems to have very little influence on the survival of spores. This fact was established by Lambrat (1903), and Schaerffenberg (1959 and 1964), in the case of B. bassiana. Spores of this fungus were able to produce a complete

infection, even after three years, when they were stored at low temperatures. However, according to Teng (1962), spores of this fungus failed to germinate after subjection to desiccation for eighteen days.

Headlee and McColloch (1913) reported a survival time of several hours for the spores of B. globulifera, even after exposure to -18° F. whether dry or wet. Furthermore, the spores which were exposed to changing temperatures occurring between January 5 to February 12 were not injured. The minimum was 15° F. and the daily mean was below 32° F. for a period of nine days. Kral and Neubaur (1956) have been able to store the spores of B. bassiana for five hundred days, in bottles at room temperature, without any effect on viability.

Headlee and McColloch (1913) have studied the effect of temperature and humidity on the longevity of the spores of B. globulifera also. As reported by them, spores exposed to 104° to 105° F. in a saturated atmosphere for twenty-four hours were not injured; those exposed for forty-eight hours were severely injured. Spores that were allowed to germinate and develop for forty-eight hours and then transferred to a temperature of 105° F. perished. Furthermore, spores which were exposed to a temperature of 205° F. for five hours under a dry atmosphere were not injured if they were subsequently exposed to normal temperature and humidity.

Influence of temperature on the longevity and subsequently on the germination of the spores has been reported by Steinhaus

(1960) in the case of B. bassiana. Spores stored at a temperature of 4° C. for one hundred and twenty-eight weeks were able to germinate; those stored at 23° C. lost their germinating power after six to thirteen weeks and after four to seven weeks at 38° C. Madelin (1963) states that low temperatures and low humidities are more conducive to long survival of the spores of fungi than are higher temperatures and humidities.

In 1965, Clerk and Madelin studied the effect of temperature and humidity on the survival of spores of B. bassiana, in addition to Paecilomyces farinosus and Metarhizium anisopliae which are also entomogenous hyphomycetes. They found that the longevity of spores of B. bassiana decreased as the temperature of storage was increased from 8° to 25° C. Furthermore, the spores lost the ability to germinate sooner at the higher relative humidity of 34.3 and 75.2 per cent than at 0.0 per cent.

The findings of workers on the longevity of the spores of B. bassiana show that higher humidities have deleterious effects whether the temperature is high or low. Spores seem to remain viable longer under dry conditions even at higher temperature. However, further investigation seems to be necessary to discover what would be the effect of different combinations of temperature and humidity that may exist in the environment (both in the field and stores) on the longevity of the spores, and also on the host insects which may yield to the infection, before attempting to utilize the fungus for microbial control. Furthermore, response

of strains of the fungus to the ecological factors and to infection of different hosts needs early attention of workers, as different strains show variations in their pathogenicity and distribution and host-specificity.

II. MATERIALS AND METHODS

B. bassiana is cosmopolitan in distribution. It is known to infect both useful and destructive insects. In view of its importance, many countries have made provisions to maintain cultures of the fungus grown on suitable artificial media for further investigations. Such a source was made use of in obtaining material for this work. In addition to this source, the culture which is used for the commercial production of the biocide of this fungus was also included in the investigation. The sources are:

No. 307	June 1964	Dr. D. M. Macleod, Insect Pathology Research Institute, Department of Forestry, Sault Ste. Ontario, Canada
NRR No. 3108	Sept.1964	United States Department of Agriculture, Agricultural Research Service, Northern Utilization Research and Development Division, Peoria 5, Illinois
No. 4007	Aug. 1964	Nutrilite Products, Inc., Buena* Park, California

*This strain was isolated and cultured from the commercial product received from Nutrilite Products Inc., California.

Out of these three strains of Beauveria, No. 307 was discontinued after some preliminary tests, as it was found inconvenient to harvest the spores.

Host insects, namely, the rice weevil, Sitophilus oryzae (Linn.) (Curculionidiae; Coleoptera), and the cowpea (bean) weevil, Callosobruchus maculatus (Fab.) were obtained from the Division of Entomology, College of Agriculture, The University of Tennessee, Knoxville.

Culture and Collection of Spores of the Fungus

Success in the large scale production of virulent spores of entomogenous fungi depends on providing a suitable environment including that of a suitable substrate for the growth and development of the fungus. Martignoni (1964) has given an excellent review on the suitable substrates used in the mass production of insect pathogens. Corn meal mush medium has been in use since Forbes' time (1895) for the production of the spores of B. bassiana (= B. globulifera), but the most frequently used material has been bran (Dresner 1949; York 1958; McCoy and Conver 1941). MacLeod (1954) carried out some nutritional studies on the fungus and Sabouraud Maltose agar was considered best, followed by Molish medium, blood agar base, Raulin-Thom and Czapeak-Dox media, potato dextrose, and corn meal agar, as suitable media for high production of spores. In consideration of the requirement of high spore production for the present investigation, Saubouraud Maltose agar was found preferable and the dehydrated Bacto Sabouruad

Maltose agar (0110-010) manufactured by Difco Laboratories, Detroit, Michigan, was used throughout. The medium was prepared according to instruction supplied by the manufacturer.

Nucleus cultures were maintained on test tube slants. Sterile Pyrex glass Petri dishes (both three and nine inches in diameter) and rectangular jars with lids (two-quart covered loaf dish) and rectangular aluminium pans with lids (15 x 10 x 3") were employed for large scale production of spores. Sufficient care was taken to prevent contamination via any other organisms by using sterile techniques at every stage during the course of the investigations. Sterile needles or sterile brushes were used for inoculation. Throughout the investigation, cultures were grown in a room which was maintained at a temperature of about 23 $\pm$ 1° C. and a relative humidity of thirty to fifty per cent.

Collection of spores. Collection of spores from containers was done by sterile needles, camel's hair brushes, or small sterile metal spatulas. The spores were stored in bottles with screw-type, air-tight lids and stored under the same conditions of temperature and humidity. Separate needles or camel's hair brushes were used for each strain. Spores six to eight weeks of age were used for all the experiments. Prior to the collection of spores from the containers, the lids were kept open for quick drying under room temperature. Care was taken to avoid scraping the substrate.

Breeding of the Host Insects

Callosobruchus maculatus (Fab.) (Coleoptera: Mylabridae) is commonly called the "cowpea weevil." It normally breeds on dried beans in grocery stores throughout the year. The life cycle takes about three weeks under normal conditions. The female glues her eggs on the seeds, and the new adults emerge after three weeks. The other stages can be seen only by breaking open the beans or when they accidentally fall from the grains in heavy infestations. The experimental insects were bred on beans (Blackeye Peas) in both glass and plastic wide-mouthed jars covered with thin cheese cloth.

Sitophilus oryzae (Linn.) (Coleoptera: Curculionidae), a serious pest of cereals, is commonly called the "rice weevil." It can be reared easily on sweet corn, rice, or wheat in the laboratory throughout the year. Each generation requires about four to five weeks with room temperatures of 23° to 25° C. and relative humidities of thirty to fifty per cent. Wheat varieties were obtained from the Division of Agronomy, College of Agriculture, The University of Tennessee, Knoxville. They were Knox. 407 (Redcoat) and Knox. 408 (Monon). Cheese-cloth covered, wide-mouthed glass jars were used for mass culture rearing.

Maintenance of Required Temperatures and Humidities

The following temperatures and humidities were selected for the investigation:

Temperatures: 5°, 20°, 25°, 30°, 35°, 40°, and $50^{\circ} \pm 0.5$ -1° C.

Humidities: Less than one, thirty to thirty-five, seventy to seventy-six, and ninety-two to 96.5 per cent. (All relative humidities were used with each temperature).

Of the seven temperatures employed, 20°, 40°, and 50° C. were maintained in regular incubators manufactured by the General Electric Company, and were accurate to $\pm 0.5^{\circ}$ C. Wooden cabinets containing fixed thermostats were used for the temperatures of 30° and 35° C. These were accurate to $\pm 1^{\circ}$ C. The cabinets were located in an air conditioned room which was maintained at a temperature of $25 \pm 1^{\circ}$ C. This room was used for 25° C. A cold room that was maintained at 5° C. $\pm 1^{\circ}$ C. was used for 5° C.

As listed above, four combinations of relative humidities were employed. The respective relative humidities were maintained in air-tight one gallon bottle-type desiccators, or in Bethlehem* dri-jars. To maintain less than one per cent relative humidity, phosphorus pentoxide was used, and for the remaining relative humidities, saturated solutions of suitable salts (100 ml.) worked out by Winston and Donald (1960) were used. The relative humidities maintained under each temperature are given in Table I.

Wherever wide-mouthed, air-tight one-gallon bottles were

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*Bethlehem Apparatus Company, Inc., Hellentown, Pennsylvania.

TABLE I

DIFFERENT RELATIVE HUMIDITIES MAINTAINED AT EACH TEMPERATURE TO STUDY THE LONGEVITY OF THE SPORES OF THE FUNGUS, *BEAUVERIA BASSIANA*

Temperature in ^{o} C.	Relative Humidities in Per Cent			
5°	<1.0	34.5	75.0	96.5
20°	<1.0	33.0	76.0	96.5
25°	<1.0	30.5	74.0	92.5
30°	<1.0	32.5	72.5	93.5
35°	<1.0	32.5	71.0	96.0
40°	<1.0	32.0	70.5	93.0
50°	<1.0	31.5	72.0	92.5

used they were equipped with locally made stainless steel tripod stands to keep the experimental materials out of the salt solutions. The tripod stand was made to rest on a small glass jar of suitable height, so that a portion of it would remain above the level of the chemical solution used to maintain the required relative humidity. The inner wall of the containers of the saturated solution, the bottom portion of the tripod stands, and the exposed portion of the glass jars upon which the metal stands rested were smeared with petroleum jelly to prevent the rise of the chemical solution. A time of one week was allowed for equilibration of the humidity inside the containers, under all temperatures, before starting the experiments.

Method Used to Determine Percentage Germination of Spores

Percentage germination of spores is one of the ways of studying the effect of temperature and humidity on the rate of their viability. All spores do not respond with exact uniformity to a given factor; they preserve a certain individuality in relation to it. Müller-Kögler (1960) and Steinhaus (1960) have adopted different methods for estimation of per cent germination. Steinhaus, who studied the effect of temperature on the viability of the spores of several strains, made counts of the germinated spores as follows.

Three different strains of *B. bassiana* were grown on potato-dextrose agar for three days, after which the spores were washed off with sterile distilled water containing a few drops of

spreader (Colloidal X-77) which gave a uniform suspension. The suspension was strained through a double thickness of cheese cloth, and a loopful was then spread on a sterile microscope slide and allowed to dry at room temperature. Such slides were stored in plastic slide boxes at scheduled temperatures. Counts of viable spores were made after certain intervals by taking a slide at random and suspending it in ten ml. of sterile saline, and transferring 0.1 ml. of the material to a potato-dextrose-agar plate. Approximately 500,000 spores were estimated to be on each slide.

The method followed by Müller-Kögler is also summarized below.

Ten mg. of spores of the fungus, B. bassiana, were mixed with a known quantity of sterile distilled water containing 0.1 per cent of a wetting agent. The mixture was strained through silk net No. 10 on a watch glass. This conidial suspension was transferred to microscopic slides covered with a thin layer of nutrient media. Only one drop was put on each slide and spread with a sterile glass rod. An area of nearly 26 x 45 mm. was covered by nutrient media on each slide. Dilution of the spore suspension for the strain used was so designed that five to ten spores occupied a microscopic field of 0.32 mm. diameter. This corresponded to about 3×10^6 spores per ml. Such slides were placed in Petri dishes provided with moist filter paper and kept at a room temperature of 20° to 22° C. Counts were taken at

intervals of twenty-four, forty-eight, and seventy-two hours. Microscopic slides covered with cover glasses were examined under a phase contrast microscope with bright illumination. Spore material was stained with Lactophenol-cotton blue three hour prior to examination. The magnification used was 10 x 40. Every spore that was present was counted. The spores showed interpretable germination after forty-eight hours, according to the worker.

In 1955, Hart and MacLeod devised an apparatus to distribute spores of this fungus on plain microscopic slides, to facilitate easy counting. Here also, spores were counted individually under the microscope, after exposing the slides to particular temperatures, with different relative humidities.

The method followed in the present investigation is entirely different from the methods of Hart and MacLeod and Steinhaus, but is somewhat similar to the technique followed by Müller-Kögler. The method follows.

Spores six to eight weeks of age were used to assess the rate of viability in all experiments. The percentage of viable spores was estimated before use in each experiment. To begin with, a required quantity of spores (one to two mg.) was put in small vials of 10 x 45 mm. These vials were loosely plugged with sterile cotton. Each strain was replicated four times. Therefore, eight vials were kept under each combination of relative humidity and temperature. After the lapse of a prescribed scheduled interval, germination percentage was estimated by the

following sampling process. A minute quantity of spores was taken from each vial with the aid of a sterile inoculating needle and transferred to a small Pyrex test tube (10 x 90 mm.) containing about fifteen drops (0.5 ml.) of sterile distilled water which contained 0.1 per cent strength of a spreading agent, Multi-film X77* (Steinhaus 1960). These test tubes were subjected to mechanical agitation for about thirty minutes after an initial lapse of fifteen minutes, to obtain good dispersion of the spores. In the meantime, Sabouraud Maltose agar medium was prepared and spread on sterile microscopic slides so as to cover two-thirds of the total slide area. This area was divided into four parts of 1 x 1/2 inches with the aid of a glass-cutter and numbered one, two, three, and four to facilitate transfer of the spore samples of the respective replications. A thin film of the medium was obtained by spreading with the same dropper tip which was used for coating the slides with the media. During these processes, the slides were kept inside sterile glass Petri dishes of nine inches in diameter. Microscopic slides were also marked with respective relative humidity percentages.

At this stage a drop of the spore suspension which was prepared as previously described was transferred to each part of the microscopic slide at the rate of four replications per slide. Therefore, each slide served for holding the samples (four) of

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*Colloidal Products Company, California.

each strain. In all cases the slides were placed in sterile glass Petri dishes, nine inches in diameter, the bottoms of which were provided with filter paper wetted with five ml. of sterile distilled water.

The dishes were kept at a room temperature of 23° to 25° C. After a lapse of twenty hours the slides were examined under a research microscope (American Optical Company), with a mangification of 20 x 45. Clear bright light was used. Both germinated and non-germinated spores that appeared within a microscopic field of 0.17 mm. were counted. An ocular square micrometer was used to facilitate counting the spores correctly. It was possible to count the spores (germinated and nongerminated) without staining. Preliminary tests indicated the need to make the first observation after an interval of twenty hours. Subsequent observations were made depending on the condition of the spores. Generally, viable spores were found swelling after fifteen to twenty hours, the length of time depending on the conditions to which they were exposed. However, slides were checked again even after observing the interpretable germination.

The same method was followed for testing the viability of spores before subjecting them to various combinations of temperature and humidity, and also while making viability tests after scheduled intervals, i. e., after exposing the spores to the respective conditions.

Method Adopted to Study the Longevity of the Host Insects Under Different Temperatures and Humidities in the Presence and Absence of Food

The experiments were conducted in plastic dishes of 35 x 95 mm. size with perforated lids. The same varieties of wheat and beans that were used for mass culture rearing were used here also. The quantity of grains used was about seventy-five grams. Observations were made once in three days up to a period of nine days. Dead insects were removed each time after counting.

Statistical Analysis

Throughout the study concerning the influence of temperature and humidity on the longevity of the spores of the fungus, a minimum of four replications was maintained, with a small quantity of spores. However, four counts were made by changing the position every time under each replication. The average of these four observations was used for each replication for analysis of the data. When studying the longevity of host insects under different temperatures and humidities, a minimum of three replications, with twenty insects selected at random irrespective of sex, in each replication, was maintained, with food and without food. Results obtained from these investigations have been analyzed statistically by following the method of "Analysis of Variance." Wherever there were more than two means and they were significant, Duncan's New Multiple Range Test was conducted to

compare them. The means which have been underscored by a common bar represent no significant difference between the means. Those that are underscored individually show a significant difference between the means.

III. RESULTS

Preliminary Observations

As previously mentioned, *B. bassiana* is one of the entomogenous fungi which can be cultured on artificial media. Both of the strains that have been used in the present investigation grow and sporulate very well on Sabouraud Maltose Agar. Temperatures of 23° to 25° C. with thirty to fifty per cent relative humidity seem to be optimum for the development and sporulation of both strains of the fungus. Sporulation appears to start after about seventy-two hours. It produces a creamy white flat powdery appearance on the surface of the culture. Hence, the spores are easy to collect either by brushing or with the aid of a spatula. As observed by MacLeod (1954) in other strains, coremial development is also seen from time to time in these strains. The shape of the spores of both strains is somewhat globose which could be seen under the dark field of a phase-contrast microscope with a magnification of 1000x. Though the surface color is creamy white in early days, a change in color to yellowish white is noticed after about ten to twelve weeks. Hence, spores which are collected when they are about three weeks old look quite white,

but after about eight weeks they look somewhat yellowish.

The number of spores per gram of each strain was estimated with the aid of a Hemocytometer. Ten milligrams of spores were diluted in 100 ml. of distilled water with a drop of spreading agent. The number of spores was found to be about 20.5×10^{10} for Strain 3108, and 21×10^{10} for Strain 4007.

Attempts to study the germination of spores with the aid of a Hemocytometer by diluting a known number of spores failed because most of the fungi require aerobic conditions for spore germination and development. Hence, the method explained under materials and methods of Section II was adopted.

With regard to time for germination of spores of Strain 3108, optimum germination occured after about twenty hours on the Sabouraud Maltose agar medium. Strain 4007 took twenty-two to twenty-three hours on the same medium. These materials were kept at a room temperature of 23° to 25° C. and a relative humidity of thirty to fifty per cent. Preliminary tests conducted at temperatures of 20° to 21°, 23° to 25°, 25° to 27°, and 30° C., to find the optimum temperature for germination and development and sporulation of both strains of the fungus showed that 23° to 25° C. is optimum for all the above activities. Light had no effect on germination and development. Therefore, production of spores and germination tests were carried out at 23° to 25° C.

<u>Longevity of the spores</u>. Observations on the longevity of

spores kept under room temperatures from June 1964 to May 1965 proved their ability to survive this duration in the laboratory. All three strains originally received from different sources (given under materials and methods in Section II) were tested for their viability. Even after transferring to the control room, which was maintained at 23° to 25° C., they were found viable when tested on December 6, 1965. At this time they were tested for the percentage of viability, though it was not done on the previous occasions. Percentage viability was as follows:

		Age of each (in months)
Strain No. 307	< 1.0 per cent	18
Strain No. 3108	73.8 per cent	15
Strain No. 4007	77.1 per cent	14

One important point to be considered may be the way in which the spores were stored. All the cultures were kept under dry conditions by plugging the tubes with sterilized cotton covered with cheese cloth. Above this there was paper tied in place with rubber bands.

IV. RESULTS OF EXPERIMENTS

Influence of Different Temperatures and Humidities on the Longevity of the Spores of the Two Strains of *B. bassiana*

Longevity of the spores of the two strains, 3108 and 4007, was studied at 50°, 40°, 35°, 30°, 25°, 20°, and 5° C. Different relative humidities, namely, less than 1.0, 30.5 to 34.5, 70.5 to

76.0 and 92.5 to 96.5 per cent, were maintained under each temperature with a difference of 0.5 to 3.5 per cent. Time intervals between observations to estimate the rate of viability of the spores varied with temperatures. It was once in three days at 50° and 40° C.; once a week at 35°, 30°, and 25° C.; and once a month at 20° and 5° C. Results obtained under each temperature with different relative humidities are discussed below. The effect of different temperatures with each percentage of relative humidity is discussed in the following paragraphs.

Influence of 50° C. With Different Relative Humidities

This experiment was conducted inside the incubator. The temperature was $50 \pm 1^{\circ}$ C. The relative humidities were maintained in desiccators and bottles as mentioned under Materials and Methods of Section II. Vials (45 x 10 mm) with small quantities of spores were kept inside these desiccators and bottles. Each desiccator or bottle had eight such vials representing four replications of each strain. Numbers 3108 and 4007 were the strains used for the experiment. Longevity was studied by conducting germination tests by following the method explained earlier (Section II). The test was started after twenty-four hours of exposure to the above conditions and repeated after twenty-four hours for the first three days. After three days, it was repeated once in three days until the spores failed to germinate. Each time a very small quantity of spores was taken from each vial with the aid of a sterile inoculating needle and

tested for viability. Detailed observations are given in Table XIX in the Appendix. The influence of the above conditions on the longevity of the spores of both strains of the fungus are graphically presented in Figure 1. Averages of the percentage of germination obtained during different periods are tabulated in Table II.

It appeared that spores of both strains were able to survive at this temperature only with less than one per cent relative humidity. Under these conditions more than fifty per cent of spores of both strains, i. e., 62.7 and 52.8 per cent of Strain 3108 and Strain 4007, were viable after six days; 3.4 per cent of Strain 3108 and 9.2 per cent of Strain 4007 were viable after twelve days. With 31.5 per cent relative humidity, 5.2 per cent of the spores of Strain 3108 were viable at three days, whereas 11.9 per cent of spores survived for three days and 4.4 per cent lived up to six days in the case of Strain 4007. There was no spore survival of Strain 3108 at seventy-two and 92.5 per cent relative humidities at three days. Only 4.7 per cent of the spores of Strain 4007 were able to survive for three days at seventy-two per cent relative humidity and none of the spores of this strain survived at 92.5 per cent relative humidity as in the case of Strain 3108.

Influence of this temperature with the above relative humidities on the spores of the strains is quite marked as can be seen from Table II. Strain 4007 was able to withstand the conditions of high humidity better than Strain 3108, but it is

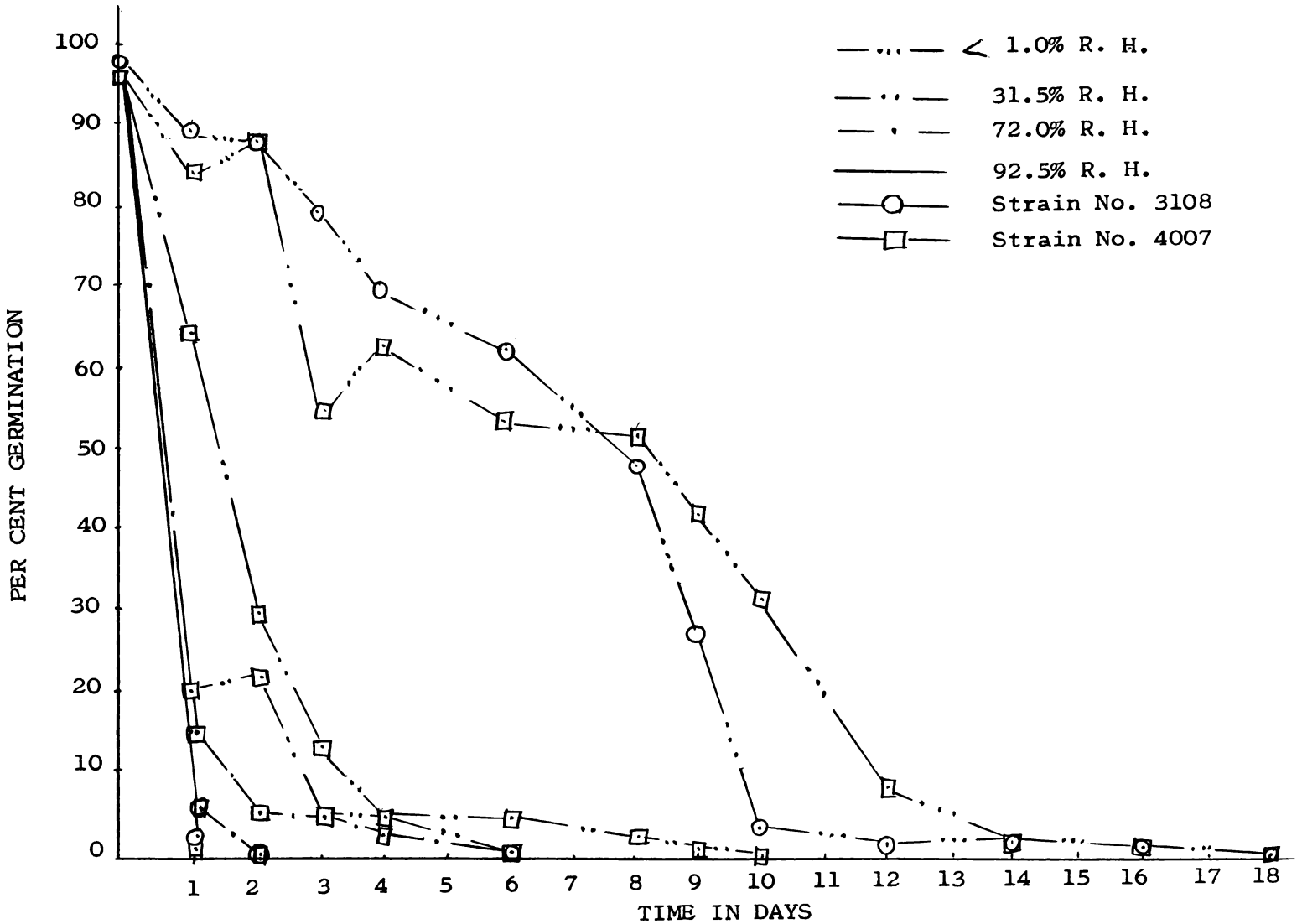

Figure 1. Effect of humidity on the lpngevity of the spores of two strains of the fungus, Beauveria bassiana, at 50° C.

TABLE II

RESULTS OF THE LONGEVITY OF THE SPORES OF TWO STRAINS OF THE FUNGUS, BEAUVERIA BASSIANA, AT 50° C. WITH DIFFERENT RELATIVE HUMIDITIES (IN PERCENTAGES)

Relative Humidity in Per Cent	Temperature 50°									
	Strain 3108					Strain 4007				
	3*	6	9	12	24	3	6	9	12	24
<1.0	79.3	62.7	27.9	3.4	0.0	54.6	52.8	41.8	9.2	0.0
31.5	5.2	0.0	0.0	0.0	0.0	11.9	4.4	0.7	0.0	0.0
72.0	0.0	0.0	0.0	0.0	0.0	4.7	0.0	0.0	0.0	0.0
92.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*In days.

clear from the table that higher relative humidities at this temperature have deleterious effects on the viability of spores.

Influence of 40° C. With Different Relative Humidities

This experiment was also carried out in an incubator with a difference of ± 0.5° C. Desiccators and bottles which were inside the incubator were maintained at different relative humidities as before. Vials with spores were kept in these containers. The same interval of three days was allowed at this temperature as at 50° C. for initial testing of the viability. Table XX (Appendix) shows the detailed observations made under these conditions at different periods. The influence of these factors on the spores of both strains are presented in Figure 2.

It may be seen from Table III and also from Figure 2 that the longevity of spores is severely affected with higher relative humidities at 40° C. But in the presence of low relative humidities, spores of both strains were found viable even after twenty-four days. However, the per cent viability varied with the period of exposure to these humidities at this temperature. More than fifty per cent of the spores of Strain 3108 survived up to twelve days under both lower relative humidities. Only 16.8 to 16.9 per cent of spores of the same strain lived up to twenty-four days. In the case of Strain 4007, more than fifty per cent of spores survived only for three days, and that only with less than one per cent of relative humidity. Of course,

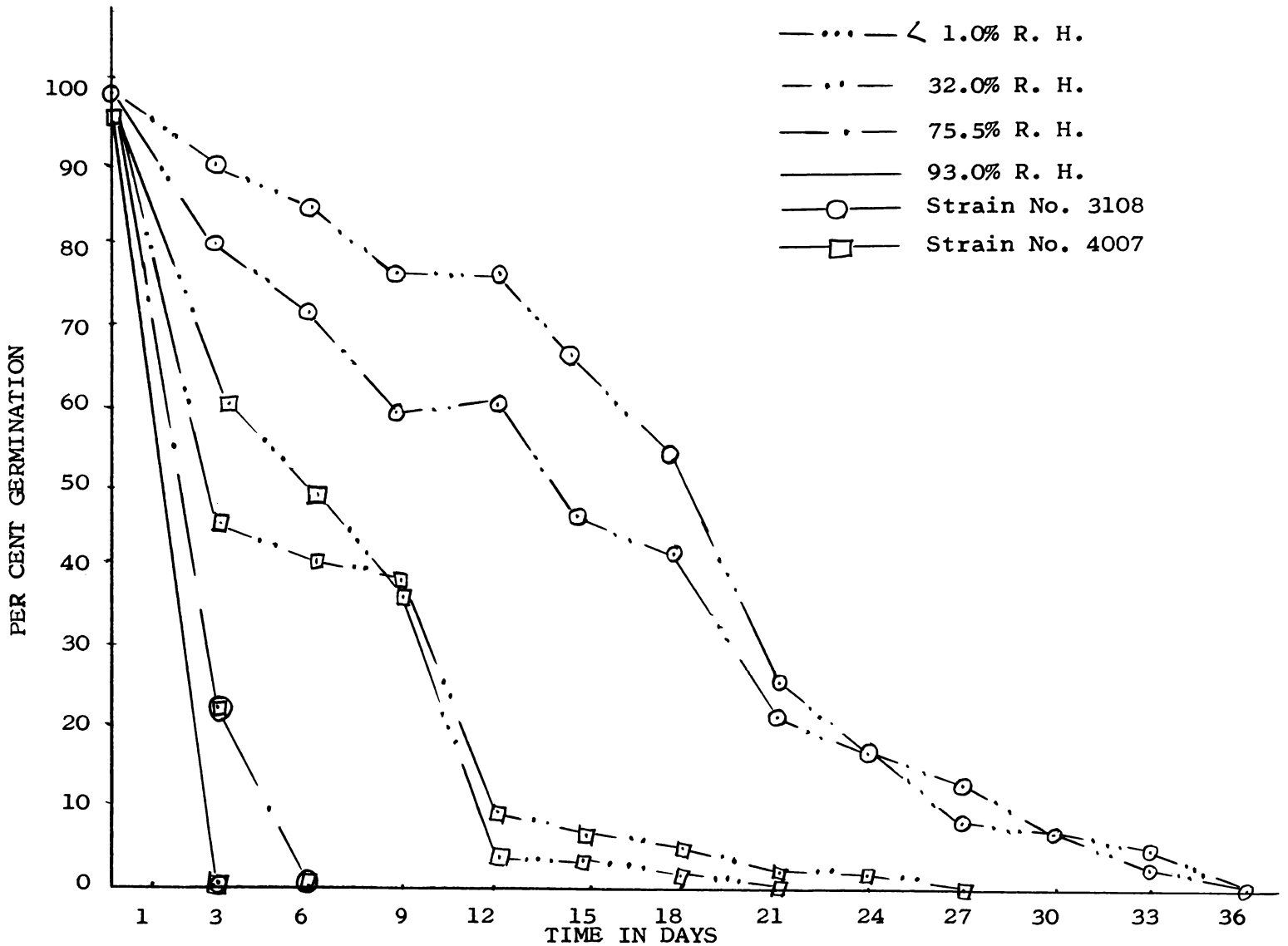

Figure 2. Effect of humidity on the longevity of the spores of two strains of the fungus, Beauveria bassiana at 40° C.

TABLE III

RESULTS OF THE LONGEVITY OF THE SPORES OF TWO STRAINS OF THE FUNGUS, BEAUVERIA BASSIANA, AT 40° C. WITH DIFFERENT RELATIVE HUMIDITIES (IN PERCENTAGES)

Relative Humidity in Per Cent	Temperature 40° C.									
	Strain 3108					Strain 4007				
	3*	6	9	12	24	3	6	9	12	24
1.0	89.0	83.9	75.0	74.6	16.9	61.0	48.6	36.9	3.4	0.0
32.0	80.6	72.9	57.8	59.5	16.8	46.5	40.7	38.9	7.5	0.6
70.5	23.4	0.0	0.0	0.0	0.0	23.0	0.0	0.0	0.0	0.0
93.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*In days.

more than twenty-five per cent of spores were viable up to nine days with both less than one and thirty-two per cent relative humidities in the case of Strain 3108. After twelve days only 3.4 and 7.5 per cent of spores were able to survive in Strain 4007 with relative humidities less than one per cent and thirty-two per cent respectively.

When spores of both strains were exposed to higher relative humidities, namely, 70.5 and 93.0 per cent, none of the spores were viable with 93.0 per cent and only 23.0 to 23.4 per cent of spores of both strains were able to germinate after three days at 70.5 per cent relative humidity.

With regard to response of the spores of both strains at this temperature there was similarity with reference to different relative humidites. However, there was variation in longevity between them. Spores of Strain 4007 lived for a shorter period when compared with spores of Strain 3108. This gives an indication that different strains of the same fungus respond differently to these environmental factors.

Effect of Higher Temperatures on the Longevity of the Spores of the Fungus, *B. bassiana*, With Different Relative Humidities

Temperatures of 50° and 40° C. were combined with almost similar percentages of relative humidity. The influence of these higher temperatures in the presence of the same percentage of relative humidity was found quite marked. This has been clearly

demonstrated graphically in Figures 3, 4, and 5. As can be seen from these figures and also from the tables, increase in temperature or higher temperature decreases the longevity of spores of both strains. Spores of Strain 3108 which were viable up to twenty-four days at 40° C. with less than one per cent of relative humidity were unable to survive at 50° C. with the same humidity (Figure 3). With relative humidities of 31.5 to 32.0 per cent, more than ten per cent of spores of both strains were viable after twelve days at 40° C. None of the spores survived for as long as twelve days at 50° C., in spite of the same percentage of humidity (Figure 4). With further increase in relative humidity, germination was observed after three days in the spores of both strains at 50° C. whereas twenty-three to 23.4 per cent of spores germinated after three days at 40° C. with 70.5 to 72.0 per cent relative humidity, as can be seen in Figures 5 and 6.

No difference in effect was recorded under both higher temperatures, when the relative humidity was 92.5 to ninety-three per cent. There was no germination of spores in either of the strains.

In order to determine whether the influence of these factors on the longevity of the spores of the two strains is significant or not, the results recorded in Tables XIX and XX (Appendix) were statistically analyzed together as both were identical in setup. They were run under almost identical percentages of relative

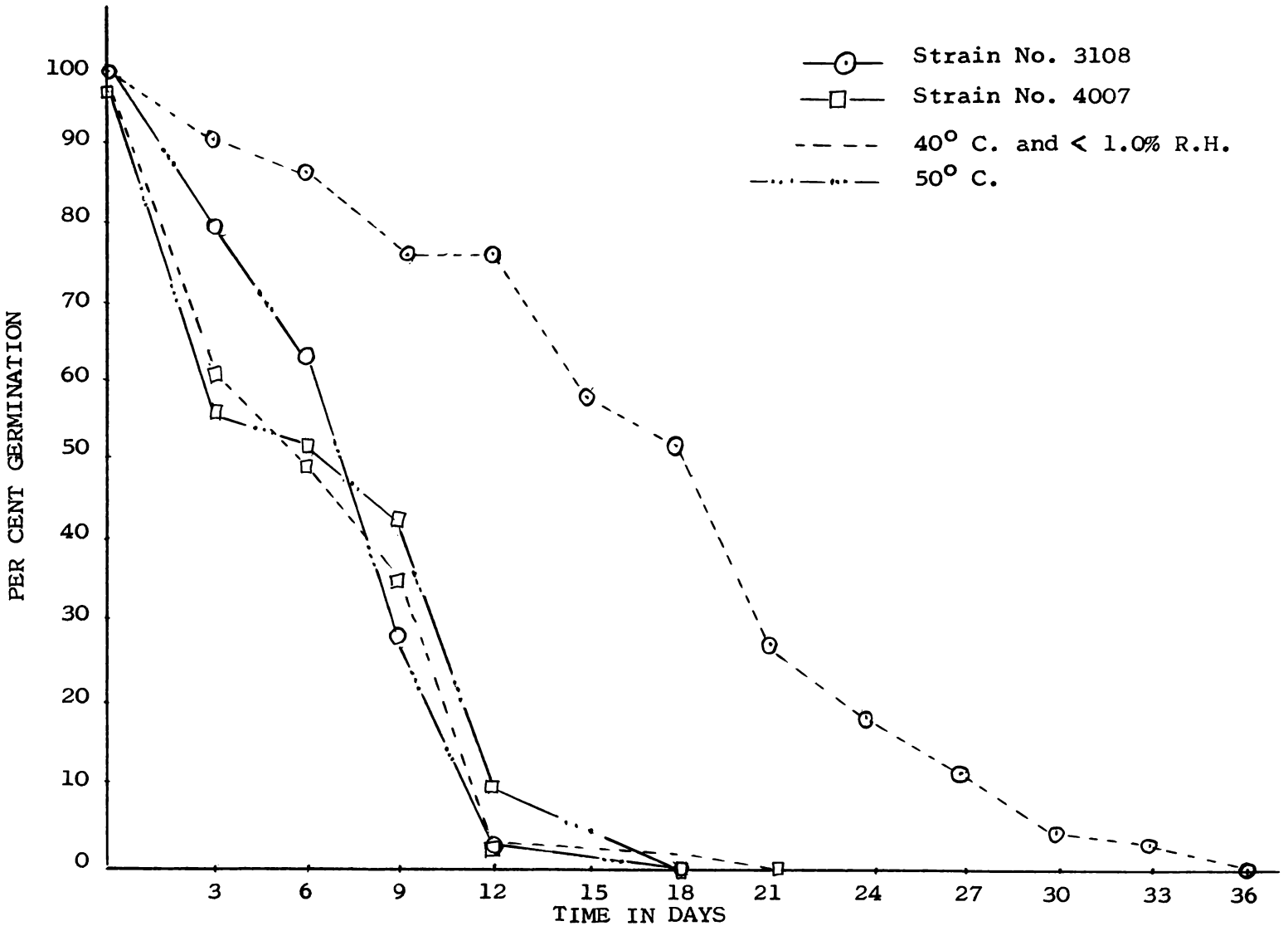

Figure 3. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana at less than one per cent relative humidity.

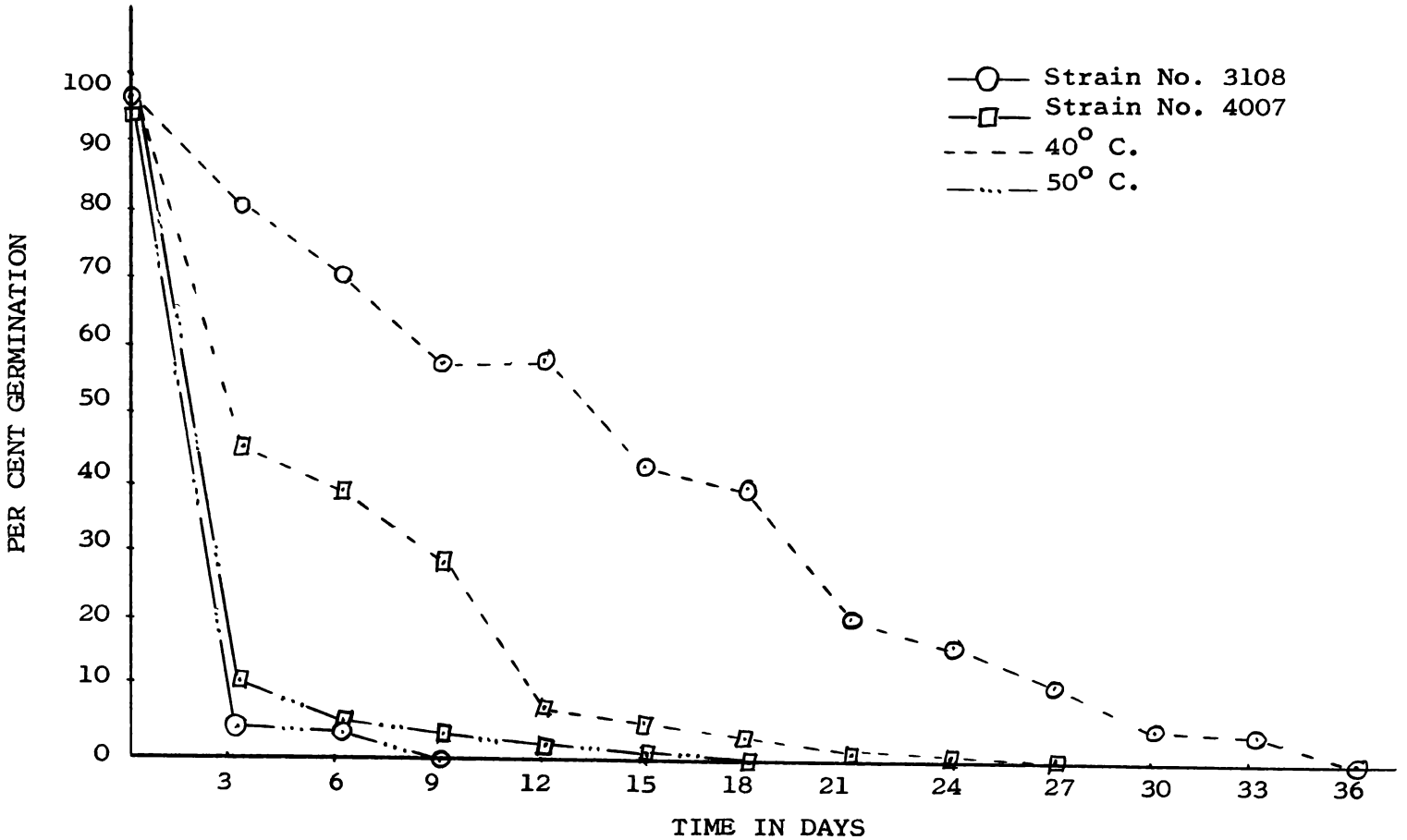

Figure 4. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 31.5 to 32.0 per cent relative humidity.

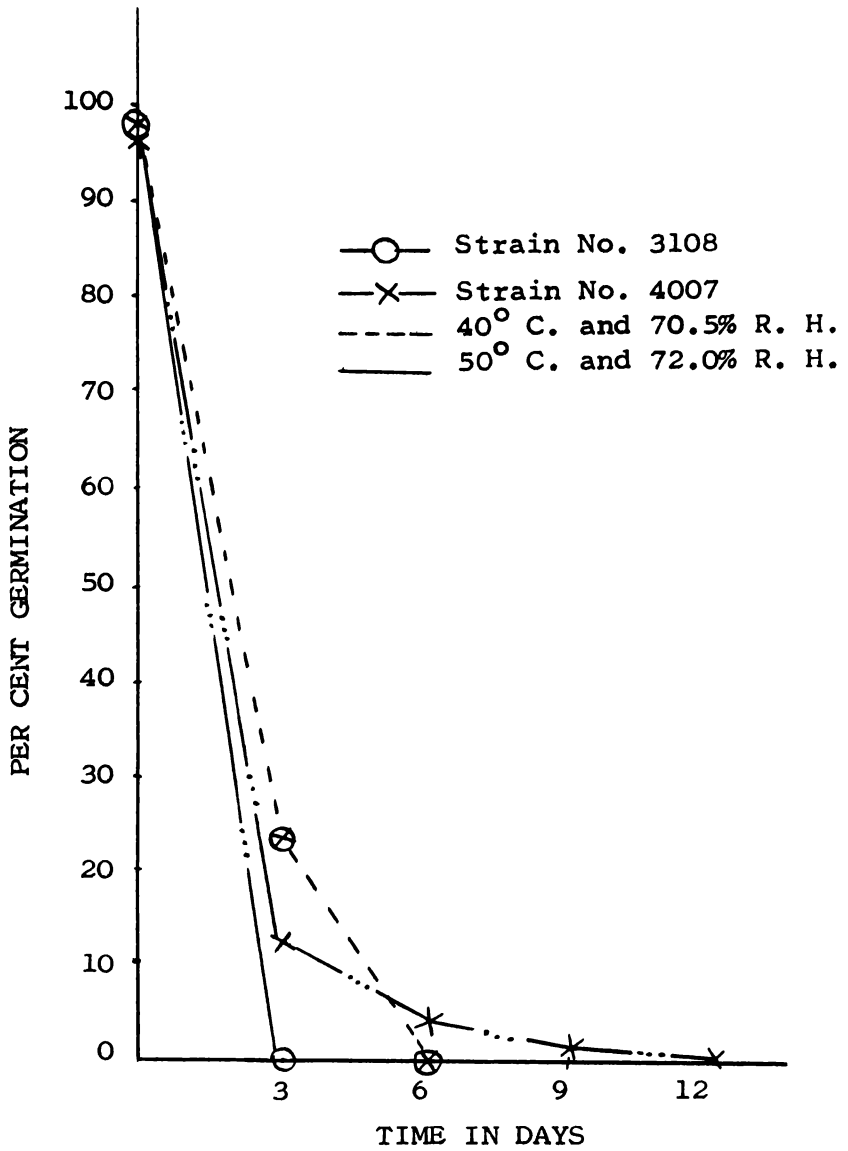

Figure 5. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 70.5 to 72.0 per cent relative humidity.

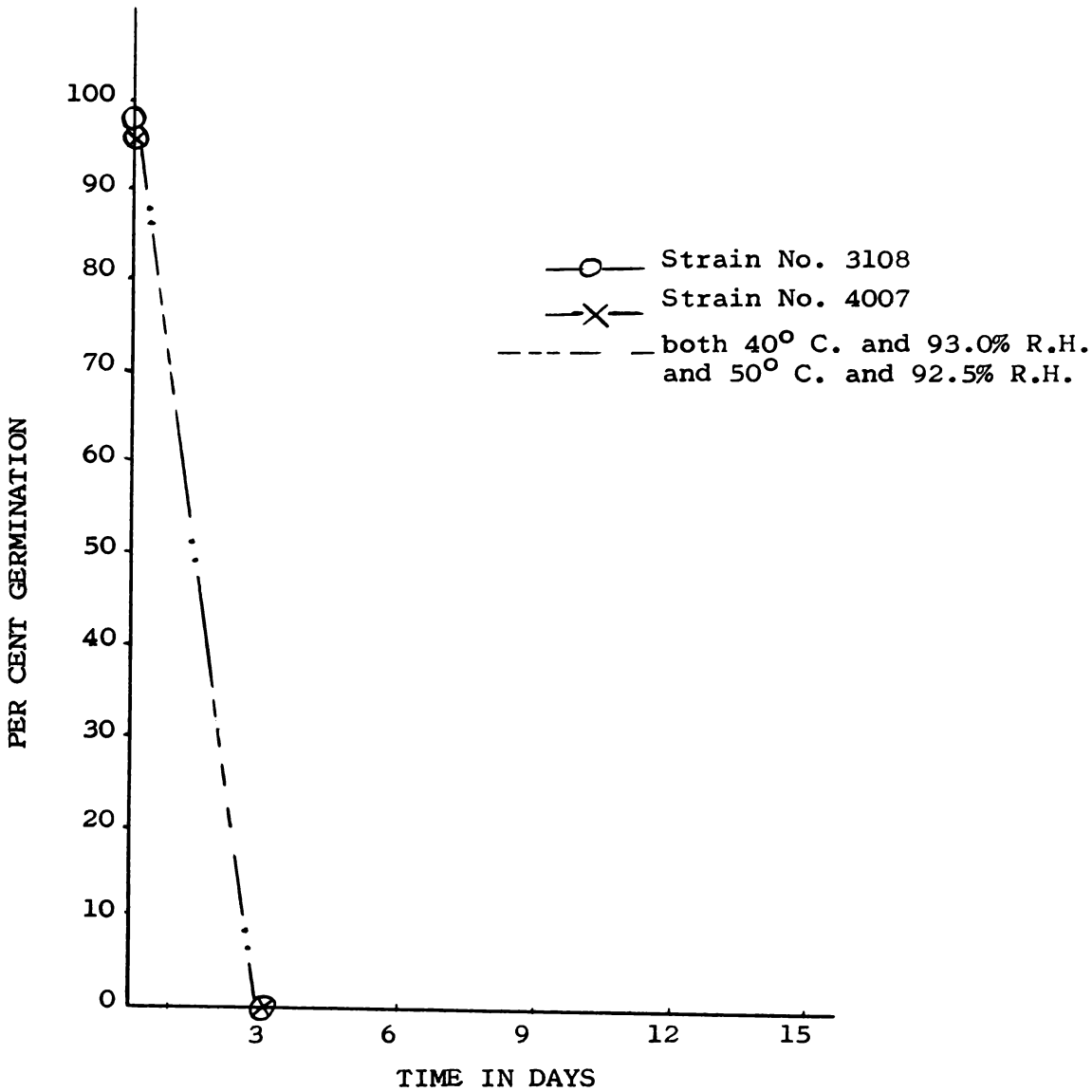

Figure 6. Effect of temperatures on the longevity of the spores of two strains of the fungus, Beauveria bassiana at 92.5 to 93.0 per cent relative humidity.

humidities. Analysis was done by following the method of "Analysis of Variance." The analyzed results are given in Table XXI in the Appendix. According to the table, the results between the temperatures, between the strains, between the different times of observation, and between the different relative humidities are significantly different at the 0.99 per cent level. In view of their significance and as there were more than two means, the results between the different times of observation and between the different relative humidities were further analyzed by applying Duncan's New Multiple Range Test for comparison. The results of analysis are presented below.

	After 24 days	12 days	9 days	6 days	3 days
Means	2.15	9.85	17.44	22.87	30.0

From the above analysis it can be seen that the percentage survival is significantly different between the different times of observation. Significant decrease in the percentage of survival of spores of both strains is seen with increase in time.

	Relative Humidity			
	92.5 to 93.0%	70.5 to 72.0%	31.5 to 32.0%	<1.0%
Means	0.00	2.56	22.21	41.09

It can be seen from the above analysis that the percentage of survival of spores at these relative humidities are significantly different at 50° and 40° C. with exception at 70.5 to seventy-two and 92.5 to ninety-three per cent. Percentage of spores survived at less than one per cent relative humidity are superior to

percentage survival of spores at other relative humidities, including 31.5 to thirty-two per cent. No significant difference is found between the relative humidities, 70.5 to seventy-two and 92.5 to ninety-three per cent. Furthermore, percentage of spore survival at these relative humidities is negligible, at these temperatures.

In conclusion it may be said that 50° and 40° C. are not suitable for the survival of spores of both strains of the fungus at any amount of relative humidity.

Influence of 35° C. with different relative humidities. The experiment at this temperature was conducted inside a wooden cabinet containing a fixed thermostat. This setup possessed an accuracy of ± 1° C. relative humidities maintained in desiccators and bottles were less than one, 32.5, seventy-one, and ninety-six per cent. After one week, a small quantity of spores from each vial, which was kept in the desiccators or bottles of different relative humidities, was taken out with the aid of a sterile inoculating needle and the percentage of germination estimated. This procedure was repeated once a week until the percentage of germination was found to be less than 0.5 per cent. The germination percentages of the spores of both strains recorded after one, three, six, nine and twelve weeks have been tabulated in Table XXII (Appendix). The longevity of the spores of these strains of this temperature with different relative humidities up to nineteen weeks of age is illustrated in Figure 7. In addition,

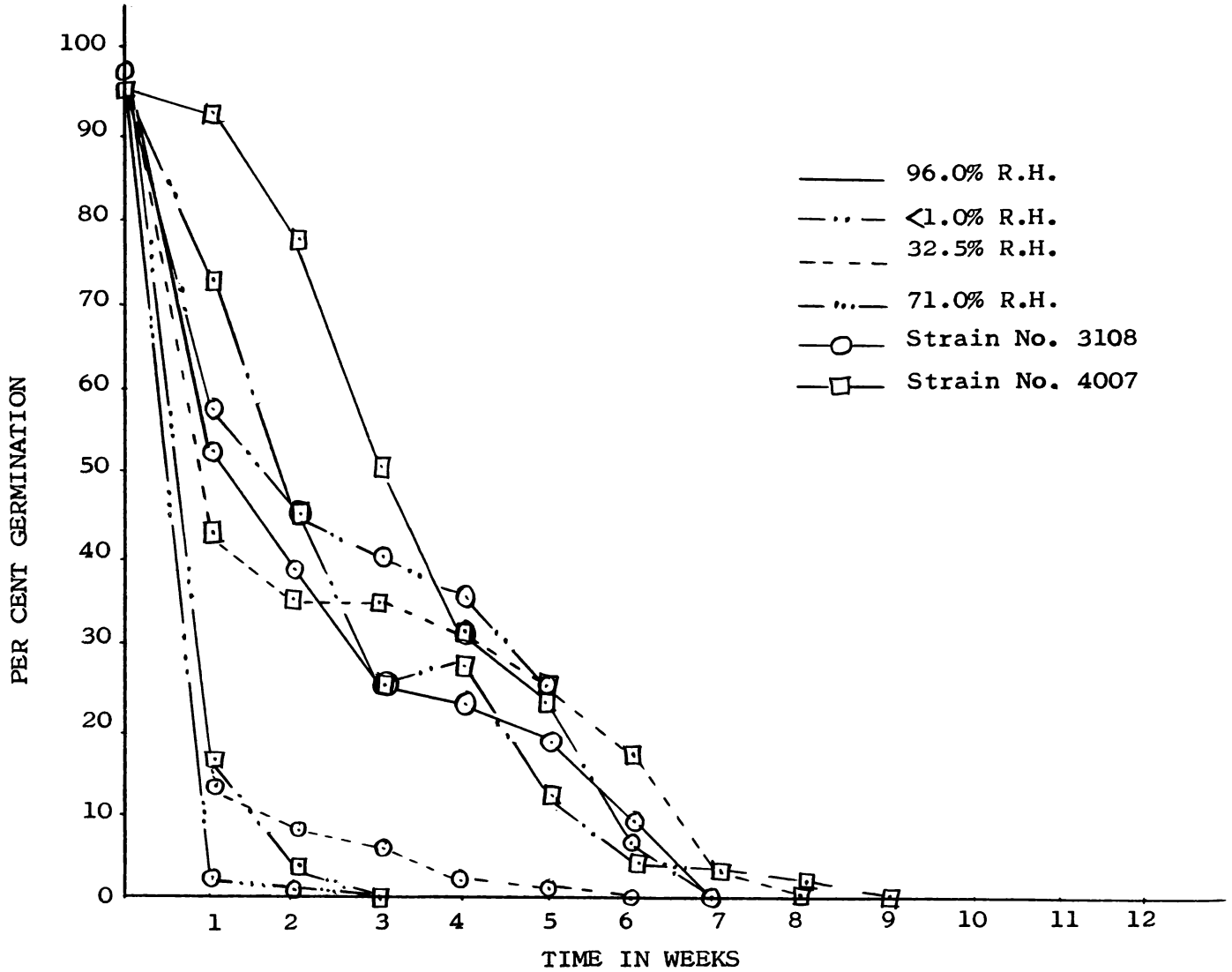

Figure 7. Effect of humidity on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 35° C.

the average percentage of spores that were viable under the above conditions during different weeks is summarized in Table IV.

It may be seen from Table IV and also from Figure 7 that the life span of the spores of both strains is as short as in the case of 50° and 40° C. with less than one and 32.5 per cent relative humidities. With seventy-one per cent relative humidity, only two and 15.6 per cent of spores of Strains 3108 and 4007 respectively, survived after one week. More than fifty-nine per cent of spores of both strains were found to germinate after one week with less than one per cent of relative humidity. Only 4.3 and 5.6 per cent of spores of Strains 3108 and 4007 were viable after six weeks with the same relative humidity. It is also quite clear from Table IV that at a relative humidity of seventy-one per cent this temperature had the same influence as it did at 40° C. Higher relative humidity showed a favorable effect at this temperature, as can be seen in Table IV. More than fifty per cent of spores of both strains was able to germinate after one week, whereas none of the spores germinated at 50° and 40° C. even after three days with almost the same per cent of relative humidity, i.e., above 92.5 per cent. This seems to indicate that spores of this fungus are able to survive at temperatures of 35° C. and below in the presence of high relative humidities (above ninety per cent).

Strain 4007 appeared to have the same response as observed at 40° C. when compared to Strain 3108, as can be observed in Table IV. A greater percentage of spores of this strain were

TABLE IV

AVERAGE PERCENTAGE OF GERMINATION OF THE SPORES OF TWO STRAINS OF *BEAUVERIA BASSIANA* AT 35° C. WITH DIFFERENT RELATIVE HUMIDITIES, FOR 12 WEEKS

Relative Humidity in Per Cent	Temperature 35° C.									
	Strain 3108					Strain 4007				
	Weeks									
	1	3	6	9	12	1	3	6	9	12
1.0	58.4	25.1	4.3	0.0	0.0	72.9	41.3	5.6	0.0	0.0
32.5	13.0	5.6	0.0	0.0	0.0	43.1	34.6	17.2	0.0	0.0
71.0	2.0	0.0	0.0	0.0	0.0	15.6	0.0	0.0	0.0	0.0
96.0	52.7	24.6	8.6	0.0	0.0	93.3	50.6	6.0	0.0	0.0

viable than in Strain 3108 with all relative humidities. In the presence of 32.5 per cent relative humidity 17.2 per cent of spores of this strain lived up to six weeks at this temperature. At six weeks time, no spores were viable in Strain 3108 and only 5.6 per cent survived to three weeks.

Influence of 30° C. with different relative humidities. The same kind of wooden cabinet was used to maintain this temperature as in the case of 35° C., with a difference of ±1° C. Less than one per cent and 32.5, 72.5 and 93.5 per cent were the relative humidities maintained at this temperature. The same kind of vials, replicated four times as in other temperatures, was utilized to expose the spores of both strains of the fungus. Germination tests, to estimate the longevity of spores, were conducted at weekly intervals, as in the case of 35° and 25° C. The results obtained under the above conditions are given in Table XXIII (Appendix). The influence of different relative humidities at this temperature is graphically illustrated in Figure 8. The average percentage germination of spores of both strains that were observed in the above conditions are tabulated in Table V.

Survival of a high percentage of spores of both strains at this temperature with low and very high relative humidities can be clearly noted from Table V. Relative humidities of 32.5 per cent appeared to be equivalent to one per cent among low relative humidities tested for both strains. However, a relative humidity of 72.5 per cent appeared to be detrimental to these strains as

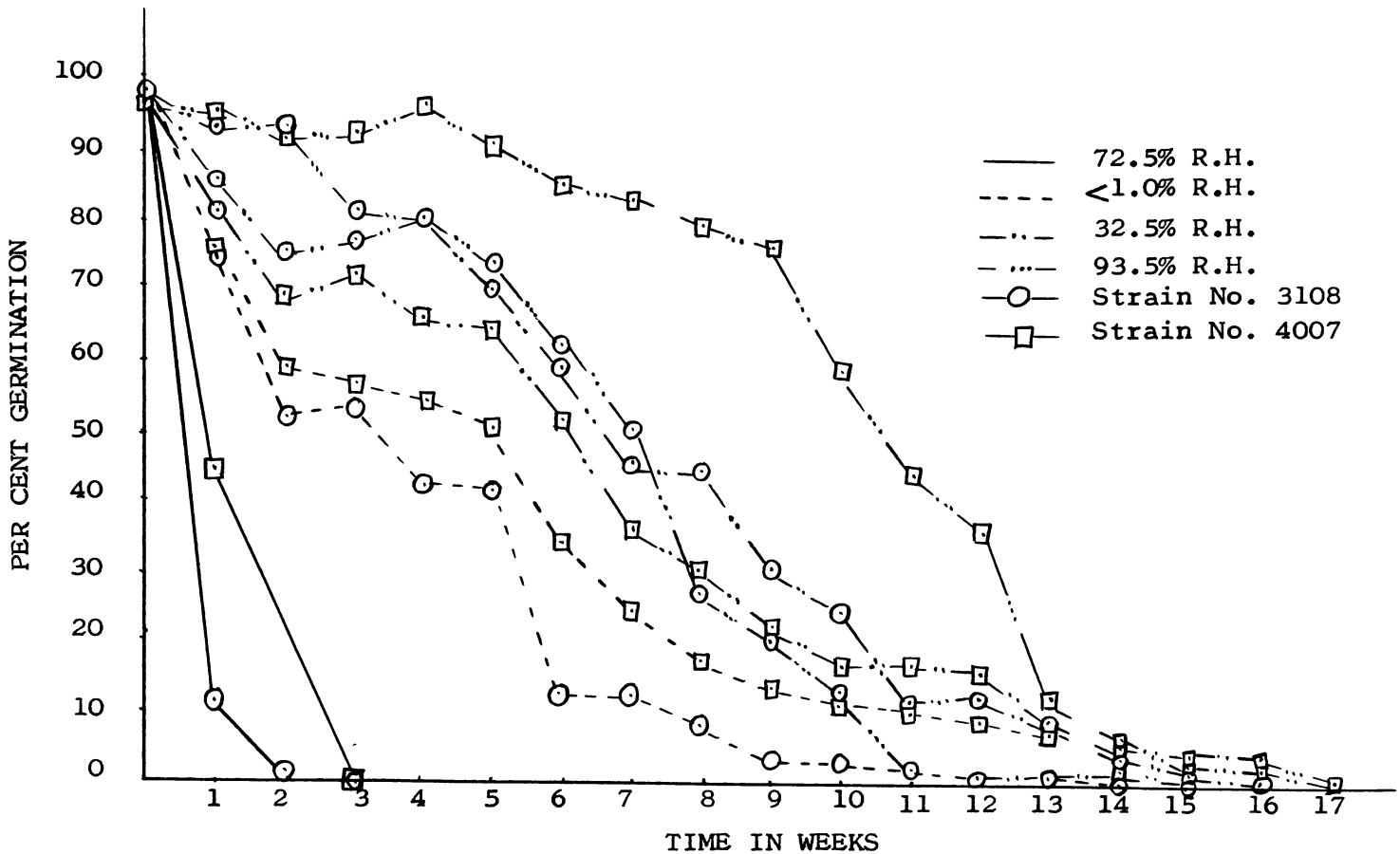

Figure 8. Effect of humidity on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 30° C.

TABLE V

AVERAGE PERCENTAGE OF GERMINATION OF SPORES OF TWO STRAINS OF *BEAUVERIA BASSIANA* AT 30° C. WITH DIFFERENT RELATIVE HUMIDITIES FOR 12 WEEKS

Relative Humidity in Per Cent	Temperature 30° C.									
	Strain 3108					Strain 4007				
	Weeks									
	1	3	6	9	12	1	3	6	9	12
1.0	81.4	53.1	11.9	3.4	1.0	76.2	56.5	33.6	13.2	8.6
32.5	86.5	77.9	59.3	28.9	11.6	74.7	65.7	51.5	21.9	15.6
72.5	10.8	0.0	0.0	0.0	0.0	44.3	0.0	0.0	0.0	0.0
93.5	93.8	80.6	67.5	20.4	1.0	95.4	92.1	84.7	76.4	35.8

can be seen in Figure 8 (page 49) and also in Table V (page 50). Less than fifty per cent of spores of both strains survived up to one week, apart from differences between strains.

It was interesting to note the higher percentage germination in the presence of a relative humidity of 93.5, at this temperature. Still more surprising was the higher rate of viability of spores of both strains at this relative humidity than at 32.5 per cent, and less than at one per cent. More than fifty per cent of spores of both strains germinated at this temperature even after six weeks, in the presence of both 32.5 and 93.5 per cent relative humidites. At less than one per cent relative humidity more than fifty per cent of the spores was able to germinate after three weeks. Less than twelve per cent of spores was viable in the case of Strain 3108 and thirty-five per cent in Strain 4007, after six weeks.

As can be seen in Figure 8 (page 49) there was a gradual decrease in the per cent survival of spores of both strains with all relative humidities tested except at 72.5 per cent. Although the percentage was very low, some spores of both strains were able to live up to sixteen and seventeen weeks in the presence of these relative humidities at 30° C.

Variation among the strains at this temperature seemed to be narrow as can be seen in Figure 8 (page 49). There is not much difference in the number of viable spores between the two strains when compared to 35°, 40° and 50° C. However, Strain 4007 showed

a higher per cent of germination than did Strain 3108, as seen in Table V (page 50).

Influence of 25° C. with different relative humidities. The experiment at this temperature was conducted in an air conditioned room maintained at 25° ± 1° C. Relative humidities of less than one, 30.5, seventy-four and 92.5 per cent were maintained as before. Small quantities of spores of both strains were exposed to the above conditions in vials. Germination tests were conducted once a week until less than 0.5 per cent of germination was obtained. Detailed results are tabulated in Table XXIV (Appendix). Longevity of the spores of both strains under these conditions is illustrated in Figure 9. The results are tabulated in a concise form with the averages of percentage of germination in Table VI.

Favor of this temperature for the survival of a higher percentage of spores of both strains is seen in Table VI. As can be seen in Figure 9, some of the spores of both strains were able to survive from fifteen to nineteen weeks in the presence of all the relative humidities tested, except seventy-four per cent where they survived only for about three weeks. More than seventy per cent in the case of Strain 3108 and more than fifty per cent in the case of Strain 4007 were able to germinate after six weeks in the presence of less than one, 30.5 and 92.5 per cent relative humidities. However, the relative humidity of seventy-four per cent at this temperature also seems to be unfavorable for the

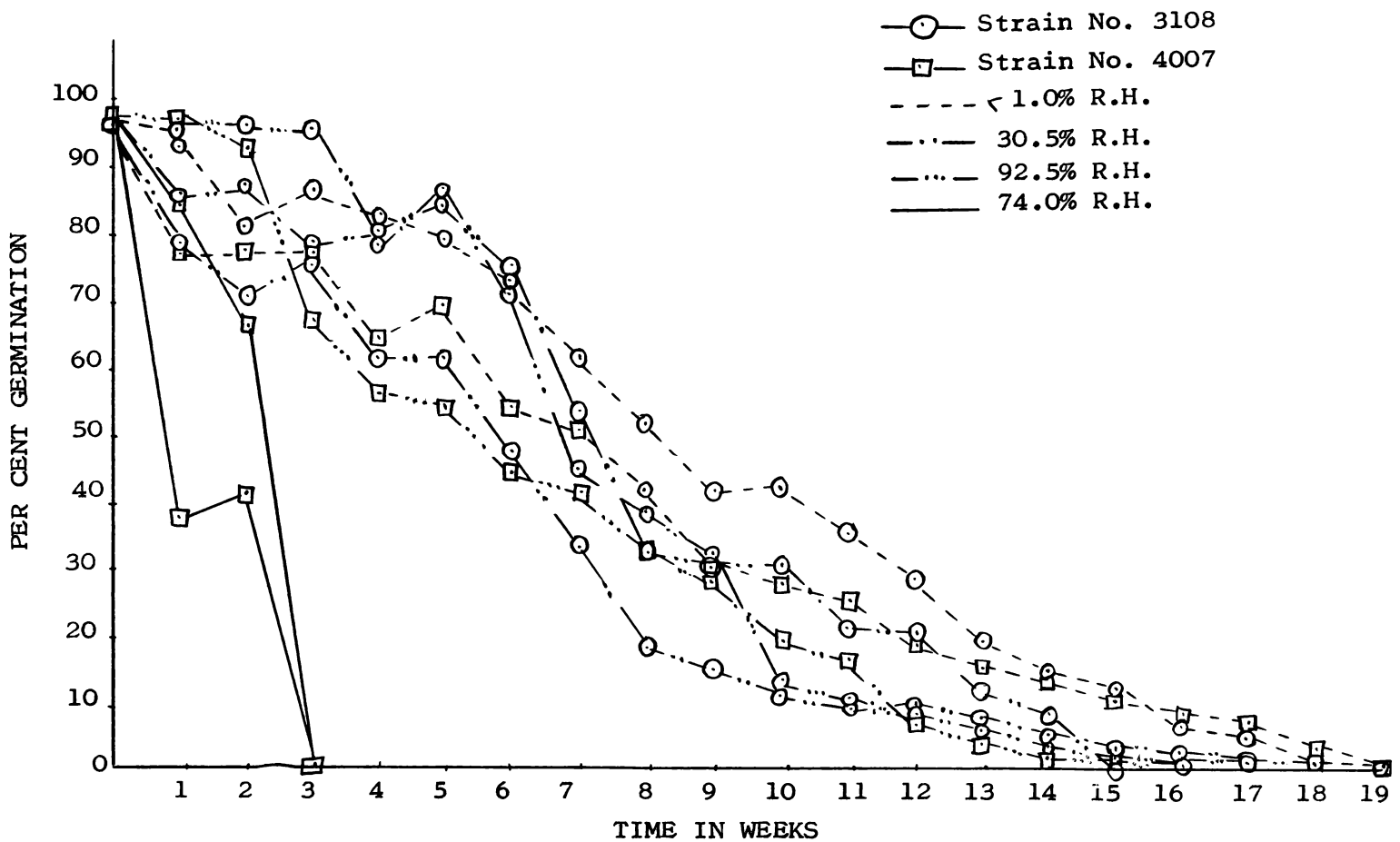

Figure 9. Effect of humidity on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 25° C.

TABLE VI

AVERAGE PERCENTAGE OF GERMINATION OF SPORES OF TWO STRAINS OF *BEAUVERIA BASSIANA* AT 25° C. WITH DIFFERENT RELATIVE HUMIDITIES FOR 12 WEEKS

Relative Humidity in Per Cent	Temperature 25° C.									
	Strain 3108					Strain 4007				
	Weeks									
	1	3	6	9	12	1	3	6	9	12
1.0	95.1	86.2	73.2	41.3	28.5	78.1	77.1	53.6	29.9	18.8
30.5	83.8	78.1	75.1	29.8	20.0	78.9	75.5	47.3	15.1	7.9
74.0	93.8	10.5	0.0	0.0	0.0	37.2	13.5	0.0	0.0	0.0
92.5	93.8	92.2	71.6	31.5	9.1	95.8	66.8	43.9	28.5	7.7

longevity of spores of these strains. As is seen in Table VI (page 53), an average of 10.5 per cent in the case of Strain 3108 and 13.5 per cent of Strain 4007 was able to germinate after three weeks.

The more favorable effect of a higher relative humidity (92.5 per cent) on the survival of a greater number of spores of these strains was observed at this temperature than at 30° C. More than seventy per cent of the spores was able to germinate after six weeks in Strain 3108 and more than sixty-six per cent for three weeks in Strain 4007. But when compared to the percentage of spores that germinated in the presence of less than one per cent of relative humidity, after twelve weeks, germination was about eight to nine per cent with 92.5 per cent relative humidity (Table VI, page 53).

Variation between strains was greater at this temperature with different humidities as can be seen in Figure 9 (page 53). This temperature seemed to be more favorable for Strain 3108 than when compared to other higher temperatures. More than seventy per cent of the spores of this strain was viable after six weeks whereas only about fifty-three per cent and below was viable in Strain 4007, at favorable relative humidities.

Effect of 25°, 30°, and 35° C. on the Longevity of the Spores of Two Strains of the Fungus at the Same Relative Humidity

It is possible to discuss the effect of temperatures, as shown in Figures 10, 11, 12, and 13, as the viability tests were

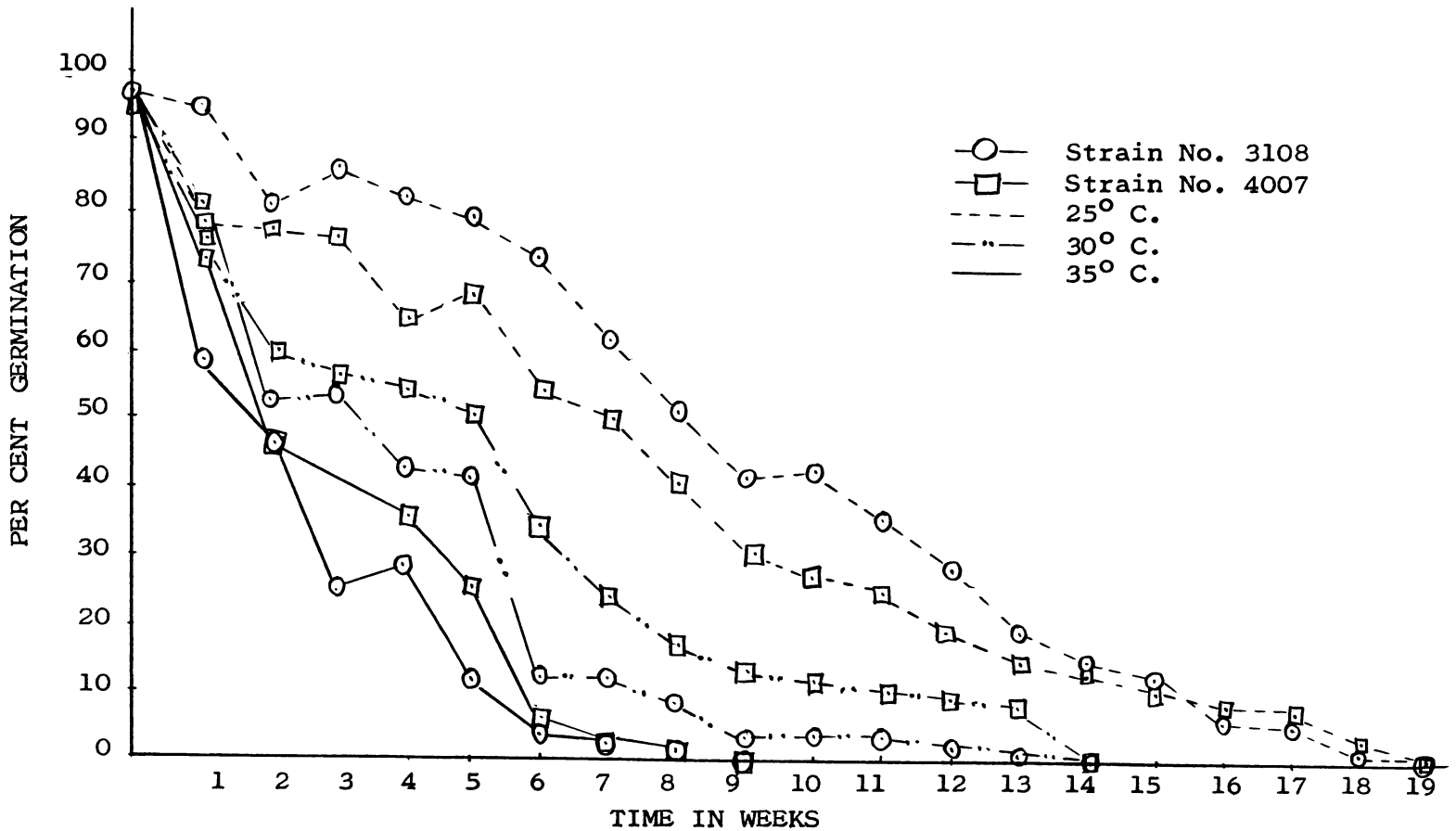

Figure 10. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at less than 1.0 per cent relative humidity.

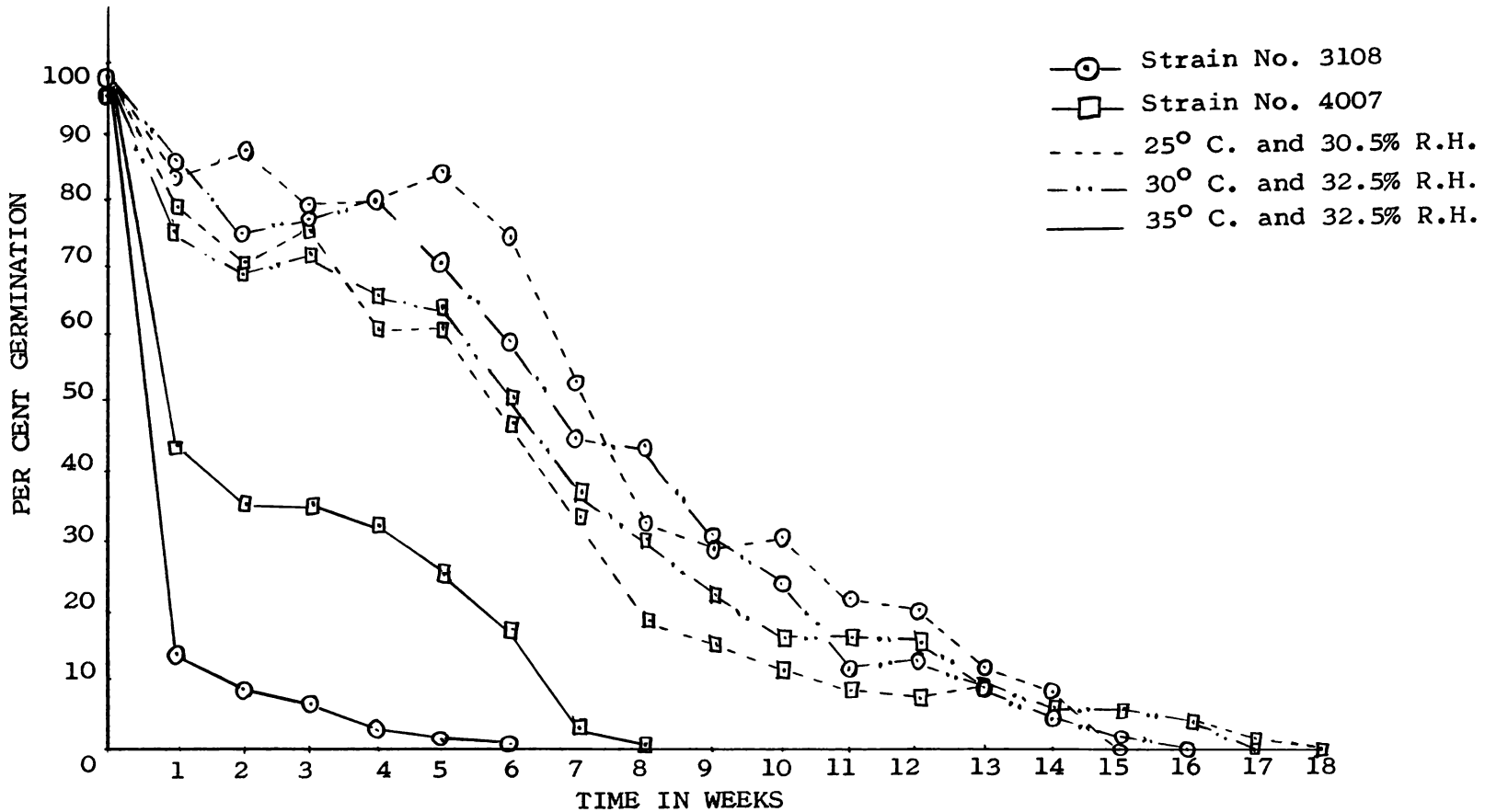

Figure 11. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 30.5 to 32.5 per cent relative humidity.

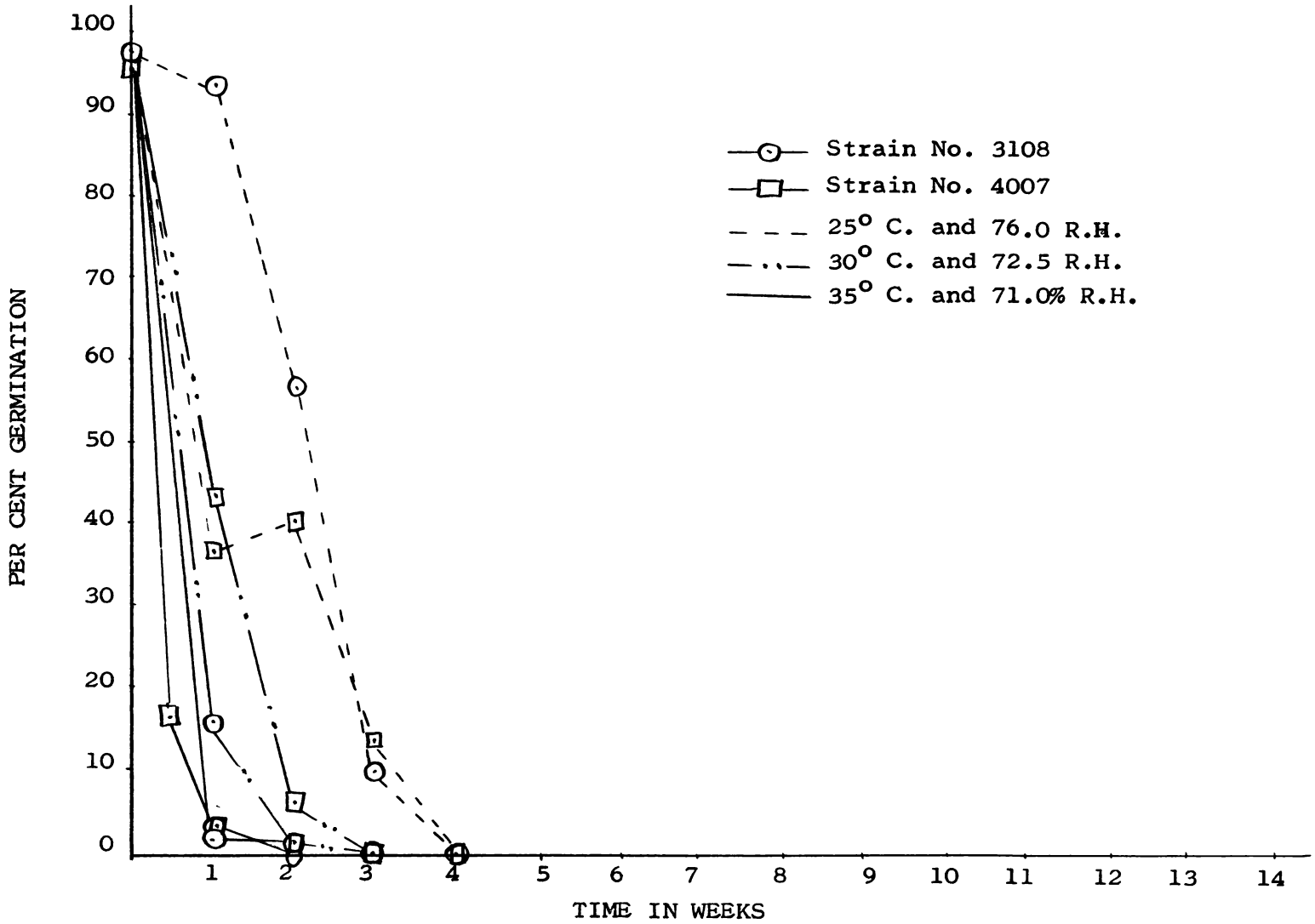

Figure 12. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 71.0 to 74.0 per cent relative humidity.

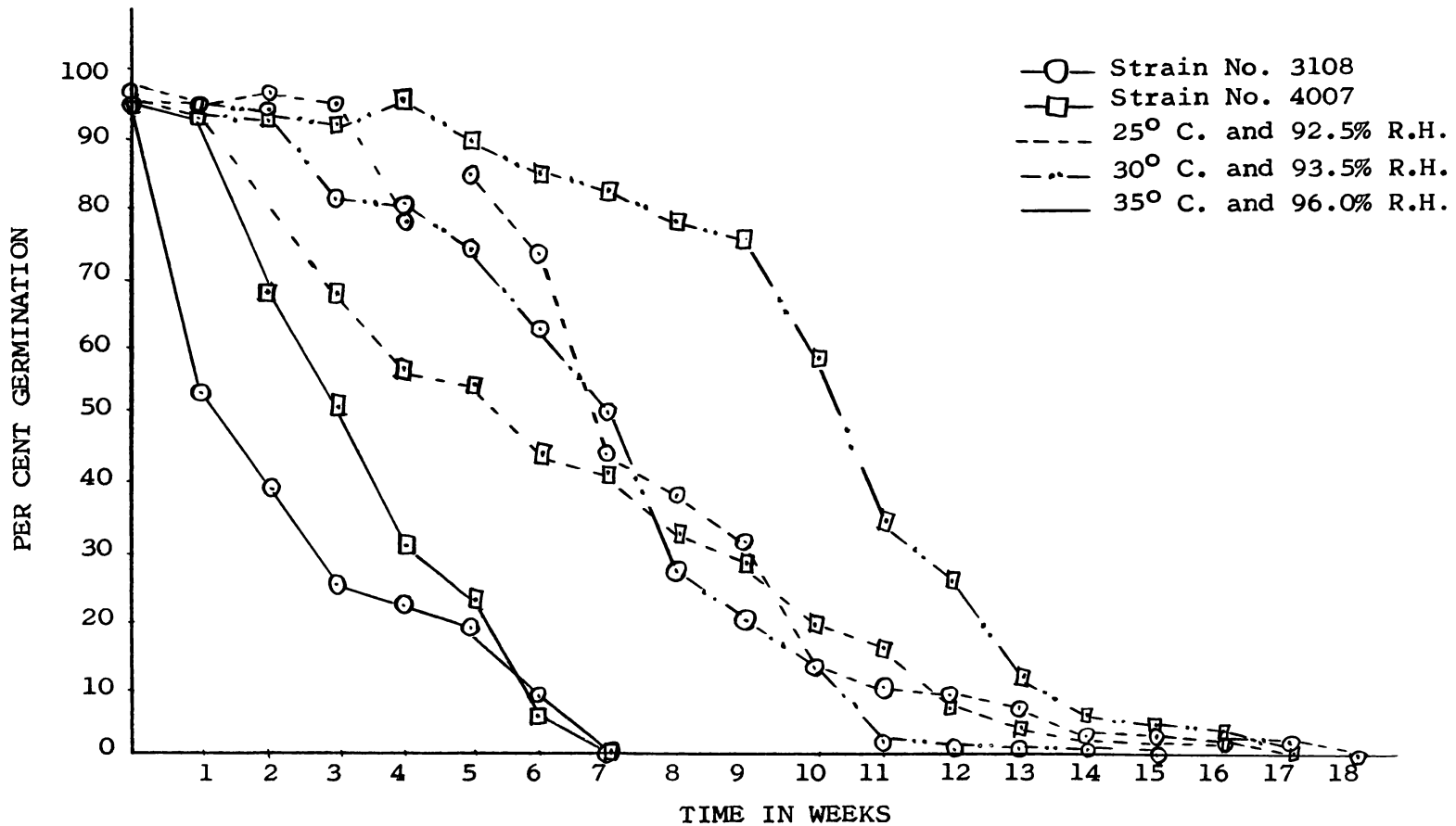

Figure 13. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 92.5 to 96.0 per cent relative humidity.

conducted at the same intervals in all these temperatures (25°, 30°, and 35° C.). Furthermore, the relative humidities maintained at these temperatures were almost identical, i.e., between 30.5 and 32.5, seventy-one and seventy-four and 92.5 and ninety-six per cent. As can be seen in Figures 9 (page 53), 10, 11, and 12 (pages 56 through 58, respectively), a gradual reduction in longevity was observed at these temperatures with each humidity except with seventy-one to seventy-four per cent, regardless of strain. Secondly, spores of both strains were able to live longer at 25° C. than at 30° or 35° C., irrespective of the humidity. Periods at which fifty per cent of the spores of both strains were able to germinate under these temperatures with each relative humidity are tabulated in Table VII. This was figured out based on Figures 10, 11, 12 and 13 (pages 56 through 59, respectively).

From Table VII, it is quite clear that each temperature has a different effect on the spores of these strains of the fungus in the presence of different relative humidities. Higher percentages of spores were viable at 25° and 30° C., at relative humidities of less than one, 30.5 to 32.5 and 92.5 to ninety-six per cent, as can be seen in Figures 10, 11, (pages 55 and 56) and 13 (page 58). Thirty degrees Centigrade seemed to have a favorable effect on these strains, particularly in the case of Strain 4007 where more than fifty per cent of spores germinated even after ten weeks (Table VII).

Results obtained at 25°, 30°, and 35° C., with different

TABLE VII

PERIOD (IN WEEKS) OF GERMINATION OF FIFTY PER CENT OF THE SPORES OF TWO STRAINS OF THE FUNGUS, *BEAUVERIA BASSIANA*, AT 25°, 30° AND 35° C., WITH DIFFERENT HUMIDITIES

Relative Humidity in Per Cent	Strain 3108			Strain 4007		
	25° C.	30° C.	35° C.	25° C.	30° C.	35° C
1.0	8.1*	3.3	1.6	7.0	5.0	1.8
30.5 to 32.5	7.2	6.6	0.6	5.8	6.1	0.9
71.0 to 74.0	2.2	0.6	0.5	0.8	0.9	0.3
92.5 to 96.0	6.8	7.0	1.2	5.4	10.6	3.1

*In weeks.

relative humidities, were statistically analyzed by the method of "Analysis of Variance" to find whether the influence of these factors is significantly different. Results tabulated in Tables XXII, XXIII, and XXIV (Appendix) were used for analysis as there was no difference in the interval of observations and there was not much variation in the relative humidities used. The analyzed data are tabulated in Table XXV (Appendix). According to the table, the results between the temperatures, between the strains, between the different times of observation, and between the different relative humidities are significantly different at the 0.99 per cent level. Wherever there were more than two significant means, Duncan's New Multiple Range Test was conducted to compare them. The results of the test are presented below.

	Temperature		
	35° C.	30° C.	25° C.
Means	14.36	38.36	44.81

From the above test it is clear that the results between these temperatures are significantly different at the 0.99 per cent level. Furthermore, it is also clear that the longevity of the spores of both strains at 25° C. is greater than at 30° and 35° C.

	After				
	12 Weeks	9 Weeks	6 Weeks	3 Weeks	1 Week
Means	6.89	14.19	29.79	46.24	65.45

From the above analysis it is seen that the results between the different times of observation are significantly different. Percentage of survival of spores of both strains is found to

decrease with increase in time of exposure to these temperatures and relative humidities.

	Relative Humidity			
	71.0 to 74.0%	30.15 to 32.5%	<1.0%	92.5 to 96.0%
Means	7.59	37.27	37.61	47.58

A significant difference between the results of different relative humidities is clearly seen in Table XXV (Appendix). However, in the above test it is seen that the effect of relative humidities of less than one and 30.5 to 32.5 per cent, on the longevity of the spores of both strains is equal. But the effects of higher humidities, seventy-one to seventy-four and 92.5 to ninety-six per cent differ from those of the lower humidities. Longevity at 92.5 to ninety-six per cent relative humidity is superior to the other three lower relative humidities tested. Longevity at seventy-one to seventy-four per cent relative humidity is minimal when compared to the results of the other three relative humidities. In conclusion, it may be said that the longevity of the spores of the two strains of the fungus is greater at relative humidities of less than one, 30.5 to 32.5 and 92.5 to ninety-six per cent than at the intermediate relative humidity of seventy-one to seventy-six per cent.

Influence of 20° C. With Different Relative Humidities

With the object of knowing the influence of humidity on the longevity of the spores at a temperature below normal (room) temperature an experiment was conducted at 20° C. An incubator

was used to maintain this temperature at an accuracy of $\pm$ 0.5^{o} C. As usual, different relative humidities were maintained in air-tight bottles. Germination tests were conducted once a month for a period of four months. Germination percentages obtained during different months in the presence of different relative humidities are presented in Table XXVI (Appendix). The longevity obtained during different periods is also illustrated in Figure 14. The average percentages of germination obtained during four months are tabulated in Table VIII.

It appears from Table VIII that the longevity of the spores of both strains is greater at a relative humidity of less than one and at 96.5 per cent than at thirty-three and seventy-six per cent. This agrees with the results obtained at 30° and 25° C. A gradual decrease is noticed in the longevity at this temperature, also (Figure 14). Moreover, at seventy-six per cent relative humidity the usual rapid fall in the percentage germination is observed with time. More than fifty per cent of spore germination is clearly seen even after four months at less than one, thirty-three, and 96.5 per cent relative humidities. Even at this low temperature, the intermediate relative humidity (seventy-six) is found to be not favorable for the fungus. Nevertheless, when compared to the longevity of spores at 30° and 25° C., more spores survived at this low temperature, particularly from Strain 3108. Moreover, there seems to be an indication of a drop in the percentage germination of the spores of both strains at thirty-three per cent relative

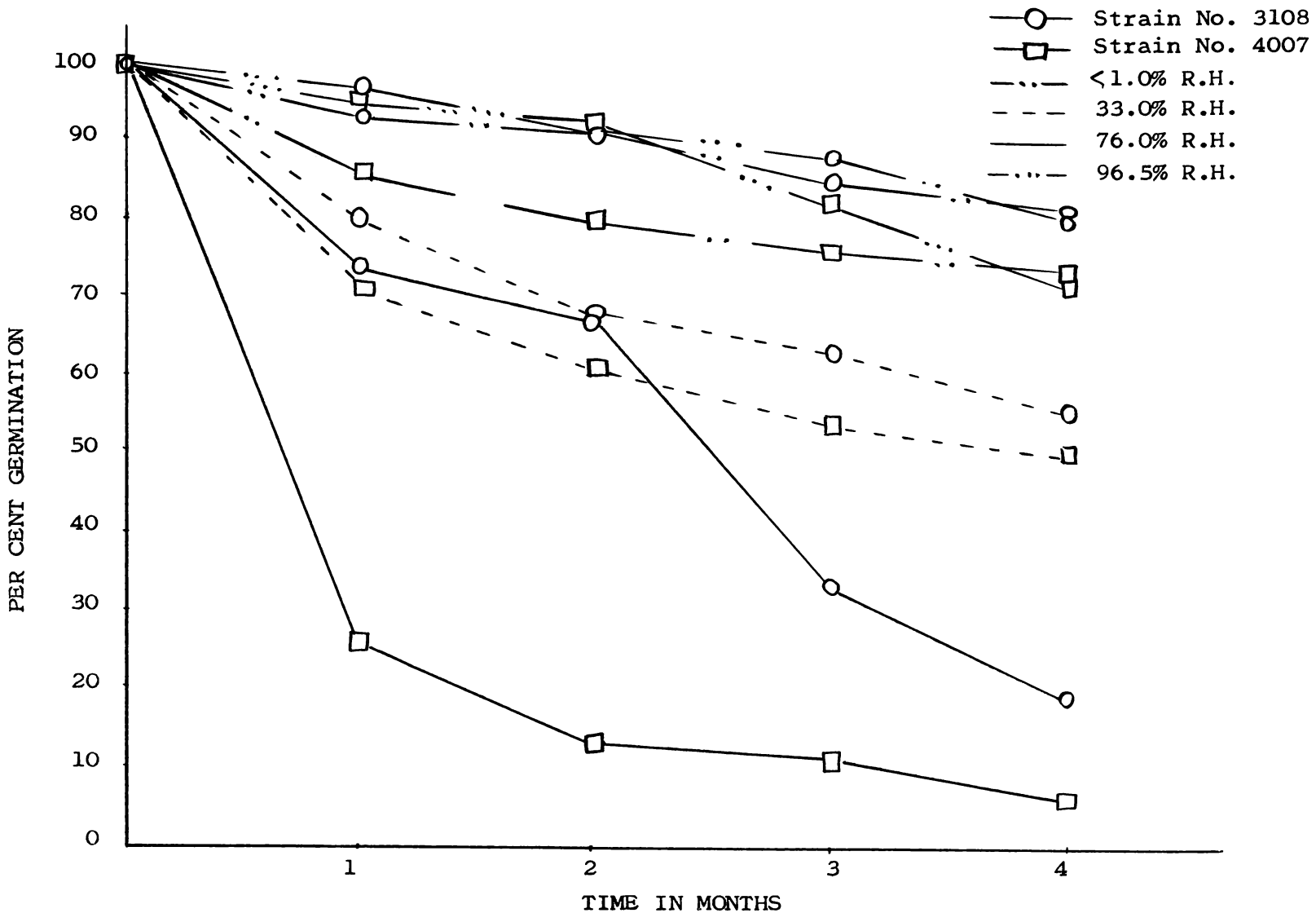

Figure 14. Effect of humidity on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 20° C.

TABLE VIII

PERCENTAGE OF GERMINATION OF SPORES OF THE FUNGUS, *BEAUVERIA BASSIANA*, AT 20° C., WITH DIFFERENT RELATIVE HUMIDITIES

Relative Humidity in Per Cent	Temperature 20° C.							
	Strain 3108				Strain 4007			
	Months							
	1	2	3	4	1	2	3	4
1.0	92.81	90.92	85.43	81.25	85.74	80.01	76.35	73.44
33.0	79.88	68.13	63.31	55.21	70.88	60.74	54.14	49.86
76.0	74.16	67.22	33.39	19.32	26.34	13.33	11.05	5.84
96.5	96.64	91.20	88.06	80.24	95.01	91.85	81.97	70.83

humidity. This seems to indicate a gradual change in the effect of intermediate relative humidity with a decrease in temperature.

Findings at this temperature agree with the response of the spores of Strain 4007 at 25° C. Spores of this strain lose their viability earlier than do those of Strain 3108, as can be seen in Table VIII (page 66). After four months, 19.32 per cent of the spores was viable in the case of Strain 3108 at seventy-six per cent relative humidity, whereas only 5.84 per cent of spores was viable in Strain 4007 under the same conditions.

Influence of 5° C. With Different Relative Humidities

The experiment at this temperature was run in a cold room maintaining this temperature with a difference of ± 1.0° C. The longevity was estimated at intervals of one month. Observations were discontinued after five months since the spores were likely to remain viable for months at a very low temperature. Detailed results are tabulated in Table XXVII (Appendix). Graphs showing the longevity of spores of both strains of the fungus at different relative humidities during these five months are presented in Figure 15. The average percentage of spores germinated at this temperature is also presented in Table IX.

Survival of a greater percentage of spores for a longer period in the presence of different relative humidities is clearly seen in Table IX. What was observed at 30°, 25°, and 20° C., regarding the higher percentage of survival at both very low and

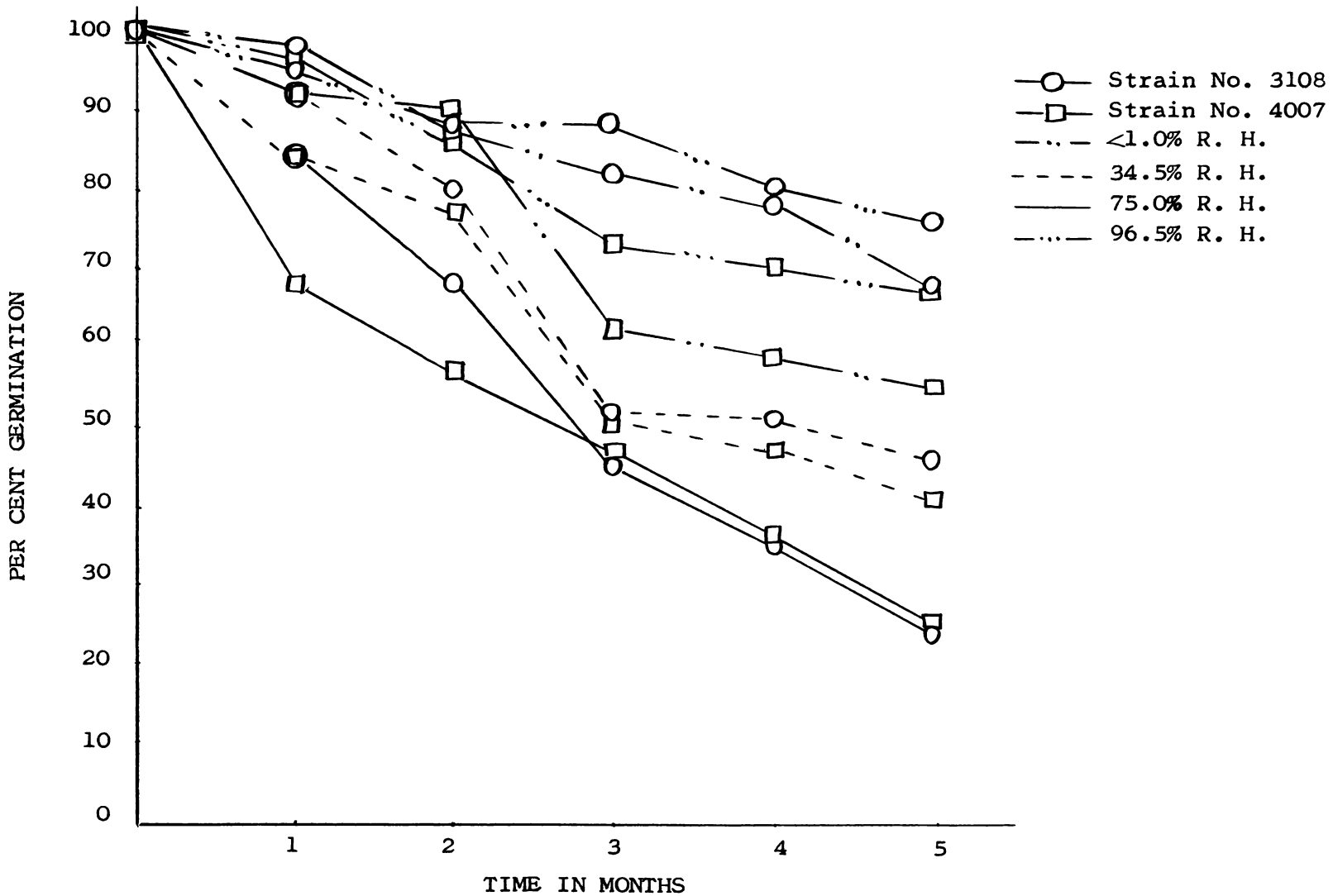

Figure 15. Effect of humidity on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 5° C.

TABLE IX

PERCENTAGE OF GERMINATION OF SPORES OF THE FUNGUS, *BEAUVERIA BASSIANA*, AT 5° C., WITH DIFFERENT RELATIVE HUMIDITIES

Relative Humidity in Per Cent	Temperature 5° C.									
	Strain 3108					Strain 4007				
	Months									
	1	2	3	4	5	1	2	3	4	5
1.0	97.95	87.10	81.79	77.90	68.35	92.13	89.78	71.58	58.83	54.85
34.5	92.47	79.92	52.19	50.84	46.04	83.94	77.00	50.96	47.13	41.24
75.0	83.30	68.09	45.18	34.73	24.15	67.77	57.35	46.56	36.26	25.36
96.5	95.27	88.02	87.55	80.25	75.85	96.46	86.11	73.40	70.03	67.13

very high relative humidity is seen at this temperature also. More than fifty per cent of the spores of both strains found viable even after five months at less than one and at 96.5 per cent relative humidities. At 34.5 per cent relative humidity more than forty-five per cent of the spores survived. However, a minimum percentage of survival was seen in the presence of seventy-five per cent relative humidity.

Spores of Strain 4007 were less tolerant of this cold temperature than were the spores of Strain 3108, as observed at 25° and 20° C. Though almost the same number of viable spores was recorded of both strains in the latter months, a marked difference in the percentage of survival between the strains was observed in the initial months.

Effect of 20° and 5° C. on the Longevity of the Spores of the Fungus, Beauveria bassiana, in the Presence of the Same Relative Humidity

As stated previously, discussion of the effect of these temperatures (20° and 5° C.) on the longevity of the spores was possible because the intervals of observation and the relative humidities were almost the same. Effects of these temperatures in the presence of each relative humidity are illustrated in Figures 16, 17, 18, and 19. In addition, a statement showing the period at which seventy-per cent of spores of both strains were able to survive under these temperatures in the presence of the same relative humidity is given in Table X, which was figured out

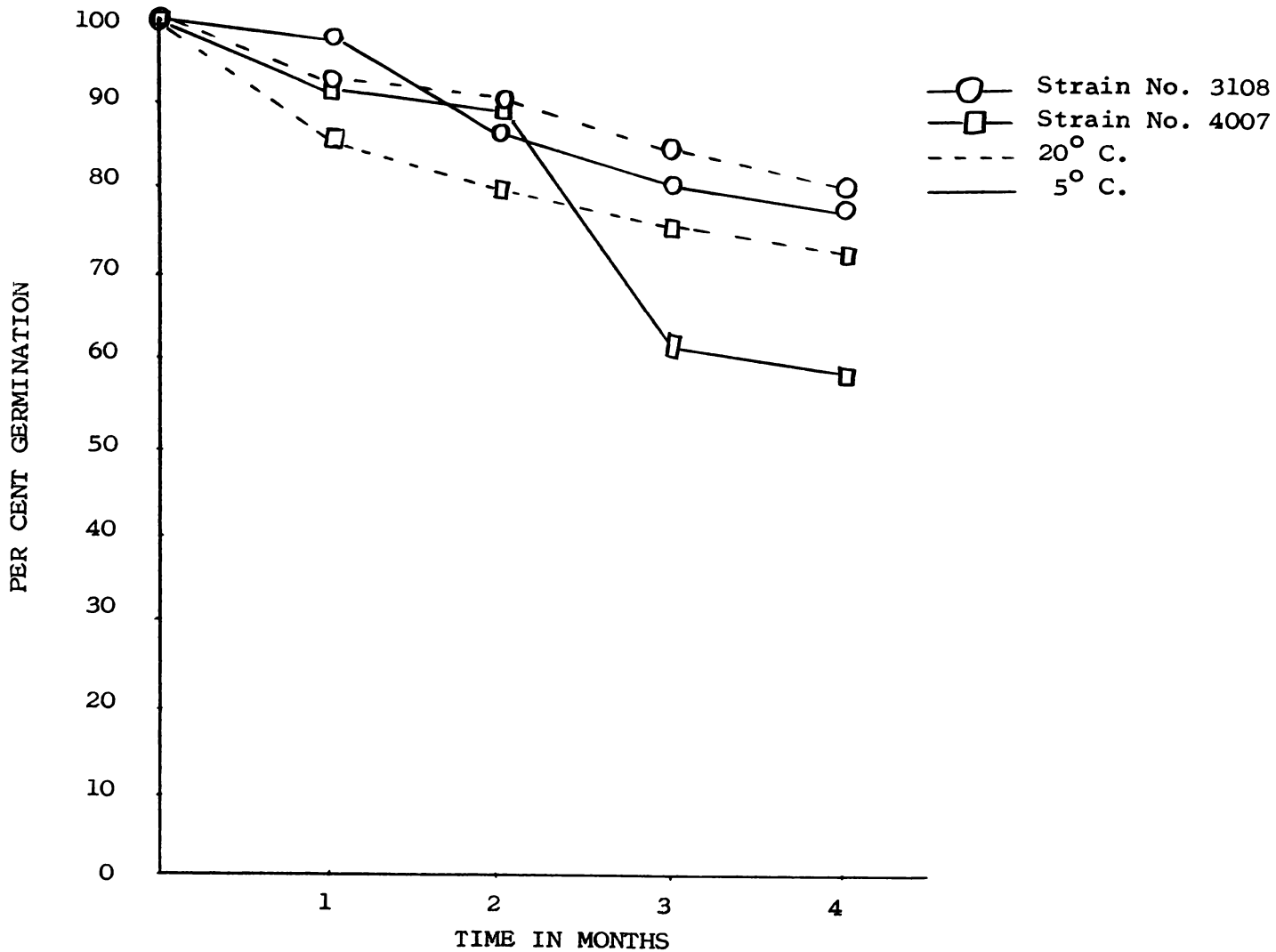

Figure 16. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at less than one per cent relative humidity.

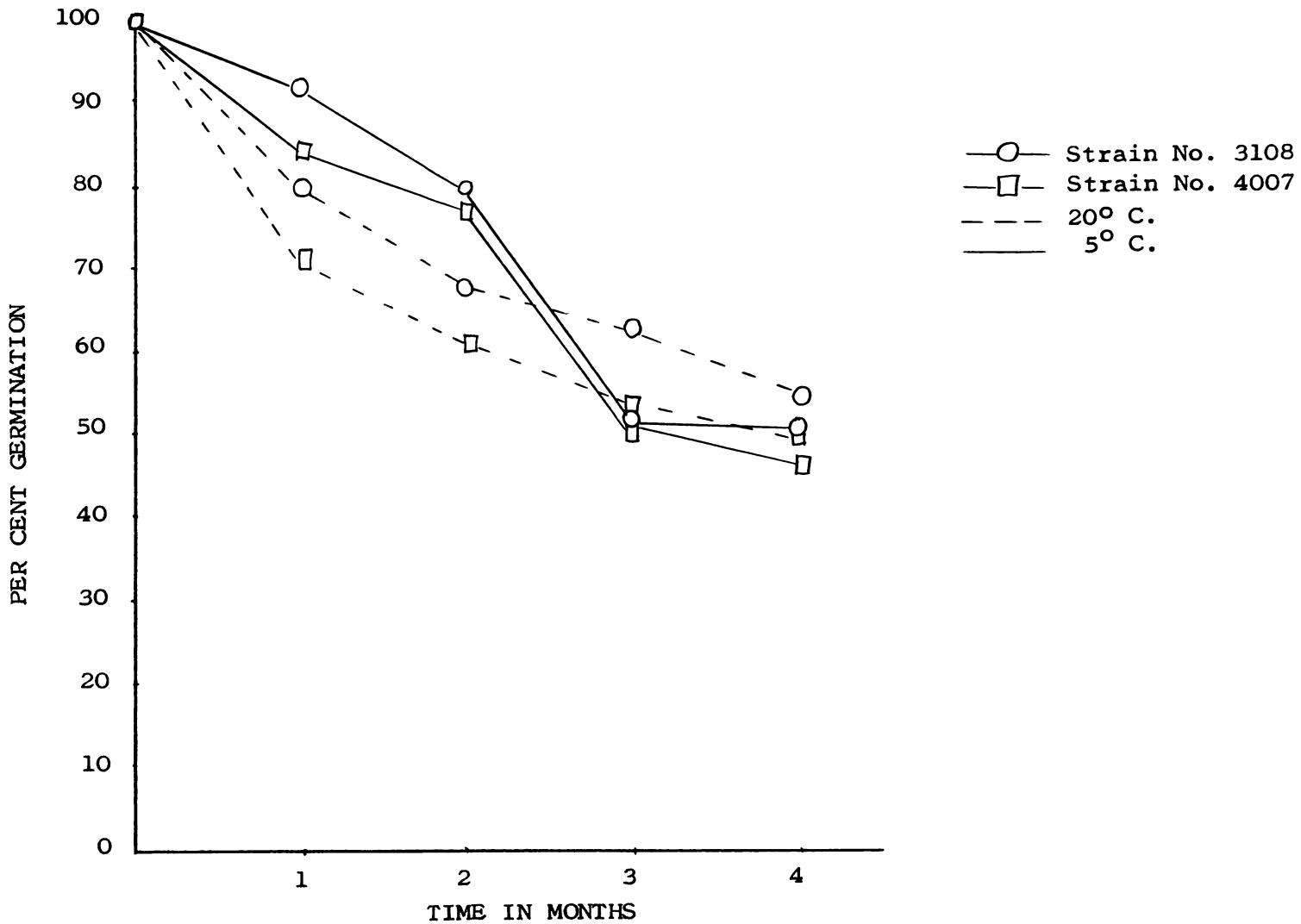

Figure 17. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 33.0 to 34.5 per cent relative humidity.

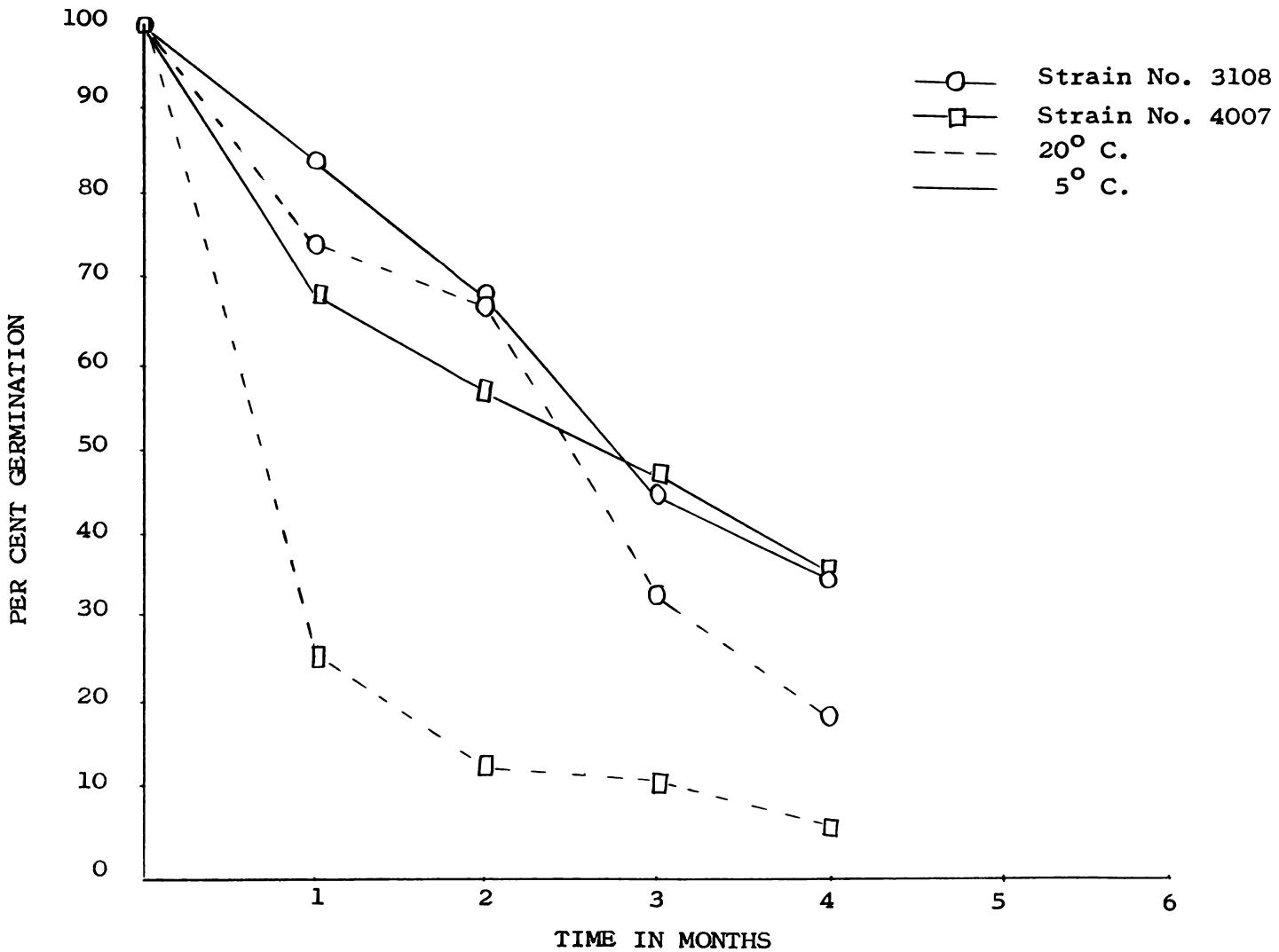

Figure 18. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 75.0 to 76.0 per cent relative humidity.

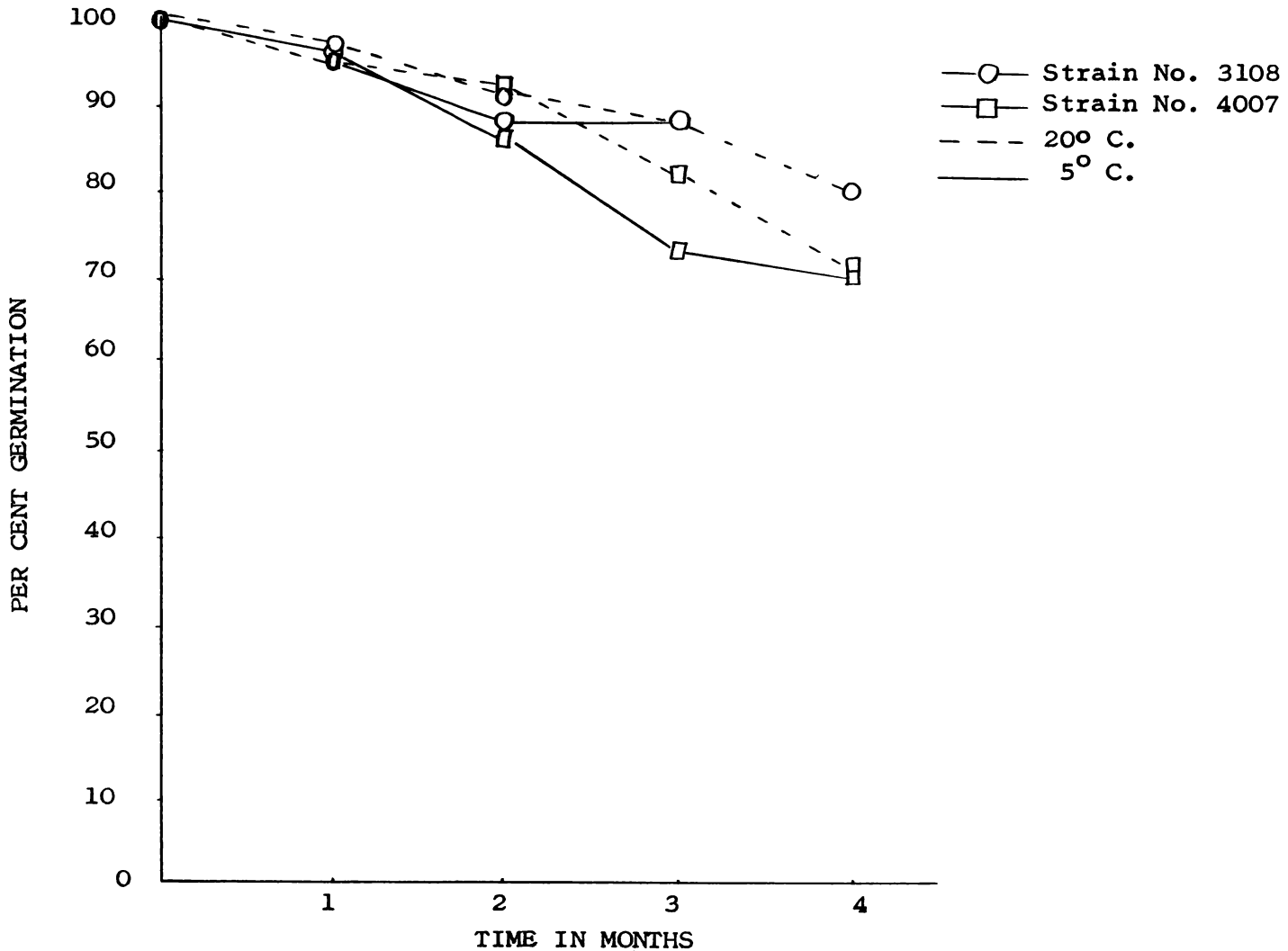

Figure 19. Effect of temperature on the longevity of the spores of two strains of the fungus, Beauveria bassiana, at 96.5 per cent relative humidity.

TABLE X

PERIODS OF SEVENTY-FIVE PER CENT GERMINATION OF THE SPORES OF TWO STRAINS OF THE FUNGUS, *BEAUVERIA BASSIANA*, AT 20° AND 5° C. WITH DIFFERENT RELATIVE HUMIDITIES (IN MONTHS)

Relative Humidity in Per Cent	Strain 3108		Strain 4007	
	20° C.	5° C.	20° C.	5° C.
1.0	4.0	4.0	3.4	2.5
33.0 to 34.5	1.4	2.2	0.9	2.1
75.0 to 76.0	0.95	1.6	0.35	0.75
96.5	4.0	4.0	3.6	2.8

from Figures 16, 17, 18, and 19 (pages 71 through 74, respectively).

From Table IX (page 68) it is clear that there is not much difference between the two temperatures, up to a period of four months, on the spores of Strain 3108 in the presence of both very low and very high relative humidities. But a marked difference was noted in the case of spores of Strain 4007. This leads to the conclusion that spores of Strain 3108 are more favored by low temperatures than are spores of Strain 4007. Variation in the length of life of spores of both strains is seen at these temperatures in the presence of both thirty-three to 34.5 and seventy-five to seventy-six per cent relative humidities.

Results obtained at 20° to 5° C. with different relative humidities were statistically analyzed to find out whether the results are significantly different. The results presented in Tables XXVI and XXVII (Appendix) were used for the analysis. The analyzed data are given in Table XXVIII (Appendix). According to the table, the results between the temperatures, between the strains, between the different months, and between the different relative humidities are significantly different at the 0.99 level. In view of their significant difference they were further analyzed by applying Duncan's New Multiple Range Test (wherever applicable) and the results of the analysis are given below.

	After			
	4 Months	3 Months	2 Months	1 Month
Means	55.39	62.04	74.80	83.25

From the above test it can be seen that the results between these different times of observation are significantly different at the 0.99 per cent level. Furthermore, the effect or, moreover, of these temperatures during different months was also significant, and a greater percentage of spores survived in the earlier months than in later months.

	Relative Humidity			
	75.0 to 76.0 %	33.0 to 34.5 %	<1.0 %	96.5 %
Means	43.18	64.78	81.69	85.81

From the result of the above analysis it can be clearly seen that the longevity of the spores of both strains are equal at both the less than one and the 96.5 per cent relative humidities. Also, these results are superior to those of the other two relative humidities (thirty-three to 34.5 and seventy-five to seventy-six per cent). The results of thirty-three to 34.5 per cent relative humidity differ significantly from those of seventy-five to seventy-six per cent relative humidity. Also, the results of thirty-three to 34.5 per cent relative humidity are superior to those of seventy-five to seventy-six per cent relative humidity.

In conclusion, it may be said that the spores of both strains of the fungus live longer at higher percentages of relative humidity and at lower temperatures than at normal and higher temperatures. Relative humidities of less than one and of 96.5 per cent seem to favor longer life of the spores.

Effect of Different Temperatures and Humidities on the Longevity of the Adults of Some Coleopterous Stored Grain Pests, in the Presence and Absence of Food

Callosobruchus maculatus (Fabr.), (Mylabridae), and Sitophilus oryzae (Linn.) (Curculionidae) are the two coleopterous insect pests that were used in the present investigation. The longevity of adults of these insects was studied under different temperatures and humidities. The object was to discover the otpimum conditions for greatest longevity. Since these insects breed in granaries, the effect of presence and absence of food on their length of life was also investigated. Experiments were conducted under different temperatures and humidities. Different temperatures and humidities employed for the investigation are given in Table XI.

As already mentioned under Materials and Methods (Section II), temperatures were maintained either in incubators, wooden cabinets or an air conditioned room. Relative humidities were maintained in wide-mouthed one-gallon glass bottles with air-tight lids. Sterile plastic dishes (95 x 35 mm.) with perforated lids were used for conducting the experiments. Wherever food was used, it consisted of seventy grams of grain. Twenty adult insects, selected at random irrespective of sex were used per replication. All the experiments were replicated three times. Observations were made once every three days for a period of nine days. This period of nine days was selected with the idea that the insects would be

TABLE XI

DIFFERENT TEMPERATURES AND HUMIDITIES EMPLOYED TO STUDY THE LONGEVITY OF THE ADULT RICE WEEVIL, *SITOPHILUS ORYZAE*, AND COWPEA WEEVIL, *CALLOSOBRUCHUS MACULATUS*

Temperature in $^{\circ}$ C.	Relative Humidities in Per Cent			
50°	< 1.0	31.5	72.0	92.5
40°	< 1.0	32.0	75.0	93.0
35°	< 1.0	32.5	75.5	96.0
30°	< 1.0	32.5	75.5	93.5
25°	< 1.0	30.5	75.5	92.5
20°	< 1.0	33.0	76.0	96.5

active in searching for their mates and suitable grains for laying eggs in the case of females. The period was thought optimum for the use of the pathogen for possible control before deposition of eggs. After each observation the dead insects were removed and counted. The results of these experiments with the two species are discussed in the following paragraphs.

Sitophilus oryzae. This weevil is commonly called the "rice weevil." Although it feeds and breeds on several different types of grain, wheat was used in the present investigation. Mortality rates observed after nine days at different temperatures and relative humidities are tabulated in Table XXIX (Appendix). Average per cent mortality obtained after nine days at different temperatures is illustrated in Figure 20, and tabulated in Table XII.

It appears that temperatures above 30° C. do not favor the longevity of this weevil, even in the presence of food, as shown in Table XII. Although some of the weevils survived at 35° C. with 75.5 and ninety-six per cent relative humidities, mortality was greater in the presence of wheat than in the absence of food. A higher percentage of weevils lived at 25° and 30° C. in the presence of food at all relative humidities except at those of less than one per cent. A greater number of weevils lived for more than nine days at temperatures of 20°, 25° and 30° C. than at other temperatures both in the presence and absence of food,

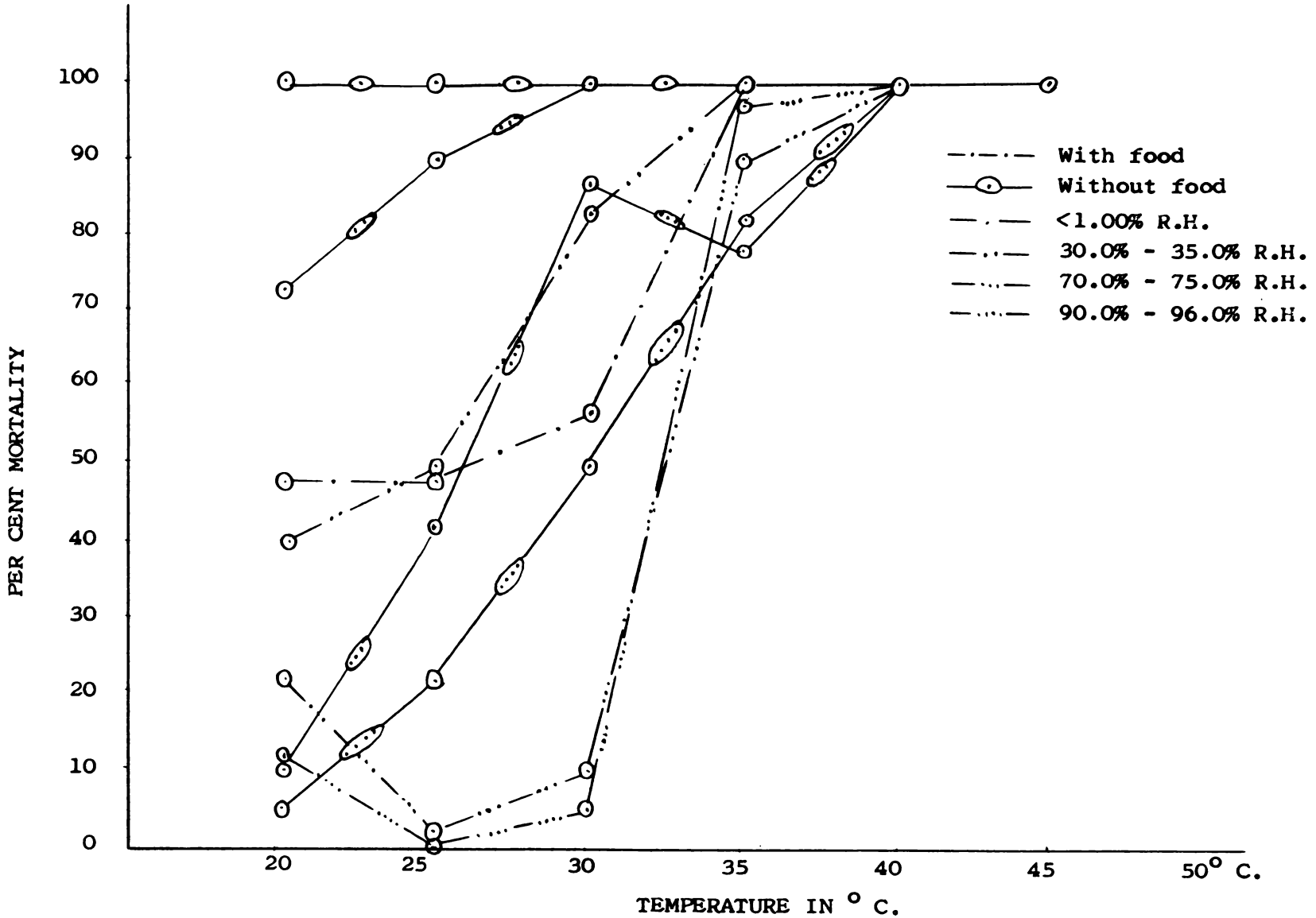

Figure 20. Effect of temperature and humidity on the longevity of the adult rice weevil, Sitophilus oryzae, in the presence and absence of food (wheat) after nine days.

TABLE XII

EFFECT OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE ADULT WEEVIL, *SITOPHILUS ORYZAE*, IN THE PRESENCE AND ABSENCE OF WHEAT AFTER NINE DAYS (IN PERCENTAGE OF MORTALITY)

Temperature °C.	Relative Humidity in Per Cent							
	<1.0		30.5 to 33.0		72.0 to 76.0		92.5 to 96.5	
	F*	WF**	F	WF	F	WF	F	WF
50°	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
40°	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
35°	100.0	100.0	100.0	100.0	90.0	78.3	96.7	81.7
30°	56.7	100.0	83.3	100.0	10.0	86.7	5.0	50.0
25°	48.3	100.0	50.0	90.0	1.7	41.7	0.0	21.7
20°	48.3	100.0	40.0	73.3	21.7	10.0	11.7	5.0

*F = With food.

**WF = Without food.

with 75.5 and 92.5 to 96.5 per cent relative humidities.

General observations made during these experiments revealed that these weevils are active at 25° and 30° C. At 20° C., they were found not active; and no damage was done to grains, whereas grains were damaged at 25° and 35° C. Hence, 25° and 30° C. were selected for studying the pathogenicity of the fungus, B. bassiana, on these weevils under Section III of this work.

Callosobruchus maculatus. This beetle is commonly called the "bean weevil." Blackeyed peas (dried beans) available in the groceries were used as food in all the experiments. Observations were taken once in three days for a period of nine days, and they have been tabulated in Table XXX (Appendix). Average percentage of mortality recorded after nine days at different temperatures and humidities is presented graphically in Figure 21. These results are also tabulated in Table XIII which shows the percentage mortality after nine days in the presence and absence of food under different conditions.

None of the beetles survived at 50° C. regardless of the percentage of relative humidity, as can be seen in Table XIII. The presence of both food and higher humidity severely affected the longevity of these beetles, both at 40° and 35° C. From Figure 20 (page 80) it is clear that 20°, 25°, and 30° C. are more favorable for the longevity of these beetles than the other temperatures in both the presence and the absence of food with

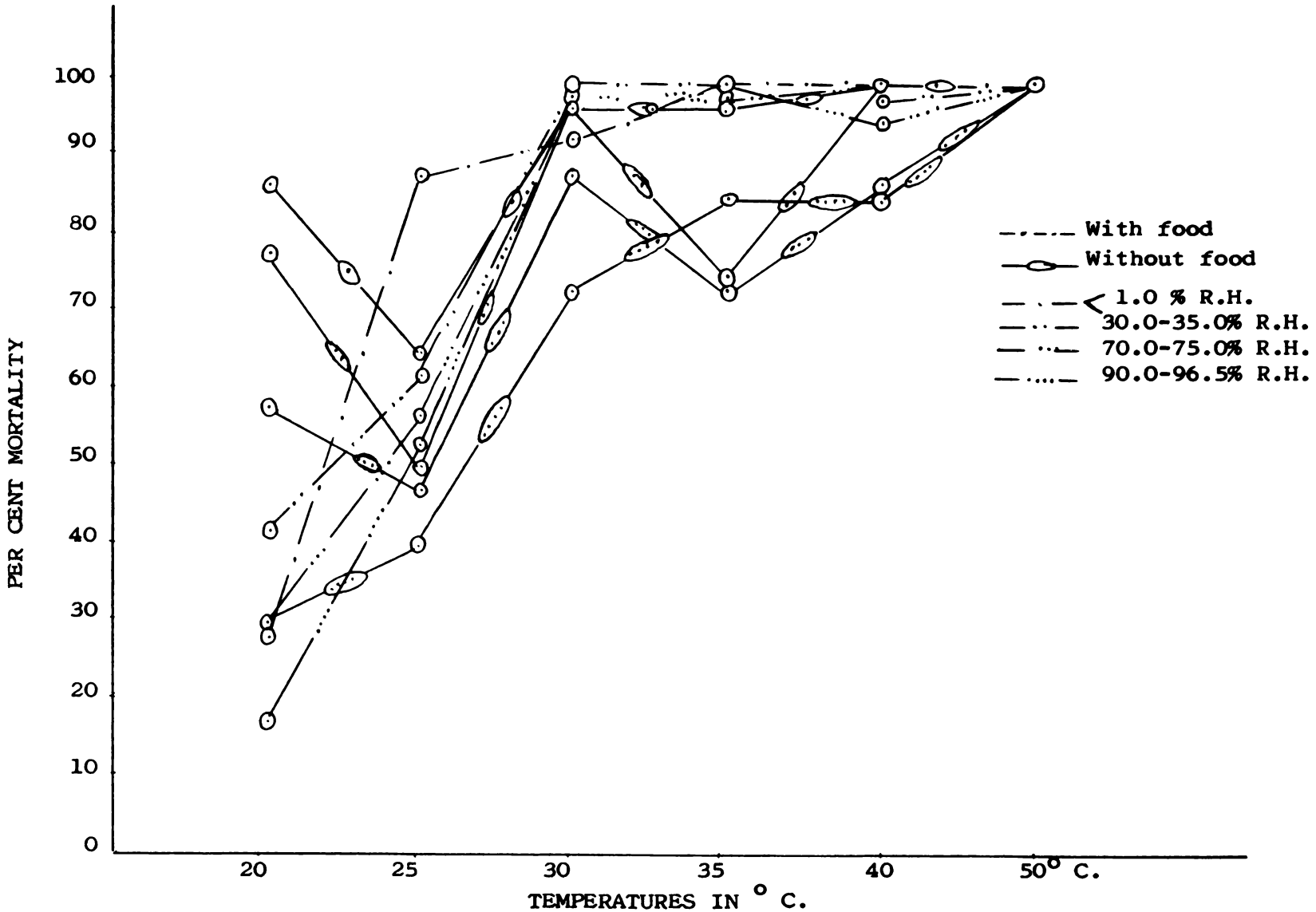

Figure 21. Effect of temperature and humidity on the longevity of the adult cowpea weevil, Callosobruchus maculatus, in the presence and absence of food (beans), after nine days.

TABLE XIII

EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE LONGEVITY OF THE ADULT WEEVIL, *CALLOSOBRUCHUS MACULATUS*, IN THE PRESENCE AND ABSENCE OF BEANS AFTER NINE DAYS (IN PERCENTAGE OF MORTALITY)

Temperature °C.	Relative Humidity in Per Cent							
	<1.0		30.5 to 33.0		72.0 to 76.0		92.5 to 96.5	
	F*	WF**	F	WF	F	WF	F	WF
50°	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
40°	100.0	100.0	98.3	100.0	100.0	86.7	95.0	85.0
35°	100.0	96.7	100.0	75.0	98.3	73.3	100.0	85.0
30°	93.3	96.7	100.0	96.7	98.3	88.3	96.7	73.3
25°	88.3	65.0	61.7	50.0	56.7	46.7	53.3	40.0
20°	28.3	87.2	41.7	78.3	30.0	58.3	16.7	30.0

*F = With food.

**WF = Without food.

all relative humidities. However, the results are inconsistent under all conditions.

In view of the fact that the insects were inactive at temperatures below 25° C., temperatures of 25° and 30° C. were selected for this insect, also.

IV. DISCUSSION

The observations made in the present investigation clearly show that both temperature and humidity have a great influence on the longevity of the spores of the fungus, B. bassiana. Similar reports have been made in the case of many other fungi which have been reviewed by Gottlieb (1950) and Cochrane (1958, 1960). Steinhaus (1960) has studied the effect of different temperatures on the spores of three strains of the fungus, B. bassiana. Clerk and Madelin (1965) have reported on the influence of both temperature and humidity on the longevity of the spores of this fungus. According to their report, the longevity of the spores of B. bassiana (in addition to two other species) decreases as the temperature of storage is increased from 8° to 25° C. Similar observations have been made by Steinhaus when the temperature was raised from 4° to 23° and 38° C. The observations made in the present investigation of the influence of temperature on the longevity of the spores of this fungus fully agree with the findings of the above workers (Figures 3, 4, 5, 6, pages 39 through 42, Figures 10 and 11, pages 55 and 56, Figure 13, page

58, Figures 16, 17, 18 and 19, pages 71 through 74).

The observations made on the influence of different relative humidities on the longevity of the spores of B. bassiana in the present investigation (Figure 1, page 32, Figure 2, page 35, Figure 7, page 45, Figure 8, page 49, Figure 9, page 53, Figure 14, page 65, and Figure 15, page 68) seem to confirm the results obtained by Clerk and Madelin at temperatures of 8°, 18° and 25° C. with 0.0, 34.3, and 75.2 per cent relative humidity in the presence of light (Table I of Clerk and Madelin, 1965). However, these workers seem not to have considered relative humidities above ninety per cent and temperatures above 25° C. at which different results have been obtained. At temperatures of 35°, 30°, 25°, 20°, and 5° C. with relative humidities of 92.5 to 96.5 per cent, the longevity of the spores of the fungus was only slightly less than the length of life with less than one per cent relative humidity. On the other hand, at temperatures of 50° and 40° C. spore longevity was very much shortened in the presence of 92.5 to ninety-three per cent relative humidity. Though none of the available literature on this aspect shows such results in the case of B. bassiana, reports made in the case of spores of some other fungi indicate similar results. For example, Teitell (1958) discovered this type of relationship, i.e., survival of a greater percentage of spores for a longer period at both low and high relative humidities and minimum at intermediate relative humidities within certain temperatures (29° and 40° C.). This was in the case of

two Aspergillus spp. Moreover, he also noticed a shift in the lethal relative humidity between seventy-five and eighty per cent with increase in temperature. However, a minimum of viability was observed at 45° and 48° C. irrespective of the percentage of relative humidity. Length of life was also short. The observations made in the present investigation of the influence of different relative humidities on the longevity of the spores of the fungus, B. bassiana, seem to show similar responses, as observed by Tietell.

The relationship observed, in the present investigation, between the longevity of the spores of the fungus, B. bassiana, with different temperatures and humidities may be theoretically hypothesized as follows.

Failure to survive for a longer period in large numbers at very high constant temperatures (50° and 40° C.), irrespective of the percentage of relative humidity, may be due to effects on the cellular activities of the spores. In the absence of humidity or in the presence of very low relative humidity, both free and bound water may be removed by desiccation which results in the death of the spores. Increased metabolic activities and exhaustion of food material due to these higher temperatures in the presence of higher relative humidities may also be responsible for early death of the spores (Cochrane, 1958; Deverall, 1965). At low temperatures the spores survive for a longer period. This may be the result of a reduced rate of metabolism.

With regard to the influence of different relative humidities on the longevity of the spores of the fungus at different temperatures, the hypothesis presented by Teitell (1958) seems to appear plausible. Presumably, the spores may spend their life in dormancy at low relative humidities. At intermediate relative humidities (seventy-one to seventy-six per cent), the metabolic activities of the spores may be changed and this may affect their state of dormancy. This may be due to absorption of sufficient moisture to break the dormant condition and interfere with the vital processes in such a way as to result in their death. The favorable influence of higher relative humidity (above ninety per cent in the case of B. bassiana) on the metabolic activity of the spore may be responsible for the higher rate of survival at both low and moderate temperatures.

It is known that when spores are dormant respiration still proceeds at a low rate. Such being the case, some of the adverse conditions of temperature and humidity may also interfere with the respiratory activities of the dormant spore and result in its early death.

Variations in the longevity of the spores of different strains of the fungus, B. bassiana, due to the influence of temperature have been reported by Steinhaus (1960). Such a significant difference in the length of life between the strains (3108 and 4007) used in the present investigation was not observed under the different temperatures and humidities tested.

Moreover, significant differences were seen in longevity at all combinations of temperature and humidity.

With these observations it may be concluded that the longevity of the spores of the fungus, B. bassiana, are influenced by both temperature and humidity. Length of life will decrease with an increase in temperature, irrespective of the percentage of relative humidity.

That humidity also has some influence can be seen in Figure 1, page 32, Figure 2, page 35, Figure 7, page 45, Figure 8, page 49, Figure 9, page 53, Figure 14, page 64 and Figure 15, page 67. Low and high relative humidities were found to be more favorable to the spores of the fungus for their survival and long life span than intermediate relative humidities (seventy-one to seventy-six per cent) at temperatures below 35° C.

Effect of Temperature and Humidity on the Longevity of the Adults of the Coleopterous Stored Grain Pests in the Presence and Absence of Food

The observations made on this aspect of the work are discussed in the following paragraphs under the respective species of insects.

Sitophilus oryzae. The (constant) higher temperatures (50°, 40°, and 35° C.) seemed not to be favorable for the longevity of the adults of this species of rice weevil. Temperatures of 50° and 40° C. were soon fatal at all percentages of

humidities tested (Table XII, page 81). The moderate temperatures (30°, 25°, and 20° C.) were favorable. The presence of less than one per cent relative humidity was detrimental to their long life. However, the presence of food (wheat containing 13.45 per cent of moisture) favored greater longevity than the absence of food at this relative humidity. The moisture present in the grains might have caused this; otherwise, they were probably subjected to desiccation at this relative humidity and killed. Presence of food and moderately high relative humidity were more favorable to the longevity of the adults at 30° and 25° C. than absence of food and lower relative humidity (less than one and 30.5 to 33.5 per cent).

Callosobruchus maculatus. The adults of this beetle lived longer at moderate temperatures than at higher temperatures. However, presence of food, and high humidity at 30° and 25° C. did not seem to favor the adult. Temperatures of 50°, 40° and 35° C. irrespective of different relative humidities (tested both in the presence and absence of food) seemed not to be favorable. Fifty degrees Centigrade was quickly fatal to the adults. Twenty degrees Centigrade, though found favorable to their longevity, was found not favorable to their activity. Temperatures of 30° and 25° were favorable (with 30.5 to 33.5 and seventy-one and seventy-six per cent relative humidities) both in the presence and absence of beans.

In conclusion, it may be said that moderate temperatures with relative humidities above 30.5 per cent are more favorable than others for the longevity of both of these coleopterous stored grain pests.

SECTION III

POTENTIALITIES OF UTILIZING THE ENTOMOGENOUS FUNGUS, *BEAUVERIA BASSIANA* (BALS.) VUILL., (FUNGI IMPERFECTI) FOR THE CONTROL OF SOME COLEOPTEROUS STORED GRAIN PESTS

I. REVIEW OF LITERATURE

Beauveria bassiana is known to be a parasite of many species belonging to several orders of insects. It has a world-wide distribution, occurring particularly wherever the silkworm, *Bombyx mori* (Linn.), is reared commercially. The disease caused by this fungus is called "muscardine" or "white muscardine." Apart from its pathogenicity on commercial insects like the silkworm, it has been recorded by many workers on both economically important and unimportant insects. Several workers have made successful attempts to utilize this fungus for microbial control. This has been reviewed by Baird (1958). Further investigations have been made to use this fungus as a microbial agent. Some investigators have attempted to use the fungus in the form of spores and also as an extract of spores called "Boverin" in combination with certain insecticides. Such workers are Blonska (1957), York (1958), Hsu et al., (1959), Telenha (1959), Smith and York (1960), Frye and Raun (1961), Smith (1961), Petrukhina (1961), Mclaughlin (1962), Zhigaev (1962 and 1963), and Dunn and Mechalas (1963). Certain

of these works have been reviewed by Tanada (1959), Sweetman (1963), Hall (1963 and 1964), and Madelin (1963 and 1966) while reviewing the prospects of microbial control.

Very few workers have either reported the occurrence of B. bassiana, or attempted to find out the potentialities of utilizing this fungus for microbial control of stored grain insect pests. Therefore, an attempt has been made to review the available literature on this subject.

Maran (1948) seems to be the first worker to report on the susceptibility of Tenebrio molitor (Linn.), Calandra granaria (Linn.), and Ephestia kuhiniella Zell., to the species of B. brumpti Langeron, which was isolated from a case of human kerato-conjunctivitis. Larvae of Tenebrio were found to succumb after nine to fourteen days; larval Ephestia after twelve to fourteen days; and imagos of Ephestia and Calandra (= Sitophilus) died after two to four days and eight to eighteen days respectively, at a temperature range of 18° to 21° C. Furthermore, development of immunity was observed in all the test insects, not only to B. brumpti, but also to B. bassiana, B. densa, B. globulifera, B. doryphorae, and B. stephanoderis. B. brumpti was transferred to the genus Tritirachium by MacLeod (1954) when he revised and reclassified the different species of Beauveria and Tritirachium, after critical investigations on morphological and cultural characteristics of both genera.

One other worker who studied the susceptibility of stored

product insects to B. bassiana is Dresner (1949). He dusted rice grains with a small quantity of pure spore material of the fungus and introduced ten adult insects of Sitophilus oryzae. At a temperature of 65° to 85° F. and a relative humidity of thirty-eight to eighty-five per cent, the whole population was killed within fifteen days. Control of Tribolium confusum was unsatisfactory.

Steinhaus and Bell (1953) seem to be the earliest workers who have made an attempt to conduct preliminary tests to find out the possibilities of using different microorganisms for the control of some of the stored grain insects. B. bassiana was one, among other microorganisms, used for their investigations. Tests were conducted in Petri dishes, half-pint cartons, and in one-gallon jars which were partially filled with wheat grains treated with different concentrations of spores of the fungus. The method of application followed was either dusting or immersing the grains in aqueous suspension. Adults of Sitophilus granarius (Linn.), S. oryzae (Linn.), Rhizopertha dominica (Fabr.), Tribolium confusum (Duv.), and Sitotroga cerealella (Oliv.), were used for infectivity tests. According to these workers, coleopterans were found susceptible to B. bassiana in varying degrees, but not to the extent of the fungus being practical for their control. Adults of S. cerealella were not found susceptible to this pathogen.

During the course of the above investigations, the same

workers also made an attempt to investigate the effect of a toxin produced during the germination of the spores of B. bassiana on the adults of S. cerealella, T. confusum, and S. granarius. The presence of a toxin was reported by Dresner (1950) in his studies on infectivity of the adults of Musca domestica; the green dock beetle, Gastroidea cyanea Melsh; and the larvae of potato tuber moth, Gnorimoschema operculella (Zeller). The authors studied the effects of two strains of B. bassiana on various stages of the test insects. None of them showed any significant toxic reaction to contact with the fungal spores.

After a decade, Dunn and Mechalas (1963) experimented with B. bassiana and the adults of S. granarius and T. confusum. Their laboratory trials involved wheat treated with spores of the fungus and was tested against the two species of insects listed above. Control was moderate to good with concentrations of 0.123 and 0.5 per cent of spore material. The trial was held at sixty-five to seventy per cent relative humidity and 26° C. These investigators are of the opinion that high dosages or massive field inoculation will be necessary for the successful use of B. bassiana.

II. MATERIALS AND METHODS

Research carried out in the laboratory frequently serves as a basis for practical studies in the field. From this point of view, investigations of any kind will have to be planned and carried out in such a way that the results may be utilized

(hopefully) in applied research. Furthermore, the combinations of factors studied in experimental work have always to be chosen with due regard to the conditions which the organism is likely to encounter in nature, if any positive correlation is to be realized.

With this idea in mind, an attempt was made to investigate the influence of different combinations of temperatures and humidity on the pathogenicity of B. bassiana on S. oryzae and C. maculatus. Following the results of these experiments, attempts were made to investigate the potentialities of using this fungus to prevent the infestation of stored wheat by S. oryzae and beans (blackeyed peas) by C. maculatus. Potential control was tested by treating the grains with different concentrations of the fungal spores.

As the same two strains of the fungus and the same species of host insects were used for the investigations, the methods of culturing and collecting of spores, rearing of host insects, and the maintenance of temperature (25° and 30° C) and humidity (0.00, 30.5 and 32.5, 72.5 and 92.5 and 93.5) were exactly the same as the ones followed in Section II. Hence the reader is referred to Materials and Methods of Section II.

Different concentrations of the pathogenic organism were prepared by using spores three to four weeks old (ninety-eight to one hundred per cent viability). Spore material was crushed and mixed well in a suitable container (conical flasks) with the aid of plastic stirring bars and an electrical Stir Plate. Alphacel,

(purified nonnutritive cellulose) manufactured by Nutritional Biochemicals Corporation, Cleveland, Ohio was used as a diluent. Different concentrations used in these investigations were chosen based on the results of previous workers (Dresner, 1949; Dunn and Mechalas, 1963). The spore material was kept in a cool place where the temperature was about 20° to 22° C. The material was stored in a sterile bottle with an air-tight screw-type lid. Whenever new spore material was used, it was tested for viability following the same method as explained in Section II.

Dosages used for both direct treatment of insects and treatment of grains were decided upon after the required preliminary tests. These tests were made by using different known quantities of the diluent along with twenty insects in Petri dishes. The size of the Petri dishes was 100 x 15 mm. On transfer of both diluent and insects to the Petri dishes, they were shaken well by hand. It was found that 25 mg. of dusting material was sufficient to cover the inside of a Petri dish as well as the bodies of the insects used. Fifteen and 20 mg. were found too low and 30 and 35 mg. were considered more than enough. The same procedure was followed to find a suitable dosage for treating the grains. One pound of grain of each type and forty insects of each species was tested separately with different dosages. One gram of dusting material was found sufficient for both species of insects.

Concentrations of pathogen used for testing the insects by direct treatment were 0.5, one and two per cent by weight.

After observing the performance of different concentrations under direct treatment, pathogenic material of 0.25, 0.5 and one per cent strength were used for treatment of grains.

A minimum of four replications per treatment was maintained throughout these experiments. Twenty insects selected at random, irrespective of sex (as it is very difficult to separate sexes when they are living), were used in both species of insects for testing by direct treatment with the pathogen; whereas grains treated with the pathogen were infested with forty insects selected at random in the same manner.

Method of Studying the Effect of the Pathogen on the Insects

Sterile disposable plastic Petri dishes, 100 x 15 mm. in size, were used for the experiments. The spores at different concentrations were put into these dishes, the dosage being 25 mg. of diluted material per dish, and the insects were transferred as early as possible. The Petri dishes were shaken well to insure contact of the insects with the pathogen. Then the plates were transferred to the respective temperatures and humidities. The control of insects were treated with only diluent material (25 mg.). Mortalities were recorded every three days for a period of nine days. The dead insects were removed following counting.

Method of Evaluation of Treated Grains Against Insect Infestation

As stated earlier, the grains were mixed with one gram of spores at several concentrations. The quantity of grains used

per bottle was one pound. The mixing was accomplished by using a specially built seed dressing drum, which is also called a "seed mixer" and operated by hand. This equipment is commonly used for mixing diluted insecticides or fungicides with seeds to prevent the attack of insects or disease causing agents. One quart size wide-mouth glass bottles with screw-type lids were used for conducting these experiments. Wheat and beans were used for *S. oryzae* and *C. maculatus*, respectively. Forty insects selected at random, irrespective of sex were introduced after a lapse of thirty minutes following mixing of the grains with the pathogen. The bottles were placed in a room which was maintained at a temperature of 25 $\pm$ 1° C. and a relative humidity varying from thirty to fifty per cent. Bottles were not shaken after introduction of the insects and the lids were left loose. The moisture content of the grains was as follows:

Wheat (Knox. 407, red coat)	13.45 per cent*
Beans (Blackeyed Peas from stores)	9.67 per cent**

The duration of the testing period differed, depending upon the length of the life-cycle of the species of insect. Both species were given time to complete two generations. Ten weeks was allowed for the granary weevil on wheat and eight weeks for the bean weevil on beans.

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*Tested in Sterilite Moisture Tester available in Agronomy Division, The University of Tennessee, Knoxville.

**Tested by Oven-dry method.

Following the scheduled period, the bottles were transferred to the cold room, which was maintained at 3° to 5° C., for killing the insects. Since it is very difficult to estimate the percentage of damage, if any, and to count the number of insects when they are active, a method of stratified sampling was adopted to study the percentage of damage. The following explains the method.

The contents of the bottle were divided into three parts by marking the outside with a wax pencil. Each part, for example the top layer, was taken out with the aid of a spoon and poured onto a white paper of 11" x 8" size. This paper was marked previously with four thick parallel lines of six inches length, drawn three inches apart. The contents of the top layer present on such a paper were mixed well and spread to cover all the four lines. Each line represented a replication, thus four samples were taken from each layer. The grains which appeared on each line formed one sample. These were taken out and the damaged and undamaged grains counted and recorded. The same procedure was followed for all the three parts of each bottle and for both types of grains.

There were differences in identifying the damage. With *S. oryzae*, grains which were obviously damaged and those showing indication of damage were considered the same and counted. With *C. maculatus*, the presence of eggs was taken into consideration, in addition to actual damaged grains. Grains showing actual damage possess a hole through which the adult emerges, or they

visibly reveal the presence of a larva or pupa. It would be wrong to count the number of insects in each layer of the bottle because many of them had come up to the surface, particularly in the case of the granary weevil. This may be due to increase of heat at the bottom, increase in moisture which had resulted in clotting of both grains and excreta and the dead insects, and absence of air. Population density may also be one of the reasons for upward migration, in addition to attraction to light. Hence, the total number of insects present in each bottle was taken into consideration. Each treatment had four replications. Each replication consisted of the average of percentage of damage of the three layers. And the percentage of damage of each layer was the average of four samples taken from each layer.

As both insects breed within the grains, other life stages were not considered in the evaluation. However, tests for their susceptibility were conducted wherever possible.

Statistical Analysis

Results were analyzed by following the method of "Analysis of Variance" and "Duncan's New Multiple Range Test," as mentioned earlier under "Statistical Analysis" of Section II.

III. RESULTS

Preliminary Observations

Preliminary tests to determine the susceptibility of the host insects to the fungus were conducted in a room which was

maintained at a temperature of 23° to 25° C. and a relative humidity of thirty to fifty per cent. Adults of both species of insects were used for the test with the two strains (3108 and 4007) of the fungus. However, in the case of the bean weevil, C. maculatus, larvae and pupae were also tested irrespective of their ages.

Ten adults of both S. oryzae and C. maculatus were directly treated with pure spores of both strains. These tests were made in sterile disposable plastic Petri dishes of 100 x 15 mm. size. Dishes were well shaken after addition of both the spores and insects in order to insure contact of the insects with the pathogen. There was one hundered per cent mortality after eight days in the treated insects of both species whereas it was only ten to twenty per cent in the controls. Similar tests were conducted with both larvae and pupae of C. maculatus. Here also ten larvae and ten pupae were treated which were collected from mass culture material. Eight adults emerged from ten pupae in the untreated dishes, whereas there was one hundred per cent mortality in the treated dishes after ten days. One hundred per cent kill was recorded in the case of treated larvae, with a twenty per cent death in the controls. In the above tests, the controls were maintained without any treatment.

Another preliminary test was conducted to test the effect of diluted materials. Twenty adult insects were used in both species. A concentration of 0.5 per cent spore material was prepared. Alphacel was used as the diluent. The dosage was

25 mg. of diluted material per Petri dish. These tests were also carried out in the plastic Petri dishes. Insects were brought into contact with the pathogen by shaking the Petri dishes. Control insects were treated with Alphacel only. Results obtained are summarized in Table XIV.

Since these preliminary tests gave an indication of the susceptibility of the adult insects to this fungus, in addition to the reports of the previous workers, investigations were made by using different concentrations. Furthermore, with a view to determine the effect of different relative humidities on the action of the fungus under favorable temperatures, experiments were run at different humidities, namely, less than one, 30.5 to 32.5, seventy-four and 72.5, and ninety-two and 93.5 per cent at 25° and 30° C., respectively. Results obtained at these conditions are presented below.

Effect of Different Relative Humidities on the Pathogenicity of the Spores of Two Strains of the Fungus on Adult Insect Pests of Stored Grains at 25° and 30° C.

Since these insect species were found to live longer and be more active at temperatures of 25° and 30° C. than at the other temperatures tested, according to the results obtained in Section II (Results), investigations were carried out only at these temperatures. The four different relative humidities, as mentioned earlier, were maintained. The larvae of the rice weevil, *S. oryzae*, and the bean weevil, *C. maculatus*, live inside the grains; there-

TABLE XIV

RESULTS OF PRELIMINARY OBSERVATIONS ON THE SUSCEPTIBILITY OF THE ADULTS OF THE RICE WEEVIL, *SITOPHILUS ORYZAE*, AND THE BEAN WEEVIL, *CALLOSOBRUCHUS MACULATUS*, TO THE SPORES OF DIFFERENT STRAINS OF THE FUNGUS, *BEAUVERIA BASSIANA*

		Mortality in Per Cent			
Insect	Treatment	4	8	12	16 days
Sitophilus oryzae	Strain 3108	0.0	5.0	70.0	80.0
Sitophilus oryzae	Strain 4007	0.0	20.0	65.0	85.0
Sitophilus oryzae	Control	0.0	0.0	35.0	70.0
Callosobruchus maculatus	Strain 3108	20.0	75.0	85.0	90.0
Callosobruchus maculatus	Strain 4007	35.0	75.0	90.0	95.0
Callosobruchus maculatus	Control	20.0	30.0	35.0	60.0

fore, only adults were used for the investigations. Concentrations used were 0.5, one, and two per cent of spores. Spores of both strains, 3108 and 4007, were tested. Twenty-five mg. of diluted spore material per Petri dish was the dosage, applied in the form of dust. Pure diluent was used for the control. Sterile disposable plastic Petri dishes of 100 x 15 mm. size were made use of for running these experiments.

A temperature of 30 $\pm 1^{\circ}$ C. was maintained in a wooden cabinet containing a fixed thermostat for regulating the temperature. An air condition room maintained at a temperature of 25 $\pm 1^{\circ}$ C. was used for this temperature. Wide-mouthed one gallon bottles were used for maintenance of the relative humidities.

Twenty insects, selected at random irrespective of sex were used per replication. Each experiment was replicated four times. As the purpose of this experiment was to find the effect of the pathogen on the insect by direct treatment, direct contact was effected by shaking the dishes by hand before transfer to the respective relative humidities. Observations were taken once in three days for a period of nine days. As stated earlier, this period of nine days was chosen after considering the time required for infection, the pre-oviposition period of the insects, and the effect of higher relative humidities on the grains. Dead insects were removed after counting.

The results obtained at both temperatures with the different relative humidities are discussed separately pertaining to each

species of insect, in the following paragraphs.

Sitophilus oryzae. The rice weevil was susceptible to the spores of both strains of the fungus, at both 25° and 30° C. with relative humidities of 92.5 and 93.5 per cent, respectively. Details of observations made after nine days are given in Table XXXI in the Appendix. Mortality with different concentrations under different conditions is illustrated in Figure 22. A consolidated statement showing the effect of both concentrations and different relative humidities on the infectivity of the fungus, after nine days is tabulated in Table XV.

It is seen from Table XV that this weevil is not able to survive long at the very low relative humidity of less than one per cent at both 25° and 30° C. Although the insect is not able to survive long at 30° C. in the presence of 32.5 per cent relative humidity, about fifteen per cent survived at 25° C. with 30.5 per cent relative humidity. About a two to five per cent rise in mortality due to this fungus was observed at 25° C., with 30.5 per cent relative humidity, when compared to the control. In the presence of 75.5 per cent relative humidity, very little control was recorded when compared to the control. A relative humidity of 92.5 per cent seemed to favor the infection by the fungus as can be seen from Table XV, at both 25° and 30° C. When compared to the control, a slight increase in mortality with treatment by the pathogen was recorded at 30° C., in the presence of 75.5 per cent relative humidity.

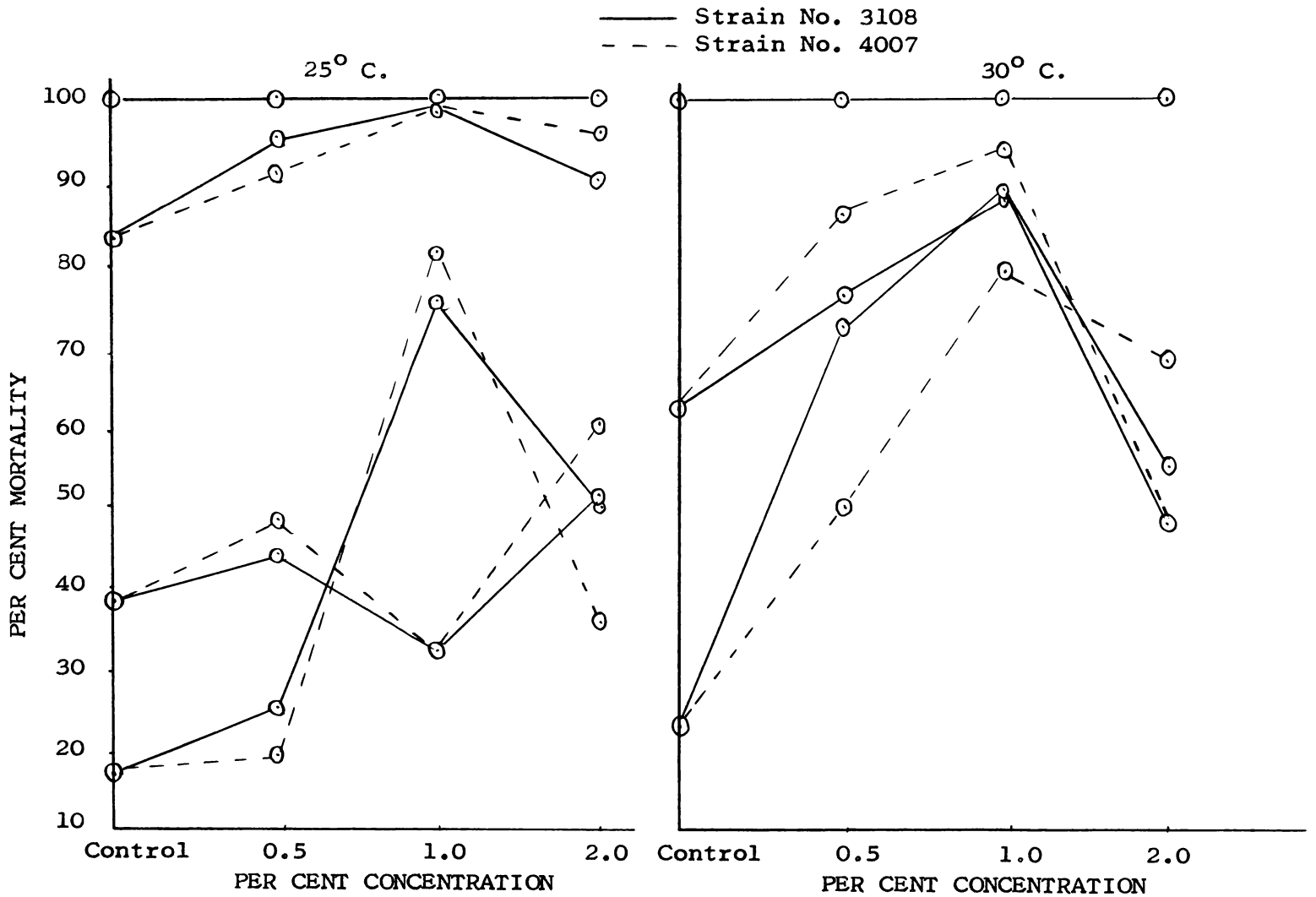

Figure 22. Effect of humidity on the pathogenicity of the spores of two strains of the fungus, Beauveria bassiana, to the adult rice weevil, Sitophilus oryzae at different temperatures (after nine days).

TABLE XV

INFLUENCE OF HUMIDITY ON THE PATHOGENICITY OF *BEAUVERIA BASSIANA* TO THE ADULT WEEVIL, *SITOPHILUS ORYZAE* AT 25° C. and 30° C., AFTER NINE DAYS (IN PERCENTAGE OF MORTALITY)

Concentration of Spores in Per Cent	Temperature 25° C.				Temperature 30° C.			
	Relative Humidities in %				Relative Humidities in %			
	1.0	30.5	75.5	92.5	1.0	32.5	75.5	93.5
				Strain 3108				
0.5	100.0	95.75	43.75	21.25	100.0	100.0	76.25	72.50
1.0	100.0	98.75	32.50	75.00	100.0	100.0	87.50	78.75
2.0	100.0	90.00	43.75	50.00	100.0	100.0	55.0	47.50
Control	100.0	83.33	38.33	46.66	100.0	100.0	64.99	23.33
				Strain 4007				
0.5	100.0	91.25	47.50	17.50	100.0	100.0	86.25	50.00
1.0	100.0	98.75	32.50	81.25	100.0	100.0	93.75	78.75
2.0	100.0	96.25	61.25	36.25	100.0	100.0	66.25	68.75
Control	100.0	83.33	38.33	16.66	100.0	100.0	64.99	23.22

Note: Mortality after nine days and expressed in per cent.

Among the different spore concentrations tested, 0.5, one and two per cent by weight) the one per cent concentration appeared optimum. No marked difference was noted between the strains tested with regard to insect mortality.

The results given in Table XXXI (Appendix) were analyzed to find whether these results are statistically significant. Results of analysis are presented in Table XXXII in the Appendix. It is seen from the table that the results between temperatures, between treatment, and between the different relative humidities are significant at the 0.99 level. It is also clear from the table that the effect of 30° C. on the pathogenicity of spores to the adult weevil is greater than at 25° C. There was no statistical significance between strains.

In view of their significance, Duncan's New Multiple Range Test was used to compare the effect of different relative humidities and different concentrations. The results of the test are presented below. The means underscored by a common bar indicate that their effects are equal. A break in the bar represents a difference between such factors.

	Relative Humidity			
	92.5 to 93.5	75.5	30.5 to 32.5	1.0
Means	47.34	58.30	96.04	100.0

According to the analysis shown above the higher relative humidities (75.5 and 92.5 to 93.5 per cent) have a different effect on the mortality of the weevils. Less than one and 30.5 to 32.5 per cent

relative humidites do not differ in their effect, but the effect of 75.5 per cent relative humidity is superior to the effect of 92.5 to 93.5 per cent. Low relative humidities are superior to higher relative humidities with regard to mortality of the adult weevils.

	Control	0.5%	2.0%	1.0%
Means	65.83	75.07	75.93	84.84

In general, the results between treatments of different concentrations are equal. Results of one per cent differ from the control and are also superior to the control. There is no difference between the results of the control group and the treatments of 0.5 and two per cent.

From the results obtained in the above experiments regarding the effects of different relative humidities and different concentrations of the spores of the fungus, B. bassiana, it is clear that the adult weevil is susceptible to the fungus. Humidities in excess of ninety per cent proved superior for infection by these strains. A concentration of one per cent was more effective than the other concentrations (0.5 and two per cent) when compared to the control.

Callosobruchus maculatus. The adult bean weevil was found to be only slightly susceptible to the spores of both strains of the fungus (Table XVI). However, detailed results are presented in Table XXXIII in the Appendix. Average percentage of mortality

TABLE XVI

INFLUENCE OF HUMIDITY ON THE PATHOGENICITY OF *BEAUVERIA BASSIANA* TO THE ADULT BEETLE, *CALLOSOBRUCHUS MACULATUS* AT 25° C. AND 30° C., AFTER NINE DAYS (IN PERCENTAGE OF MORTALITY)

Concentration of Spores in Per Cent	Temperature 25° C. Relative Humidities in %				Temperature 30° C. Relative Humidities in %			
	1.0	30.5	75.5	92.5	1.0	32.5	75.5	93.5
				Strain 3108				
0.5	86.25	90.0	86.25	81.25	96.25	83.75	78.75	98.75
1.0	100.00	98.75	100.00	100.00	96.25	95.00	85.00	100.00
2.0	95.00	96.25	77.50	100.00	100.00	98.75	92.50	100.00
Control	76.66	71.25	58.75	68.33	99.16	81.66	84.58	97.50
				Strain 4007				
0.5	98.75	88.75	72.50	88.75	91.25	76.25	77.50	100.00
1.0	100.00	96.25	96.25	100.00	97.50	80.00	91.50	100.00
2.0	98.75	96.25	87.75	100.00	100.00	97.50	95.00	97.50
Control	76.66	71.25	58.75	68.33	99.16	81.66	84.58	97.50

Note: Mortality after nine days and expressed in per cent.

obtained after nine days at both temperatures (25° and 30° C.) with different relative humidities is illustrated in Figure 23. From Table XVI (page 112) and Figure 23 it is clear that the percentage mortality in the control itself is more than fifty-eight per cent at 25° C. and more than eighty per cent at 30° C., regardless of humidity. Regarding the effect of different spore concentrations, mortality was high with one per cent material at 25° C., in the presence of 92.5 per cent of relative humidity. With 75.5 per cent relative humidity the mortality was low by comparison. In the presence of even lower humidities there was more than seventy-one per cent mortality in the control. However, there was a fifteen per cent greater mortality in the treated groups than in the control.

Results at 30° C. were inconsistent. When compared to the mortality of the control with different relative humidities, very little increase in the mortality with the pathogen was recorded.

There was no difference between strains in degree of infection on the adult beetle.

With a view to confirm the results of these observations, the results given in Table XXXIII (Appendix) were statistically analyzed. Results of the analysis are given in the Appendix, Table XXXIV. The results between temperatures, between the different relative humidities, and between the treatments were found significantly different at the 0.99 per cent level. The effect of the pathogen at 30° C. is superior to the effect of 25°

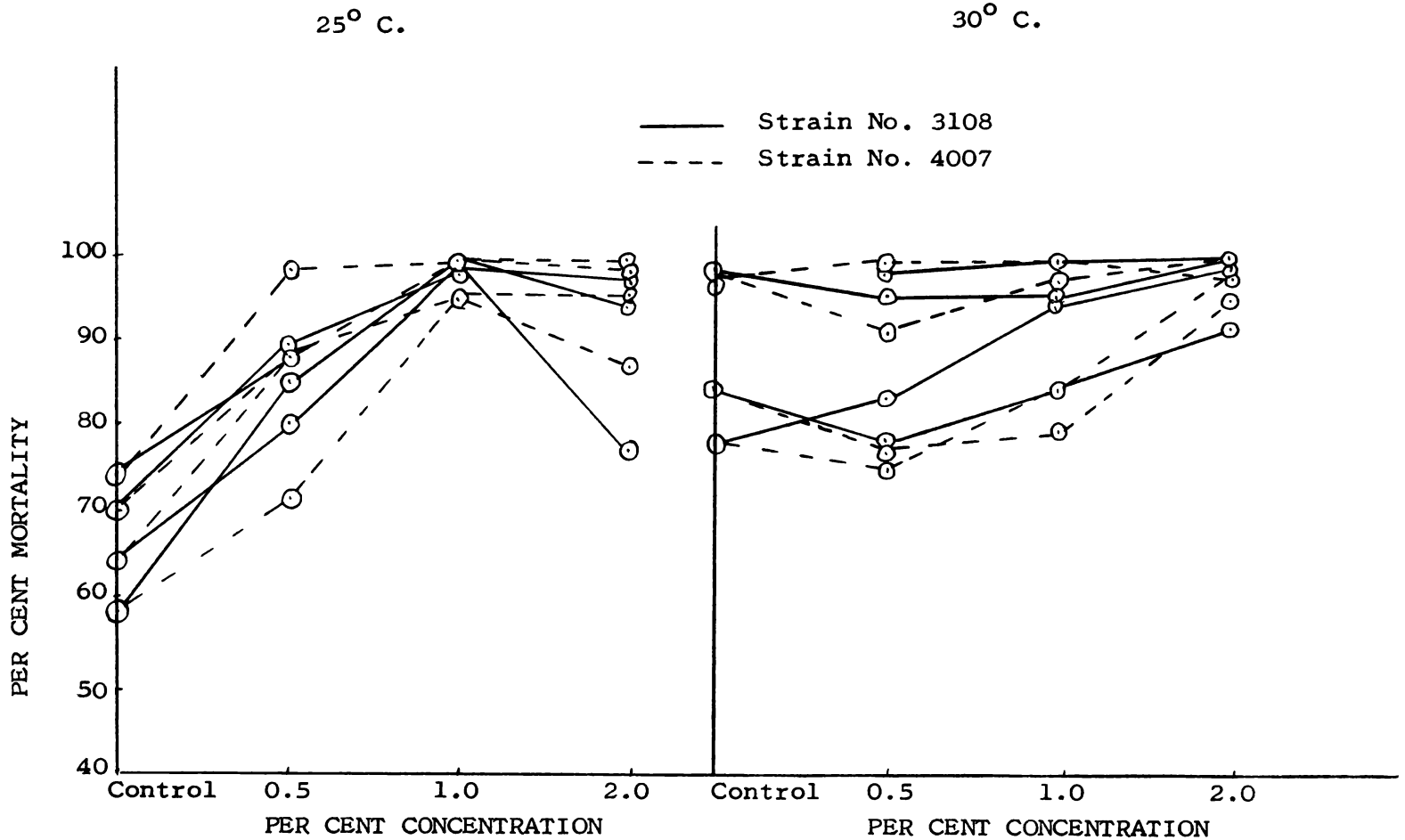

Figure 23. Effect of humidity on the pathogenicity of the spores of two strains of the fungus, Beauveria bassiana, to the adult cowpea weevil, Callosobruchus maculatus, at different temperatures (after nine days).

C. In view of their significant differences, the results were further tested by running Duncan's New Multiple Range Test, wherever applicable. The results are given below. As stated earlier, means underlined by a common bar represent that they are equal in their effects. Broken bars indicate significant differences.

	75.5	30.5 to 32.5	92.5 to 93.5	<1.0
Means	82.91	87.71	93.62	94.63

From the above analysis it is clear that relative humidities of less than one per cent, 30.5 to 32.5, and 92.5 to 93.5 per cent are similar in their effects. The results of 75.5 per cent relative humidity differ from the results of less than one and 92.5 per cent. In other words, the percentage of mortality observed with less than one and 92.5 to 93.5 per cent relative humidities are definitely superior (higher) to 75.5 per cent relative humidity.

	Control	0.5	2.0	1.0
Means	79.74	87.34	95.78	96.02

Results of the above analysis show that the different concentrations are equal. The results of the control and 0.5 per cent spore material are equal, and the results of the control differ from the concentrations of one and two per cent. In other words, there is no difference in the rate of mortality

(statistically) between the different concentrations. However, mortality in the control is low when compared to the insects treated with one and two per cent concentrations.

General observations. During these experiments clotting of dust material was noticed in the Petri dishes kept in the bottles maintained at a humidity of 92.5 to 93.5 per cent, at both temperatures. Furthermore, in the case of *C. maculatus*, development of a yellow saprophytic fungus was frequently noticed at both temperatures with 92.5 to 93.5 per cent relative humidity. The fungus was later identified as *Aspergillus* species by Dr. R. H. Petersen, Department of Botany, The University of Tennessee, Knoxville.

Possibilities of Utilizing the Spores of the Fungus *Beauveria bassiana* (*Bals.*) *Vuill.*, in Preventing the Attack of Some Coleopterous Pests of Stored Grains and Legumes

These experiments were conducted in an air conditioned room maintained at a temperature of $25^{\circ} \pm 1^{\circ}$ C. and relative humidity of thirty to fifty per cent. Sterile clear bottles of one quart size were used for the tests. These had wide-mouths with screw-type lids. The grains used were wheat and blackeyed peas (beans) for *S. oryzae* and *C. maculatus*, respectively. Spore material was applied in the form of dust. Alphacel was used as a diluent. Proper mixing of the grains with the pathogenic material was accomplished with the aid of a seed treating drum

(mixing drum), as explained under "Materials and Methods" in Section III. Dosage used per pound of grains was one gram of diluted material.

Forty insects, selected at random irrespective of sex were introduced into each bottle. The bottles were not disturbed after introducing the insects. Each experiment was replicated four times. Appropriate controls were used in all tests. After an initial scheduled lapse of time, allowing for completion of two generations in both species of insects, percentage of damage, if any, was estimated by following the method narrated under "Materials and Methods" (Section III).

In general, none of these experiments revealed any high degree of control of the stored grain insects tested, with either strain of the fungus. However, results pertaining to each insect species tested are summarized in the following paragraphs.

Sitophilus oryzae. The bottles infected with this species of weevil were disturbed after an interval of ten weeks, during which time they had completed two generations. Percentage of damage to the grain was estimated. The results are tabulated in Table XXXV (Appendix). The average per cent of damage estimated with different concentrations of spores of both strains is presented in Table XVII.

It appeared that there was a slight degree of control of the weevil due to the fungus. It is also clear that spore

TABLE XVII

EFFECT OF DIFFERENT CONCENTRATIONS OF THE SPORES OF TWO STRAINS OF THE FUNGUS, *BEAUVERIA BASSIANA*, IN PREVENTING DAMAGE TO STORED WHEAT BY THE RICE WEEVIL, *SITOPHILUS ORYZAE* AT 25° C., WITH 30.0 TO 50.0 PER CENT RELATIVE HUMIDITY

Treatment Concentration in Per Cent	Strain 3108	Strain 4007
0.25	23.78*	28.75
0.5	23.16	17.91
1.0	20.82	13.11
Control	28.23	28.23

*Average per cent of damage.

concentrations of 0.5 and one per cent were more effective than 0.25 per cent. Strain 4007 was more effective than Strain 3108. According to Table XVII, more than a fifty per cent reduction in damage was observed in the case of grains treated with one per cent spores of Strain 4007, when compared to the 0.25 per cent spore concentration and the control. Approximately seven per cent of reduction in damage was observed in the case of Strain 3108, with a one per cent spore concentration. With the 0.5 per cent spore concentration, there was a five and eleven per cent reduction of damage with Strain 3108 and 4007 respectively, when compared to the control. Very little control resulted from the use of 0.25 per cent spore concentrations with either strain.

The results were statistically analyzed to determine whether they are significantly different. Results tabulated in Table XXXV (Appendix) were used for the analysis. Analyzed data are furnished in Table XXXVI in the Appendix. There was no significant difference between the strains even at the 0.95 per cent level. However, there was a significant difference between the treatments and the control at the 0.99 per cent level. In view of the difference between the treatments, Duncan's New Multiple Range Test was conducted to compare their effects on the percentage of damage. Details of the test are given below. The means of the treatments underscored by a common bar indicate their equality. A break in the bar represents a significant difference.

	Treatments			
	1.0	0.50	0.25	Control
Means	16.96	20.53	26.28	28.23

From the above test it is clear that the results of the control and the 0.25 per cent concentration are equal, as are the 0.5 and one per cent concentrations. The results of the control and the 0.25 per cent concentrations not only differ from the results of the 0.5 and one per cent concentrations, but are also superior to them. In other words, damage done by the weevils in the control and with the 0.25 per cent concentration is higher than with the 0.5 and 1.0 per cent concentrations. Since there is no significant difference between the effects of the 0.5 and one per cent concentrations, it may be concluded that they are more effective than the 0.25 per cent concentration.

Callosobruchus maculatus. After a period of eight weeks, the bottles of beans which were infested with this beetle were examined for damage. Symptoms of damage were observed in all bottles. Therefore, percentage damage was estimated by following the same procedure as in the case of the rice weevil, *S. oryzae*. Results of estimation of damage are tabulated in Table XXXVII in the Appendix. The average percentage damage with different concentrations of both strains of spore material and the control is tabulated in Table XVIII.

As can be seen from Table XVIII, the effect of the spores of the fungus on this beetle were inconsistent. However, concen-

TABLE XVIII

EFFECT OF DIFFERENT CONCENTRATIONS OF THE SPORES OF TWO STRAINS OF THE FUNGUS, *BEAUVERIA BASSIANA*, IN PREVENTING DAMAGE TO STORED BEANS BY THE COWPEA WEEVIL, *CALLOSOBRUCHUS MACULATUS*, AT 25° C., AND WITH 30.0 TO 50.0 PER CENT RELATIVE HUMIDITY

Treatment Concentration in Per Cent	Strain 3108	Strain 4007
0.25	97.85*!	94.55
0.5	69.56**	83.19
1.0	77.66*	82.29
Control	98.03**	98.03

*Average of four replications.

!Average per cent of damage.

**Average of three replications as there was very low percentage of damage which is due to early death of the beetles.

trations of 0.5 and one per cent, show an indication of some effect on the beetle when compared to the control.

This low degree of control and the inconsistency in the control was confirmed by statistical analysis. Results of the analysis are furnished in Table XXXVIII in the Appendix.

According to the analyzed results, there is no significant difference between the strains. Furthermore, even the results between the treatments and the control are not significantly different. In conclusion, it may be said that the fungus is not effective on this beetle under the conditions in which the tests were conducted, even though there are indications of its infective capacity.

General observations. In the case of *S. oryzae*, clotting of grains with dead weevils and excreta was observed in all the control bottles, as well as in the bottles containing grains treated with the 0.25 per cent spore material. Such a condition had not occurred with *C. maculatus* in any treatment, including the control. This condition of clotting and accumulation of powdery material in the bottles infested with weevils was observed only in the later part of the second generation.

IV. DISCUSSION

The observations made in the present investigation regarding the potentialities of utilizing the fungus, *B. bassiana* for the

control of the rice weevil, S. oryzae, and the cowpea weevil, C. maculatus are discussed in the following paragraphs.

Effect of Humidity on the Pathogenicity of the Fungus, B. bassiana on some Coleopterous Stored Grain Pests at 25° and 30° C.

Data obtained in the present investigation, where the adults of both S. oryzae and C. maculatus were tested by direct treatment confirmed the general opinion that high relative humidity is necessary for infection by the fungal spores. This appears to be particularly true, if the fungus has to infect by gaining entry through the integument. A high percentage of mortality due to infection by the fungus was observed in the presence of high relative humidity, in both species of insects (Table XV, page 108 and Table XVI, page 112). High mortality of S. oryzae at very low relative humidity (less than one per cent at 25° C. and less than one per cent and 32.5 per cent at 30° C.) appeared to be due to desiccation of the insects rather than to their infection by the fungus. In the case of C. maculatus, very low relative humidity (less than one per cent) seemed unfavorable for the long survival of the adults of both temperatures tested. They may also have died of desiccation.

Among the different concentrations tested, use of very high spore concentration (two per cent) seemed unnecessary as concentrations below that level give the same results (Table XV, page 108, and Table XVI, page 112). However, a minimum of 0.5

per cent spore material seemed insufficient to obtain an effect equal to that of the one per cent concentration. A concentration of one per cent appeared to be as effective as one of two per cent. The two strains of the fungus used in this investigation appeared to have the same effect on the insects.

Potentialities of Utilizing This Fungus for Control of These Stored Grain Insects

Observations made regarding the percentage of damage done to the grains treated with different concentrations of the spores of the fungus are discussed in the following paragraphs.

Sitophilus oryzae. Dresner (1949), Steinhaus and Bell (1953), and Dunn and Mechalas (1963) have studied the treatment of stored grains with spores of *B. bassiana*. The earlier worker used rice whereas the latter workers used wheat for treating the grains with spores of the fungus. None of the investigators estimated the percentage of damage to the grains, if any, with different concentrations. All applied the pathogen in the form of a dust. Dresner used the rice weevil, *S. oryzae*, for his work. Dunn and Mechalas used the granary weevil, *S. granarius*. The same species were used by Steinhaus and Bell, in addition to other coleopterous pests of wheat. According to Steinhaus and Bell, both species of *Sitophilus* are susceptible to this fungus. In the present investigation, the data obtained regarding the percentage of damage done by *S. oryzae* to the grains treated with

different concentrations of the spores seem to agree with their findings regarding the pathogenicity to these weevils. However, as observed by Steinhaus and Bell, this weevil also does not appear to show a high degree of susceptibility to this fungus with the dosage used, and under the conditions studied. Dunn and Mechalas conducted tests with different dosages and are of the opinion that higher dosages or massive inoculation will be necessary for the successful use of this fungus. Such an attempt was not made to test higher dosages in the present investigation with the presumption that it would be neither economical nor advisable. The dosage used in the present investigation was about 0.22 gm. of diluted spore material per one hundred grams of grain (one gram per 456 gm. of grain) and this gave a good coverage to both the grains and the container.

Out of the three concentrations of spore material tried (0.25, 0.5, and one per cent), 0.5 and one per cent gave equal control in the present investigation. In other words, the percentage of damage was significantly less in wheat treated with 0.5 and one per cent of spore material. The percentage of damage in the case of grains treated with 0.25 per cent of spore material and the grains treated with alphacel only (control) was equal. This observation agrees with the observations of Dresner (1949) who reported that concentrations above 0.3 per cent would give effective results. There was no significant difference between the two strains used in the test.

Callosobruchus maculatus. The adults of this insect showed a slight degree of susceptibility to infection by the fungus, *B. bassiana*, both in the preliminary tests and when treated directly with different concentrations of the spores. The estimation of damage done to the grains treated with different concentrations of the spores of the fungus does not seem to show or give any indication of its effect on the infestation by this beetle. This leads to the hypothesis that unless proper or optimum relative humidity is present in the surroundings, the spores of the fungus may not be able to infect the insects as observed in the earlier experiments of the present investigation. Otherwise, the spores have to be ingested by the insects along with their food in order for infection to occur. Since the adult rice weevil, *S. oryzae*, feeds on the grains during all of its life stages, ample opportunity exists for the spores to get into the alimentary system. Thus, infection may occur in the absence of proper environmental conditions which would allow penetration through the body wall. The adults of the cowpea weevil (beetle) do not feed in the adult stage on the dried grains. Of course, when they start their activity in the field they are known to feed on tender pods, petals, and flower buds. In the stores their main activity is to mate, in the case of the males, and lay eggs in the case of females and thus perpetuate the species. Under these circumstances, the adults of this beetle may not be infected through the integument because of insufficient humidity

for the germination of spores on the body surface. In the absence of this situation, spores should get the chance of gaining entry into the beetles through the food upon which they feed. But, since this beetle does not feed in the stores on the grains as explained earlier, the chances of getting them through the food are also remote. Therefore, circumstances which favored the host insect and not the parasite may be responsible for failure to get effective results in the case of the cowpea weevil on beans in the present investigation. These observations seem to support the findings of Rozpal (1930), Ferreire (1943), Steinhaus and Bell (1953), Schaerffenberg (1957a), and York (1958).

SECTION IV

SUMMARY

The influence of temperature and humidity on the longevity of the spores of the fungus, Beauveria bassiana, was studied. Two strains, 3108 and 4007, were used as potential pathogens. As a prerequisite to an investigation of the potentialities of utilizing this fungus, studies were made to discern the influence of different combinations of temperature and humidity on the longevity of the spores. Temperatures of 50°, 40°, 35°, 30°, 25°, 20°, and 5° C. were tested. Relative humidities of less than one, 30.5 to 34.5, 70.5 to seventy-six, and 92.5 to 96.5 per cent were combined with each temperature. The adults of the rice weevil, Sitophilus oryzae and the cowpea weevil, Callosobruchus maculatus, were used as host insects to test their susceptibility to the pathogen at 30° and 25° C. with the above relative humidities. Spore concentrations of 0.5, one, and two per cent were tested by direct treatment. Grains treated with 0.25, 0.5, and one per cent spore material were tested against both insect species at 25° C. with thirty to fifty per cent relative humidity. Results of the investigations are summarized in the following paragraphs.

1. Spores of the fungus, kept in test tubes on dried culture media, survived fourteen to eighteen months of laboratory conditions, when stored in the presence of negligible moisture.

2. The influence of both temperature and humidity on the longevity of the spores of both strains of the fungus was, in general, quite significant. The longevity of the spores decreased significantly with an increase in temperature, in the presence of all relative humidities tested.

3. The survival of the spores of both strains of this fungus was minimal at 50° and 40° C., regardless of the amount of humidity.

4. At 35°, 30°, 25°, 20°, and 5° C., the relative humidities that were most favorable for spore survival were less than one and greater than ninety per cent.

5. Although there was no significant difference in the maximum longevity between the two strains of spores tested, there was a marked difference in mean survival time. Spores of Strain 4007 remained viable for longer periods at 35° and 30° whereas lower temperatures were more favorable for Strain 3108. In general, a gradual decrease in per cent survival occurred under favorable temperatures and humidities.

6. The longevity of the adults of *S. oryzae* and *C. maculatus* was studied under the same combinations of temperature and humidity as those stated in the first paragraph, with the exception of 5° C. Presence or absence of food was also included in the study. None of the adults of both species of insects was able to survive beyond nine days at 50° and 40° C. regardless of different relative humidities and the presence or absence of food.

Very few insects survived beyond nine days at 35° C., regardless of other conditions. A greater number of adults of both species survived and remained active after nine days at 30° and 25° C. with relative humidities of thirty to ninety-three per cent, in the presence of food. However, the percentage that survived in the absence of food was also high at these temperatures when compared to the percentage surviving at other temperatures. Though the insects possessed an enhanced longevity at 20° C., their activity was limited at this temperature.

7. From preliminary observations it appeared that the adults of the rice weevil and the adults, larvae, and pupae of the cowpea weevil are susceptible to the spores of both strains of the fungus under laboratory conditions.

8. The presence of high relative humidity (above ninety per cent) gave a greater mortality of the insects with the pathogen than did that of the other relative humidities tested. A temperature of 30° C. favored more infection than did one of 25° C., in the presence of high relative humidity. A spore concentration of one per cent showed a greater effect than did that of 0.5 and two per cent, when compared to the control, under direct treatment.

9. Though there was not a complete prevention of attack of the treated wheat by the rice weevil, a significant difference in the percentage of damage was observed between the treated and untreated grains. In other words, there was a certain degree of

control by the pathogen. Among the different concentrations tested, one and 0.5 per cent gave equal control. Damage resulting from the use of 0.25 per cent spore material was almost identical to that of the control. There was no significant difference in the percentage of damage between the treated and untreated beans, whereas adults, larvae, and pupae of the cowpea weevil, *C. maculatus*, showed varying degrees of susceptibility to the spores of both strains of the fungus.

10. Regarding the pathogenicity of the two strains tested, no significant difference was found in their infection of the adults of both the rice weevil and the cowpea weevil.

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APPENDIX

TABLE XIX

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE SPORES OF BEAUVERIA BASSIANA

Relative Humidity in Per Cent	Temperature 50° C.							
	Strain 3108				Strain 4007			
	*R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
				After Three Days				
<1.0	74.43	75.60	85.74	81.50	58.97	47.99	52.44	58.96
31.5	4.16	1.04	6.25	9.37	13.62	9.63	14.17	10.23
72.0	0.00	0.00	0.00	0.00	7.13	6.44	2.50	2.80
92.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				After Six Days				
<1.0	65.83	61.22	62.22	61.55	50.83	56.51	46.93	57.00
31.5	0.00	0.00	0.00	0.00	4.90	4.82	3.49	4.50
72.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				After Nine Days				
<1.0	26.99	28.89	28.61	27.01	43.03	42.35	41.08	40.77
31.5	0.00	0.00	0.00	0.00	1.40	0.00	1.56	0.00
72.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				After Twelve Days				
<1.0	2.68	3.46	4.58	3.04	9.47	13.13	5.79	8.42
31.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
72.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				After Twenty-Four Days				
<1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
72.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*R = Replication.

TABLE XX

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE SPORES OF BEAUVERIA BASSIANA

Relative Humidity in Per Cent	Temperature 40° C.							
	Strain 3108				Strain 4007			
	*R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
				After Three Days				
<1.0	94.78	89.00	84.76	90.26	57.12	64.31	56.38	66.36
32.0	80.85	82.44	78.71	80.55	41.81	53.95	39.97	50.11
70.5	19.63	22.40	25.30	26.41	21.22	24.69	26.56	19.58
93.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				After Six Days				
<1.0	93.58	73.42	83.56	84.97	43.31	47.69	51.94	51.39
32.0	70.70	72.92	67.76	80.06	34.47	36.98	42.43	48.76
70.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
93.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				After Nine Days				
<1.0	82.99	74.75	70.13	72.42	36.07	34.52	45.10	32.09
32.0	64.43	61.42	48.48	56.80	39.92	38.09	37.29	40.16
70.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
93.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				After Twelve Days				
<1.0	80.90	65.86	70.77	80.91	3.18	4.09	3.61	2.66
32.0	54.91	58.41	64.48	60.24	8.22	12.96	3.44	5.33
70.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
93.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				After Twenty-Four Days				
<1.0	18.70	10.10	27.15	11.51	0.00	0.00	0.00	0.00
32.0	20.97	21.12	9.65	15.65	1.77	0.38	0.00	0.46
70.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
93.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*R = Replication.

TABLE XXI

ANALYSIS OF VARIANCE, FOUR-WAY CLASSIFICATION
EFFECT OF TEMPERATURE AND HUMIDITY ON THE
LONGEVITY OF THE SPORES OF TWO STRAINS
OF BEAUVERIA BASSIANA

TEMPERATURES: 40° AND 50° C.

Source	df	Mean Square	F	(0.99 level) Significance
Total	319			
Temperature	1	17972.26	11316.12	Yes
Strains	1	5863.17	3691.71	Yes
Times	4	7583.21	4774.72	Yes
Humidities	3	29437.35	18535.04	Yes
Temp. X Strains	1	5978.28	3764.18	Yes
Temp. X Times	4	638.23	401.86	Yes
Temp. X Humidities	3	6435.38	4051.10	Yes
Strains X Times	4	276.44	174.06	Yes
Strains X Hum.	3	2188.52	1377.98	Yes
Times X Hum.	12	2522.36	1588.19	Yes
Temp. X Strain X Times	4	331.96	209.01	Yes
Temp. X Times X Hum.	12	568.18	357.75	Yes
Strain X Times X Hum.	12	113.00	71.15	Yes
Temp. X Strain X Hum.	3	1881.07	1184.40	Yes
Temp. X Strain X Time X Hum.	12	223.19	185.99	Yes
Error	240	1.59		

TABLE XXII

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE SPORES OF BEAUVERIA BASSIANA

Relative Humidity in Per Cent	Temperature 35° C.							
	Strain 3108				Strain 4007			
	*R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
				After One Week				
<1.0	54.86	50.70	79.43	48.65	78.02	71.46	66.98	75.09
32.5	12.24	6.41	17.71	15.83	45.33	30.74	45.07	51.28
71.0	2.08	1.04	3.00	1.83	16.69	15.11	12.85	17.63
96.0	65.53	52.02	53.58	39.56	91.66	92.93	96.49	92.20
				After Three Weeks				
<1.0	20.70	24.80	27.98	26.94	40.27	39.71	41.68	43.39
32.5	11.27	4.78	3.46	2.98	36.21	32.81	33.46	35.85
71.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
96.0	33.41	31.19	14.55	19.35	57.99	50.60	53.47	40.28
				After Six Weeks				
<1.0	1.95	2.83	6.56	5.72	5.33	4.41	5.72	6.94
32.5	0.00	0.00	0.00	0.00	14.78	19.21	16.86	18.19
71.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
96.0	8.19	7.38	9.41	9.44	4.32	7.92	5.66	6.36
				After Nine Weeks				
<1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
32.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
71.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
96.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				After Twelve Weeks				
<1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
32.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
71.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
96.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*R = Replication.

TABLE XXIII

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE SPORES OF BEAUVERIA BASSIANA

Relative Humidity in Per Cent	Temperature 30° C.							
	Strain 3108				Strain 4007			
	*R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
				After One Week				
<1.0	81.64	76.90	87.08	79.99	67.78	85.74	81.74	69.46
32.5	84.28	80.00	87.30	94.38	79.27	69.45	73.57	76.70
72.5	6.94	11.11	15.98	9.04	41.41	43.54	46.30	46.12
93.5	90.46	93.08	94.16	97.50	95.23	98.08	92.80	95.64
				After Three Weeks				
<1.0	64.02	55.54	48.89	43.90	66.76	60.46	47.66	51.04
32.5	77.08	74.65	72.07	84.58	67.44	60.44	68.84	66.16
72.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
93.5	73.18	75.83	84.64	88.91	91.72	93.16	89.82	93.65
				After Six Weeks				
<1.0	11.57	8.26	7.87	19.93	32.69	33.00	32.28	36.27
32.5	51.11	63.59	66.75	55.82	52.87	55.88	48.27	49.04
72.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
93.5	65.34	60.47	74.97	69.26	81.94	84.83	87.67	84.21
				After Nine Weeks				
<1.0	2.23	2.04	3.69	5.83	17.31	11.96	13.22	10.44
32.5	25.95	27.10	34.34	28.58	24.24	25.86	16.72	20.95
72.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
93.5	16.88	14.93	24.88	24.71	79.43	71.04	75.61	79.54
				After Twelve Weeks				
<1.0	1.92	1.35	0.00	0.58	9.29	8.64	9.17	7.21
32.5	14.63	14.96	5.03	11.74	14.75	15.24	17.14	15.23
72.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
93.5	0.91	1.11	0.00	1.80	32.61	34.23	33.66	42.72

*R = Replication.

TABLE XXIV

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE SPORES OF BEAUVERIA BASSIANA

Relative Humidity in Per Cent	Temperature 25° C.							
	Strain 3108				Strain 4007			
	*R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
				After One Week				
<1.0	97.02	93.83	95.00	95.14	78.95	77.87	89.00	66.61
30.5	95.14	82.09	74.11	83.86	75.46	78.61	78.34	83.35
74.0	95.00	99.08	92.50	88.54	45.89	40.44	35.26	27.37
92.5	94.54	86.28	95.83	98.75	97.52	94.33	95.37	95.84
				After Three Weeks				
<1.0	90.78	77.44	82.98	93.56	76.09	78.76	82.44	71.20
30.5	80.84	75.36	74.10	81.94	81.92	68.25	74.70	77.10
74.0	12.96	7.87	10.53	10.47	13.14	14.14	13.10	13.73
92.5	96.68	97.50	96.43	90.14	75.56	67.00	63.43	61.13
				After Six Weeks				
<1.0	87.30	62.09	67.67	75.83	38.70	46.84	62.49	66.30
30.5	82.78	73.75	79.58	64.38	47.06	52.66	46.84	42.57
74.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92.5	68.56	70.24	71.37	76.25	47.12	40.64	45.38	42.46
				After Nine Weeks				
<1.0	54.58	28.16	41.06	41.57	29.26	27.46	34.80	28.33
30.5	30.14	29.78	29.20	30.03	17.02	15.48	13.87	14.00
74.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92.5	36.05	32.94	29.40	27.46	26.00	30.70	30.65	26.74
				After Twelve Weeks				
<1.0	33.02	28.29	31.80	20.77	23.01	15.49	17.88	18.78
30.5	22.77	14.70	20.81	21.83	7.61	10.91	7.02	6.00
74.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92.5	8.64	10.46	8.34	8.90	8.61	7.56	7.45	7.14

*R = Replication.

TABLE XXV

ANALYSIS OF VARIANCE, FOUR-WAY CLASSIFICATION
EFFECT OF TEMPERATURE AND HUMIDITY ON THE
LONGEVITY OF THE SPORES OF TWO STRAINS
OF BEAUVERIA BASSIANA

TEMPERATURES: 25°, 30° AND 35° C.

Source	df	Mean Square	F	(0.99 level) Significance
Total	479			
Temperatures	2	41185.61	2053.12	Yes
Strains	1	352.62	17.58	Yes
Times	4	54553.28	2719.50	Yes
Humidities	3	35873.40	1788.30	Yes
Temp. X Strain	2	5703.72	284.33	Yes
Temp. X Times	8	1505.57	75.05	Yes
Temp. X Hum.	6	4597.84	229.20	Yes
Strain X Times	4	131.04	6.53	Yes
Strain X Hum.	3	615.09	30.66	Yes
Times X Hum.	12	2693.36	134.26	Yes
Temp. X Strain X Times	8	730.58	36.42	Yes
Temp. X Times X Hum.	24	788.93	39.33	Yes
Strain X Times X Hum.	12	201.92	10.06	Yes
Temp. X Strain X Hum.	6	671.81	33.49	Yes
Temp. X Strain X Times X Hum.	24	477.60	23.81	Yes
Error	360	20.06		

TABLE XXVI

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE SPORES OF BEAUVERIA BASSIANA

Relative Humidity in Per Cent	Temperature 20° C.							
	Strain 3108				Strain 4007			
	R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
	After One Month							
<1.0	93.66	92.77	91.71	93.09	85.34	84.46	86.85	88.32
33.0	81.55	81.77	78.31	77.89	71.43	72.76	68.96	70.37
76.0	70.83	75.60	76.59	73.65	25.12	26.50	27.59	26.14
96.5	95.40	97.36	98.53	95.30	95.02	93.49	97.22	94.32
	After Two Months							
<1.0	92.10	92.46	88.12	91.00	78.17	82.00	81.53	78.37
33.0	67.44	69.76	69.80	65.53	60.72	60.26	64.24	57.73
76.0	66.16	69.02	65.06	68.64	13.40	12.12	11.68	16.14
96.5	91.63	92.33	90.27	90.68	93.16	91.67	91.73	90.84
	After Three Months							
<1.0	85.73	86.84	86.07	83.08	80.07	77.24	75.29	72.79
33.0	64.35	64.87	62.09	61.95	53.05	54.25	53.76	55.49
76.0	34.82	33.37	33.38	31.99	11.10	9.66	12.97	10.47
96.5	88.50	88.97	85.58	89.21	84.24	83.34	79.89	80.42
	After Four Months							
<1.0	82.79	80.07	81.43	80.72	74.53	75.34	71.45	72.44
33.0	58.33	52.05	54.51	55.95	57.63	49.07	51.95	50.98
76.0	22.10	17.34	19.30	18.52	4.77	6.59	6.07	5.94
96.5	79.74	81.16	80.03	80.04	71.36	69.56	71.51	70.91

TABLE XXVII

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE SPORES OF BEAUVERIA BASSIANA

Relative Humidity in Per Cent	Temperature 5° C.							
	Strain 3108				Strain 4007			
	R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
				After One Month				
<1.0	98.44	98.68	98.38	96.32	92.36	91.32	91.52	93.31
34.5	92.60	93.56	92.65	91.10	83.52	82.65	83.87	85.72
75.0	81.34	78.57	90.00	87.32	69.04	68.68	67.51	65.86
95.6	95.55	95.39	94.59	95.56	96.61	95.62	96.26	97.95
				After Two Months				
<1.0	81.57	90.95	84.25	91.63	86.46	86.79	95.87	90.00
34.5	80.97	79.84	78.11	80.75	84.25	68.22	87.35	68.20
75.0	69.04	68.13	67.27	67.94	57.61	69.46	50.66	51.68
95.6	85.56	89.44	81.25	95.83	85.71	88.08	89.42	81.24
				After Three Months				
<1.0	78.64	83.36	80.91	84.26	63.38	66.98	64.56	50.44
34.5	50.72	54.67	51.68	51.69	47.40	40.04	55.45	51.96
75.0	43.10	49.62	30.38	57.63	47.23	53.50	33.78	51.73
95.6	93.98	82.38	85.74	88.08	72.10	78.64	75.14	67.73
				After Four Months				
<1.0	77.01	78.58	79.38	76.64	53.83	50.80	51.74	56.55
34.5	52.48	51.27	50.24	49.38	49.42	45.57	46.74	46.80
75.0	36.76	35.39	38.23	28.55	43.37	35.65	31.82	34.22
95.6	77.59	78.36	80.84	84.22	71.99	62.84	78.30	66.99

TABLE XXVIII

ANALYSIS OF VARIANCE, FOUR-WAY CLASSIFICATION
EFFECT OF TEMPERATURE AND HUMIDITY ON THE
LONGEVITY OF THE SPORES OF THE FUNGUS,
BEAUVERIA BASSIANA
TEMPERATURE: 20° C. AND 5° C.

Source	df	Mean Square	F	(0.99 level) Significance
Total	255			
Temperature	1	1993.40	149.21	Yes
Strain	1	6963.48	521.22	Yes
Time	3	10025.31	750.39	Yes
Relative Humidity	3	24071.02	1801.72	Yes
Temp. X Strain	1	700.46	52.42	Yes
Temp. X Time	3	422.66	31.64	Yes
Temp. X Rel. Hum.	3	2495.04	186.75	Yes
Strain X Time	3	20.72	1.55	No
Strain X Rel. Hum.	3	768.65	57.53	Yes
Time X Rel. Hum.	9	294.68	22.06	Yes
Temp. X Strain X Time	3	177.16	13.26	Yes
Temp. X Time X Rel. Hum.	9	132.44	9.91	Yes
Temp. X Strain X Rel. Hum.	9	348.01	26.05	Yes
Strain X Time X Rel. Hum.	3	872.12	65.28	Yes
Temp. X Strain X Time X Rel. Hum.	9	61.83		
Error	192	13.362		

TABLE XXIX

EFFECT OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE ADULT WEEVIL, *SITOPHILUS ORYZAE*, IN THE PRESENCE AND ABSENCE OF FOOD (WHEAT)

		Relative Humidity											
		< 1.0%			30.5% to 33.0%			72.0% to 76.0%			92.5% to 96.5%		
Temp.	Treatment	After 3 Days	After 6 Days	After 9 Days	After 3 Days	After 6 Days	After 9 Days	After 3 Days	After 6 Days	After 9 Days	After 3 Days	After 6 Days	After 9 Days
50° C.	With Food	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00*!
	No Food	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
40° C.	With Food	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	No Food	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
35° C.	With Food	100.00	100.00	100.00	68.33	96.66	100.00	50.00	85.00	90.00	46.66	93.33	96.66
	No Food	100.00	100.00	100.00	85.00	85.00	100.00	15.00	68.00	78.33	6.66	41.66	81.66
30° C.	With Food	10.00	41.66	56.66	58.33	76.66	83.33	0.00	3.33	10.00	0.00	3.33	5.00
	No Food	98.33	100.00	100.00	55.00	100.00	100.00	20.00	48.33	86.66	0.00	8.33	50.00
25° C.	With Food	10.00	18.33	48.33	26.66	45.00	50.00	0.00	0.00	1.66	0.00	0.00	0.00
	No Food	96.66	100.00	100.00	71.66	75.00	90.00	1.66	20.00	41.66	0.00	1.66	21.66
20° C.	With Food	0.00	30.00	48.33	10.00	26.66	40.00	8.33	16.66	21.66	3.33	5.00	11.66
	No Food	11.66	100.00	100.00	20.00	30.00	73.33	0.00	8.33	10.00	0.00	3.33	5.00

* All are averages of three replications.

! Percentage of dead ones.

TABLE XXX

EFFECT OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF THE ADULT BEETLE *CALLOSOBRUCHUS MACULATUS* (FABR.) IN THE PRESENCE AND ABSENCE OF FOOD (BEANS)

		Relative Humidity											
		< 1.0%			30.5% to 33.0%			72.0% to 76.0%			92.5% to 96.5%		
Temp.	Treatment	After 3 Days	After 6 Days	After 9 Days	After 3 Days	After 6 Days	After 9 Days	After 3 Days	After 6 Days	After 9 Days	After 3 Days	After 6 Days	After 9 Days
50° C.	With Food	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00*!
	No Food	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
40° C.	With Food	95.00	98.33	100.00	95.00	96.66	98.33	93.33	100.00	100.00	91.66	98.33	95.00
	No Food	90.00	100.00	100.00	86.66	88.33	100.00	71.66	80.00	86.66	73.33	80.00	85.00
35° C.	With Food	91.66	100.00	100.00	91.66	100.00	100.00	80.00	95.00	98.33	88.33	100.00	100.00
	No Food	68.33	91.66	96.66	46.66	60.00	75.00	68.33	73.33	73.33	18.33	61.66	85.00
30° C.	With Food	56.66	86.66	93.33	66.66	100.00	100.00	65.00	96.66	98.33	71.66	90.00	96.66
	No Food	55.00	90.00	96.66	78.33	93.33	96.66	51.66	66.66	88.33	26.66	56.66	73.33
25° C.	With Food	55.00	68.33	88.33	26.66	68.33	61.66	36.66	51.66	56.66	25.00	45.00	53.33
	No Food	8.33	43.33	65.00	8.33	40.00	50.00	8.33	30.00	46.66	8.33	36.66	40.00
20° C.	With Food	16.66	28.33	28.33	21.66	31.66	41.66	15.00	23.33	30.00	3.33	13.33	16.66
	No Food	53.33	85.00	87.25	40.00	68.33	78.33	20.00	46.66	58.33	8.33	21.66	30.00

* All are averages of three replications.

! Percentage of dead ones.

TABLE XXXI

EFFECT OF TEMPERATURE AND HUMIDITY ON THE PATHOGENICITY OF THE FUNGUS, *BEAUVERIA BASSIANA*, ON THE ADULT RICE WEEVIL, *SITOPHILUS ORYZAE*, AFTER NINE DAYS

Relative Humidity in Per Cent	Temperature 25° C.							
	Strain 3108				Strain 4007			
	R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
				Untreated (Control)				
<1.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30.5	86.6	83.3	85.0	78.3	86.6	83.3	85.0	78.3
75.5	36.6	45.0	35.0	36.6	36.6	45.0	35.0	36.6
92.5	18.3	13.3	13.3	21.6	18.3	13.3	13.3	21.6
				Treated With 0.5% of Spores				
<1.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30.5	90.0	95.0	100.0	95.0	85.0	85.0	100.0	95.0
75.5	40.0	30.0	50.0	55.0	60.0	35.0	45.0	50.0
92.5	15.0	20.0	25.0	25.0	50.0	25.0	25.0	15.0
				Treated With 1.0% of Spores				
<1.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30.5	100.0	95.0	100.0	100.0	100.0	100.0	100.0	95.0
75.5	25.0	25.0	35.0	45.0	50.0	20.0	30.0	30.0
92.5	75.0	75.0	80.0	70.0	100.0	85.0	70.0	70.0
				Treated With 2.0% of Spores				
<1.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30.5	95.0	95.0	85.0	85.0	95.0	95.0	100.0	95.0
75.5	50.0	50.0	40.0	35.0	65.0	65.0	70.0	45.0
92.5	15.0	55.0	40.0	90.0	30.0	40.0	30.0	45.0

TABLE XXXI (continued)

Relative Humidity in Per Cent	Temperature 30° C.							
	Strain 3108				Strain 4007			
	R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
	Untreated (Control)							
<1.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
75.5	75.0	68.3	56.6	18.3	75.0	68.3	56.6	60.0
92.5	18.3	33.3	18.3	23.3	18.3	33.3	18.3	23.3
	Treated With 0.5% of Spores							
<1.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
75.5	80.0	80.0	85.0	60.0	75.0	95.0	85.0	90.0
92.5	75.0	80.0	55.0	80.0	45.0	65.0	40.0	50.0
	Treated With 1.0% of Spores							
<1.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
75.5	85.0	90.0	80.0	95.0	95.0	95.0	100.0	85.0
92.5	80.0	80.0	85.0	70.0	80.0	80.0	75.0	80.0
	Treated With 2.0% of Spores							
<1.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
75.5	45.0	60.0	70.0	45.0	70.0	65.0	75.0	95.0
92.5	55.0	40.0	70.0	25.0	65.0	75.0	80.0	55.0

TABLE XXXII

ANALYSIS OF VARIANCE, FOUR-WAY CLASSIFICATION EFFECT OF TEMPERATURE AND HUMIDITY ON THE PATHOGENICITY OF THE FUNGUS, *BEAUVERIA BASSIANA*, ON THE ADULT RICE WEEVIL, *SITOPHILUS ORYZAE*, AFTER NINE DAYS

Source	df	Mean Square	F	Significance .95	Significance .99
Total	255				
Temperature	1	12586.60	211.89	Yes	Yes
Strains	1	93.85	1.58	No	No
Treatment	3	3864.23	65.05	Yes	Yes
Humidity	3	45026.36	758.02	Yes	Yes
Temp. X Strain	1	11.81	0.19	No	No
Temp. X Treat.	3	511.01	8.60	Yes	Yes
Temp. X Hum.	3	3019.01	50.82	Yes	Yes
Strain X Treat.	3	154.26	2.60	Yes	No
Strain X Hum.	3	180.31	3.03	Yes	No
Treat. X Hum.	9	2071.60	34.87	Yes	Yes
Temp. X Strain X Treat.	3	44.63	0.75	No	No
Temp. X Treat. X Hum.	9	901.33	15.17	Yes	Yes
Strains X Treat. X Hum.	9	88.29	1.49	No	No
Temp. X Strain X Hum.	9	8.17	0.14	No	No
Temp. X Strain X Treat X Hum.	9	179.53	3.02	Yes	Yes
Error	192	59.40			

TABLE XXXIII

EFFECT OF TEMPERATURE AND HUMIDITY ON THE PATHOGENICITY OF THE FUNGUS, BEAUVERIA BASSIANA, ON THE ADULT COWPEA WEEVIL, CALLOSOBRUCHUS MACULATUS, AFTER NINE DAYS

Relative Humidity in Per Cent	Temperature 25° C.							
	Strain 3108				Strain 4007			
	R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
	Untreated (Control)							
<1.0	68.3	68.3	83.3	86.6	68.3	68.3	83.3	86.6
30.5	63.3	65.0	73.3	83.3	63.3	65.0	73.3	83.3
75.5	61.6	66.6	58.3	48.3	61.6	66.6	58.3	48.3
92.5	73.3	76.6	71.6	51.6	73.3	76.6	71.6	51.6
	Treated With 0.5% of Spores							
<1.0	95.0	80.0	85.0	95.0	100.0	100.0	100.0	100.0
30.5	90.0	90.0	85.0	95.0	95.0	65.0	100.0	95.0
75.5	85.0	90.0	90.0	80.0	75.0	75.0	80.0	60.0
92.5	80.0	80.0	80.0	85.0	85.0	95.0	85.0	90.0
	Treated With 1.0% of Spores							
<1.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30.5	95.0	100.0	100.0	100.0	95.0	100.0	95.0	95.0
75.5	100.0	100.0	100.0	100.0	95.0	100.0	100.0	90.0
92.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Treated With 2.0% of Spores							
<1.0	85.0	95.0	100.0	100.0	100.0	100.0	100.0	95.0
30.5	100.0	90.0	95.0	100.0	100.0	100.0	90.0	95.0
75.5	70.0	80.0	80.0	80.0	85.0	95.0	80.0	90.0
92.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

TABLE XXXIII (continued)

Relative Humidity in Per Cent	Temperature 30° C.							
	Strain 3108				Strain 4007			
	R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
	Untreated (Control)							
<1.0	100.0	100.0	100.0	96.6	100.0	100.0	100.0	96.6
30.5	83.3	81.6	81.6	80.0	83.3	81.6	81.6	80.0
75.5	88.3	85.0	76.6	88.3	88.3	85.0	76.6	88.3
92.5	98.3	93.3	100.0	98.3	98.3	93.3	100.0	98.3
	Treated With 0.5% of Spores							
<1.0	100.0	95.0	90.0	100.0	90.0	95.0	90.0	90.0
30.5	80.0	85.0	85.0	85.0	90.0	75.0	85.0	55.0
75.5	75.0	65.0	90.0	85.0	65.0	80.0	75.0	90.0
92.5	95.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Treated With 1.0% of Spores							
<1.0	100.0	95.0	95.0	95.0	100.0	100.0	90.0	100.0
30.5	90.0	100.0	90.0	100.0	80.0	65.0	75.0	100.0
75.5	95.0	80.0	85.0	80.0	100.0	95.0	90.0	80.0
92.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Treated With 2.0% of Spores							
<1.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30.5	100.0	100.0	100.0	95.0	100.0	95.0	100.0	95.0
75.5	90.0	85.0	95.0	100.0	100.0	100.0	90.0	90.0
92.5	100.0	100.0	100.0	100.0	100.0	95.0	95.0	100.0

TABLE XXIV

ANALYSIS OF VARIANCE, FOUR-WAY CLASSIFICATION EFFECT OF TEMPERATURE AND HUMIDITY ON THE PATHOGENICITY OF THE FUNGUS, *BEAUVERIA BASSIANA*, ON THE COWPEA WEEVIL, *CALLOSOBRUCHUS MACULATUS*, AFTER NINE DAYS

Source	df	Mean Square	f	(0.99 level) Significance
Total	255			
Temperature	1	1745.05	44.68	Yes
Strain	1	7.91	0.20	No
Treatment	3	3876.30	99.23	Yes
Relative Humidity	3	1913.69	48.99	Yes
Temp. X Strain	1	61.03	1.56	No
Temp. X Treat.	3	2253.05	57.68	Yes
Temp. X Rel. Hum.	3	422.61	10.82	Yes
Strain X Treat.	3	31.14	0.80	No
Strain X. Rel. Hum.	3	71.97	1.84	No
Treat. X Rel. Hum.	9	111.16	2.84	Yes
Temp. X Strain X. Treat.	3	17.29	0.44	No
Temp. X. Treat. X. Rel. Hum.	9	149.97	3.84	Yes
Strain X Treat X Rel. Hum.	9	63.14	1.61	No
Temp. X. Strain X. Rel. Hum.	3	63.64	1.62	No
Temp. X Strain X Treat. X Rel. Hum.	9	54.61	1.40	No
Error	192	39.06		

TABLE XXXV

POTENTIALITIES OF UTILIZING THE SPORES OF THE FUNGUS, *BEAUVERIA BASSIANA* FOR THE CONTROL OF THE RICE WEEVIL, *SITOPHILUS ORYZAE*, ON THE STORED WHEAT

Treatment	Strain 3108				Strain 4007			
	*R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
0.25	24.58**	29.81	18.98	21.76	27.32	29.61	28.55	29.54
0.50	20.98	25.72	21.31	24.61	13.45	19.99	19.09	19.12
1.00	17.00	22.79	27.67	15.81	15.00	12.83	11.01	13.61
Control	27.11	29.93	26.93	28.96	27.11	29.93	26.93	28.96

Note: Studied at 25° C. and thirty to fifty per cent relative humidity.

*R = Replication.

**Average Percentage of damage obtained out of three layers, each having the average of four samples.

TABLE XXXVI

ANALYSIS OF VARIANCE: TWO-WAY CLASSIFICATION (OF TABLE XXXV) EFFECT OF DIFFERENT CONCENTRATIONS OF TWO STRAINS OF *BEAUVERIA BASSIANA*, IN PREVENTING THE DAMAGE OF STORED WHEAT BY THE RICE WEEVIL, *SITOPHILUS ORYZAE*

Source	df	Mean Square	F	(0.99 level) Significance
Total	31			
Strains	1	31.80	3.423	No
Treatments	3	214.85	23.12	Yes
St. X TMT	3	63.78	6.86	Yes
Error	24	9.29		

TABLE XXXVII

POTENTIALITIES OF UTILIZING THE SPORES OF THE FUNGUS, BEAUVERIA BASSIANA, FOR THE CONTROL OF THE BEAN WEEVIL, CALLOSOBRUCHUS MACULATUS ON THE STORED DRY BEANS

Treatment	Strain 3108				Strain 4007			
	*R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4
0.25	95.51**	97.95	98.21	99.73	94.12	97.35	88.79	97.95
0.50	62.94	x9.50	65.52	80.24	86.16	59.38	88.19	99.03
1.00	64.78	96.91	52.74	96.21	97.06	55.78	91.51	84.81
Control	98.62	x17.78	97.15	98.33	98.62	x17.78	97.15	98.33

*R = Replication.

**Average percentage of damage obtained out of three layers, each having the average of four samples.

xThere was very low percentage of damage which may be due to early death of beetles.

Note: Studied at 25° C. and thirty to fifty per cent of relative humidity.

TABLE XXXVIII

ANALYSIS OF VARIANCE: TWO-WAY CLASSIFICATION (OF TABLE XXXVII) EFFECT OF DIFFERENT CONCENTRATIONS OF THE SPORES OF TWO STRAINS OF THE FUNGUS, *BEAUVERIA BASSIANA*, IN PREVENTING THE DAMAGE TO STORED DRY BEANS BY THE BEAN WEEVIL, *CALLOSOBRUCHUS MACULATUS*

Source	df	Mean Square	F	(0.99 level) Significance
Total	31			
Strains	1	449.17	0.675	No
Treatments	3	1035.21	1.556	No
St. X TMT.	3	415.32	0.624	No
Error	24	665.45		