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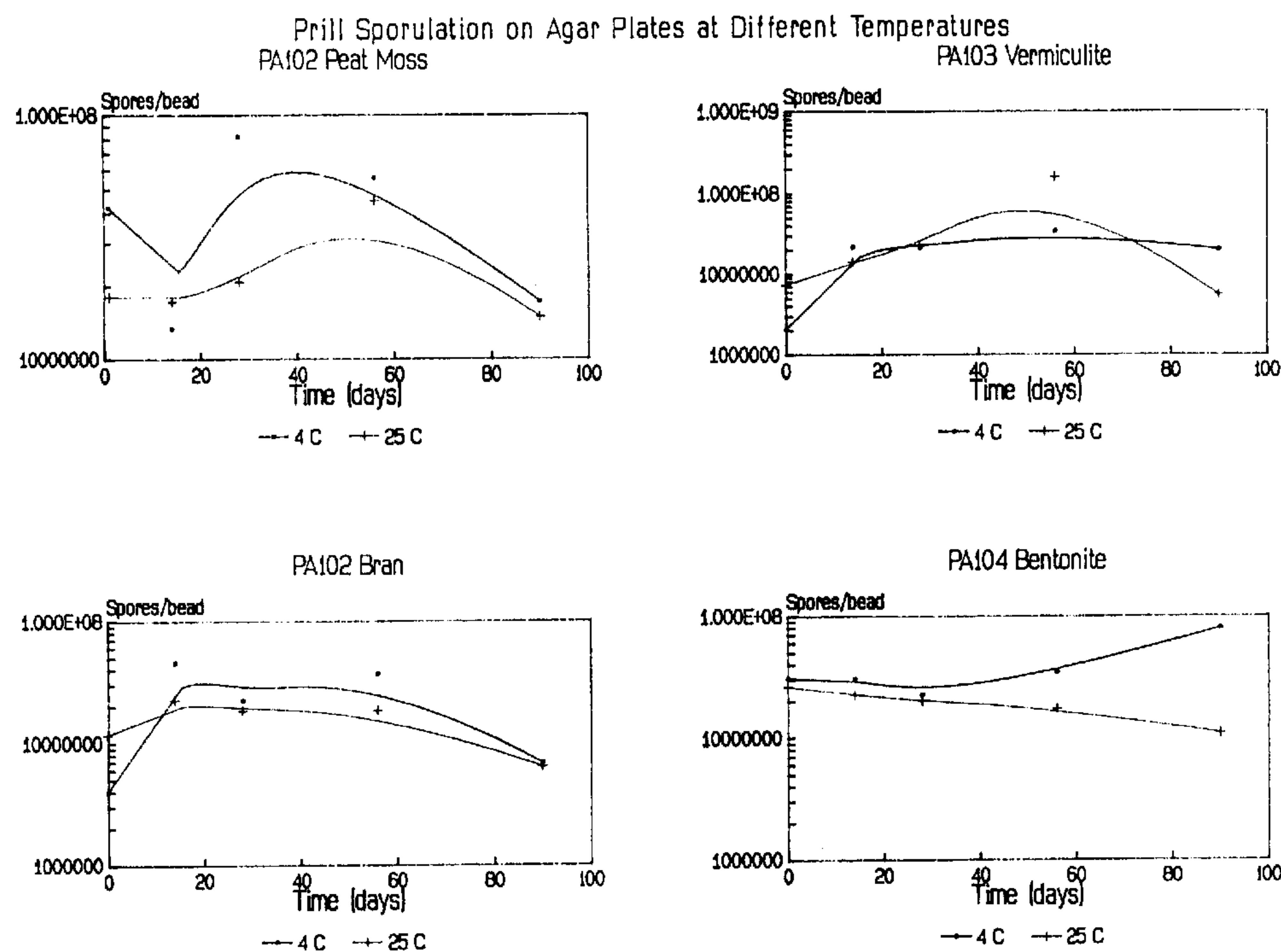
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(54) Titre : UNE METHODE ET UN PROCEDE POUR L'OBTENTION ET L'UTILISATION D'UNE COMPOSITION
FONGIQUE PATHOGENE COMME AGENT PESTICIDE

(54) Title: A PROCESS AND METHOD FOR PRODUCTION AND USE OF PATHOGENIC FUNGAL PREPARATION
FOR PEST CONTROL



(57) Abrégé/Abstract:

The present invention provides a process, formulations, and method of using a new biopesticide which comprises a prilled formulation comprising a carrier and pathogenic fungal mycelium.



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Abstract of the Disclosure

The present invention provides a process, formulations, and method of using a new biopesticide which comprises a prilled formulation comprising a carrier and pathogenic fungal mycelium.

This application is a continuation-in-part application of copending U.S. Serial No. 639,641, filed January 10, 1991.

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TECHNICAL FIELD

10 The present invention relates generally to a novel method for the economic and efficient preparation of biological pesticide agents comprising pathogenic fungus in mass quantities. These biopesticide agents are particularly useful in controlling pests which cause a significant amount of damage to plants (e.g., plant pests living in the soil or on the plants, particularly insects). A preferred entomogenic fungal preparation is 15 *Paecilomyces fumosoroseus* ATCC 20874.

20 The invention also provides methods for growing and preserving biopesticides effectively, methods for preparing and applying such biopesticide preparations in order to get maximum efficacy in controlling different soil pests, plant pests, mosquitos and soil nematodes. Methods for effective quality control are provided.

BACKGROUND

25 Chemical pesticides have been used intensively to control pests for many years. An awareness of recent problems in the use of pesticides and concern about their adverse effects on man and his environment have resulted in more commercial attention being given to biological control alternatives. Certain entomogenic fungi have 30 been recognized by researchers to be pathogenic to different pests; particularly the use of entomogenic fungi has been widely studied as biological control agents in the USSR and in Europe. Update reviews on the different pathogenic fungi and their use and status can 35 be found in: Carlo M. Ignoffo and B. Handava "Handbook of

Natural Pesticides", vol. V, part A., C. W. McCoy et al.
"Microbial Insecticide", CRC Press, 1988, and M. N. Burge
"Fungi in Biological Control Systems", Manchester
University Press, 1988. Unlike insect pathogenic
5 bacteria or other microorganisms (e.g., viruses or
protozoa) which must be ingested by the insect to
initiate diseases, entomogenic fungi normally invade
through the host's cuticle.

Entomogenic fungi infect pests, usually insects, by
10 a parasitism disease mechanism. The infection process
development is believed to consist of the following
steps:

1. Attachment - The conidium of the entomogenic fungi
15 spore is attached to the insect
cuticle.
2. Germination - The conidia spore is germinated on
the insect cuticle to form a germ
tube.
3. Penetration - The germ tube penetrates directly
20 into the cuticle. It is believed
that the cuticular invasion involves
both enzymatic and physical
activities.
4. Growth - The fungus grows in the hemocoel as
25 mycelium or blastospore. The fungi
overcome the host by invasion of
organs.
5. Saprophytic Growth -
30 The fungi grows on the outside of the
insect and produces aerial conidia.

Some entomogenic fungi overcome their host before
extensive invasion of organs takes place, presumably by
production of toxins. Although toxic compounds have been

5 reported from culture filtrate of mycelium of several
entomogenic fungi (e.g., *Paecilomyces fumosoroseus* was
shown to produce the toxin Beauvericin (Ferron, Annual
Review of Entomology 23:409-442 (1978), and a peptide
toxin known as destruxin A has been isolated from culture
broth of *Metarhizium anisoplia* (Y. Kodaira, Agr. Biol.
Chem. Chem. 26-36 (1962)), only in few cases it was
reported that toxins have been detected in insects
infected with the entomogenic fungi (Suzuki et al.,
10 Agric. Biol. Chem. 35:1641-1643 (1971)).

The use of the entomogenic fungi for control of
different pests is not itself a new idea. Entomogenic
fungi such as *Metarhizium*, *Beauveria*, *Hirsutella*,
Vermicillium or *Paecilomyces* have been studied for
15 development as pest control agents. Solid state
fermentation has been widely examined because this method
allows the production of the infectious bodies of the
entomogenic fungi, e.g., conidia spore. However, it has
been found that conidia of the various entomogenic fungi
20 are very sensitive to drying processes and the conidia
spores lose their viability very quickly.

Consequently, submerged fermentation seems to have
great potential for mass production. However, it too
seems to have significant problems to overcome. Most of
25 the entomogenic fungi grown in submerged culture produce
mostly blastospores with some mycelium. Blastospores are
not stable during storage or drying processes. Attempts
to formulate blastospores result in negative effects and
reduced efficacy and stability.

30 In order to overcome the above stability problems
associated with conidia and blastospore preparations,
several processes have been disclosed in the literature
for the production of biocontrol agents based on biomass

obtained from submerged culture fermentation. These techniques generally involve growing fungi in liquid media followed by inoculation of a solid media or an inert carrier, such as vermiculite, on which the conidia are produced. For example, Kybal (1976) discloses a process for incubating biomass containing mycelium, blastospores and other fungal stages in shallow, aerated vessels to produce conidia directly on the surface of the coated vessel surface. However, this process is very labor intensive and the conidia obtained is only on the vessel surface. The surface area per apparent volume tends to be small and the efficiency is low. In another process McCabe et al., (U.S. Patent 4,530,834), discloses the use of entomophthoralean mycelium produced in submerged culture for the production of resting conidia spores. This process is a diphasic system whereby production of spores or blastospores is bypassed. First, the biomass produced by liquid fermentation is dried with protective agents, and then the dry biomass mat obtained is milled to a dry powder form. Next, the dry powder preparation is rewetted and applied to the target pest. However, this method still has the following associated problems. The powder product needs to be stored below 4°C to maintain its viability. Even under these storage conditions the product is only stable for 64 days. The conidia produced from reconstituted powder are short lived and fragile.

Dried mycelium particles obtained from liquid fermentation of different entomogenic fungi have been produced in similar ways by different investigators. For example, McDonald et al. (Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Aus, p. 147) disclose a similar basic process for the

entomogenic fungus *Culicinomyces clavisporus* for mosquito control. This process involves growing the fungus in liquid culture for 6 days, harvesting the suspension by filtration and adding sucrose followed by air drying.

5 The dried mycelial mat was then ground in a hammer mill and sieved through a 3.5 μ m sieve. Upon addition of these mycelium particles to water approximately 5×10^6 conidia/mg of dried particle were produced. Dried

10 mycelium particles could be stored for 1-5 weeks at 4°C without losing activity. A similar approach has been used by Roberts et al. (Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Aus, p. 336) for the use of dried mycelium particles of *Metarhizium anisopliae* isolate ARSEF 2457 to control the

15 Japanese beetle and other pests. Based on the Roberts et al. results it has been found that lyophilized *Metarhizium* mycelium has a longer shelf life in soil than conidia.

Bayer A.G. (EP application 0268117 A2, 1987) has

20 reported on a similar method which is based on the production of mycelium and blastospores of *Metarhizium anisopliae* in a fermenter. During the course of fermentation, the mycelium/blastospore aggregate to form pellets in the size of 0.1 mm up to 1.5 mm in diameter.

25 At the end of the submerged culture fermentation, the mycelium/blastospore aggregates are harvested and dried in a fluidized bed dryer to form a final product which consists of dry mycelium/blastospore granules with 0.5 to 1.5 mm diameters. The granules can be applied to soil

30 where they can form infectious conidia. However, this method suffers from several problems. First, the fungal pellets formed in the fermenter result in a very low biomass yield. Therefore, such a fermentation process is

not economical. Also, the final granular product obtained must be stored under vacuum and at a low temperature. It seems also that the conidia formed after activation in the soil have a short life. This is probably because the granules do not contain any nutrients which can stimulate or promote growth.

Another approach, which has been used widely for the delivery of different fungal pathogens (mainly conidia and chlamydospores) against plant diseases, is encapsulating fungal spores in alginate prills (Lewis et al., Proceed. Intern. Symp. Control. Recs. Bioact. Mater, 12:341-3 (1985), Fravel et al., Phytopathology, 27:3341-8 (1982), and J. J. Morois, U.S. Patent 4,724,147). This method has been ignored in general for the delivery of entomogenic fungi for insect and other pest control. Few publications on the use of alginate prill for delivery of entomogenic fungi are known. For example, Enrique A. Cabanillar (Ph.D. thesis) "Factors Influencing the Efficacy of *Paecilomyces lilacinus* in Biocontrol", 1987, North Carolina State University, described this method to deliver conidia spores of *Paecilomyces lilacinus* produced on solid media (solid state fermentation) against Root-knot nematode. Roberts, et al. (Entomopathogenic Fungi: Recent Basic & Applied Research; Matha, V. et al. (ed) Biopesticides Theory & Practice Proc. Conf., Sept. 25-28, 1989, Ceske, Budejovice, Czechoslovakia 11-30 pp) disclose a process for using alginate pellets which incorporate the mycelium biomass of *Beauveria bassiana* & *Metarhizium anisopliae* with pre-gelatinized starch as a basic nitrogen supply. None of the above publications disclose any method of using activated prill for control of soilborne pests or for culturing conidia spores directly from a prill carrier formulation for controlling soil or plant pests.

SUMMARY OF THE INVENTION

This invention provides a process and formulation for the production of biopesticides comprising mycelium of entomogenic fungi. The process offers a new opportunity to use entomogenic fungi in the control of soil pests as well as plant pests, nematodes, and mosquitos. This method has been developed particularly for use with the entomogenic fungus *Paecilomyces fumosoroseus* ATCC 20874 (U.S. Patent 4,942,030).

However, this process and formulation can be used with other fungi for controlling various pests.

This invention provides a process for preparing a biopesticide useful for controlling or preventing pest infestation. The process includes fermenting one or more species of fungus effective for control of pests in a culture medium by submerged fermentation to produce biomass such that at least about 80%, preferably 90% and most preferably 95% of the biomass is in the form of mycelium. The mycelium is then harvested and mixed with a carrier. The biomass/carrier mixture is formed into prill and dried. The prill can be either used directly as a dry formulation or the prill can be used as a carrier for sporulation of conidia spores (reactivated prill) or the harvested conidia can be used or the harvested conidia can be pregerminated and then used.

Generally, this method comprises the production of mycelium prills of the entomogenic fungi. The process for forming prills is divided into three main stages as follows:

- Stage I: Production of fungal biomass in very high yield in fermenter in the form of filamentous mycelium.
- Stage II: Harvesting the filamentous mycelium,

formulating the wet mycelium, and
encapsulating the formulated wet mycelium
in calcium alginate prills.

Stage III: Drying the formulated prills and packaging
the product.

The method can further comprise the following
stages:

Stage IV: Activating and cultivating the prill.

Stage V: Harvesting and use of the infective
conidia from the reactivated prill.

The method can further comprise the following stage:

Stage VI: Pregermination and use of the infective
conidia from the reactivated prill.

For purposes of this discussion, the term
"biopesticide" shall mean the use of mycelium of the
pathogenic fungi which can be formulated in a carrier and
can be used in the control or prevention of plant, soil
or water-borne pest infestation by adversely affecting
the existence or growth of the target pest, particularly
insects. Such control can comprise a complete killing
action, eradication, arresting in growth, reduction in
number, induction of plant resistance or the production
of phytoalexins or any combination of these actions. The
term "entomogenic" as employed in the specification and
claims of this invention shall mean pathogenic to insects
specifically but can also be construed broadly to mean
pest pathogen. The term "control" or "biocontrol" as
employed in the specification and claims of this
invention is to be construed as meaning protecting
plants, soil or water from pest, particularly insect,
damage by use of the biopesticides of this invention. By
"pesticidally effective amount" or "effective amount" is

meant the amount of biopesticide prill sufficient to exert pest, particularly insect, control.

As described in the background, at present there is no effective stable delivery system available for entomogenic pesticides.

The primary purpose of this invention is to provide a process for producing an economical commercial biopesticide product. The process is intended to produce a biocontrol material that is easy to handle and to apply in either horticultural or agricultural settings. The dried product produced as described herein is easily produced, handled, stored, shipped, and applied in controlling plant, soil, and water pests. The product can be stored at room temperature, preferably between 10 to 25°C, for extended periods (more than a year without losing conidiation activity and prill/spore viability).

Still another objective of this process is to convert an actively growing culture of the entomogenic fungus into a formulated biopesticide which is more easily handled and applied. By using formulated prill, it is easy to maintain biological activity of the product until the time of application. Without the prill formulation, the mycelium could not survive under such conditions. Upon application to soil, seed or root and upon rewetting, the dried prill are reconverted into a biologically active form, in which mycelium budding from the prill occurs less than 48 hours after wetting. As the mycelium budding begins consuming the nutrients provided by the prill formulation, conidiation begins, resulting in the production of conidia spores. Conidia spores are the biologically active form which is pathogenic to pests.

Another objective of this invention is to provide a

reactivated carrier for efficiently sporulating a culture of the entomogenic fungus which produces conidia in higher concentrations than presently known methods. The conidia produced from the reactivated carrier have high viability and infectivity. Upon harvest, the reactivated prill contain mostly conidia spores. The conidia spores can be applied directly to plant, soil, seed or root with or without formulation material. Either activated or non-activated prill can be applied to soil. Further, the conidia harvested from activated prill can be transferred into a aqueous suspension and applied to the soil or foliage of a plant.

Another objective is to provide a method to pregerminate the active conidia spores harvested from the prill and apply the pregerminated spores directly to plant soil, seed or root with or without formulation material.

Another objective of this invention is to provide a biopesticide formulation/carrier that is useful in the prevention or control of pest infestation, including, but not limited to, plant and soil-borne insects and nematodes and water pests, such as mosquitos. An additional objective of the present invention is also to provide an alternative to chemical pesticides.

It is a further objective of this invention to provide a method for utilizing as a biopesticide a selected strain of the entomogenic fungal, species *Paecilomyces fumosoroseus* ATCC 20874 (PFR) which has a high level of infectivity for different plant pests. It is a still further object of this invention to provide a biopesticide containing a selected strain of the fungal species PFR which can be readily mass produced as

required for horticultural and agricultural applications.

Another aspect of this invention is to provide a biopesticide preparation comprising a fungal mycelium preparation of *Paecilomyces fumosoroseus* which is produced in submerged culture. The mycelium is then combined within a novel formulation which provides good quality control, high stability, shelf life, high infectivity, and especially specificity in infecting various plant pests.

Another aspect of the invention is to provide an economical method for efficiently culturing spores for field or greenhouse application. This method enables the production of large volumes of biopesticide which can be used in an easy and convenient manner.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents spore counts for different prill (=bead) formulations stored at different temperatures.

Figure 2 compares the biological control of adult whiteflies using formulation of the invention vs. chemical treatments.

Figure 3 compares the ability of the formulation of the invention with that of chemical treatments to effect whitefly scales.

Figure 4 compares the ability of the formulation of the invention with that of chemical treatments to effect whitefly eggs.

DETAILED DESCRIPTION OF THE INVENTION

The invention disclosed herein is a process and method for preparing a biopesticide agent which can be applied to, plants or, in and/or on soil for the control

of plant pests, particularly insects, or on the surface of water to control pests such as mosquitos. The pathogenic fungi which are useful for the purpose of this invention are preferably the fungal species from the taxonomic classes as described by Ainsworth et al. "The Fungi", vol. 4 a,b., Academic press (1973). The major Taxa which contain the entomogenic fungal species are from the following subdivisions: Zygomycotina, Mastigomycotins, Ascomycotina, Basidiomycotina, and Deuteromycotina. These different subdivision can be presented also by the different classes: Chytridiomycetes, Oomycetes, Zygomycetes, Plectomycets, Pyrenomcetes, Loculoascomycets, Teliomycetes, Coelomycetes, and Hyphomycetes.

Fungi from these classes which produce pathogenic spores that are infective to pests can be used. For example, the following entomogenic fungi are considered to be the most suitable for pest control: *Aspergillus*, *Aschersonia*, *Massospora*, *Beauveria*, *Metarhizium*, *Verticillium*, *Paecilomyces*, *Hirsutella*, *Nomuraea*, *Hymenostilbe*, *Cordyceps*, *Coelomomyces*, *Lagenidium*, *Leptolegnia*, *Conidiobolus*, *Zoopthora*, *Culicinomyces*, and *Tolypocladium*. Numerous strains of these entomogenic fungi exhibit pathogenic activity against plant pests, mosquitos, and other animal pests. Most preferred are the entomogenic fungi *Paecilomyces*, particularly the species *Paecilomyces fumosoroseous* ATCC 20874. This particular strain is well suited for use as a biopesticide according to this invention.

Fermentation

According to the invention the biopesticides are prepared for delivery by growing the selected fungal

strain or strains, particularly the preferred *Paecilomyces fumosoroseous* strain ATCC 20874, in submerged culture. Although this description refers to preparation of a single fungus, it will be appreciated that a mixture of genera or species may be desirable in some applications. An inoculum of the preferred fungus or fungi may be prepared by a standard surface culture method in which the preferred strain is grown on an agar slant, and the agar content is used to inoculate shake flasks containing agar nutrient under standard conditions. After 48 hours of incubation the shake flask contains mainly blastospores. This inoculum is used to inoculate fermenters using standard methods. Other inoculations methods may be used.

The fermentation is conducted in such a way that the biomass in the fermenter substantially comprises filamentous mycelium, at least about 80% mycelium, preferably at least about 90% mycelium, and most preferably at least about 95% mycelium. This can be achieved by supplying an excess of complex carbon and complex nitrogen sources. The complex carbon source can be naturally occurring mixtures such as molasses, and the complex nitrogen source can be corn steep liquor and/or cotton seed flour. Other known complex carbon and complex nitrogen sources can also be used. Supply of pure sugars such as sucrose, dextrose, glucose or other sugar sources will result in the formation of mainly blastospores and are therefore not preferred. The composition of the nutrient media can be varied over a wide range. However, the preferred nutrient solution contains about 4 to 8 wt% complex carbon source and about 0.5 to 5 wt% complex nitrogen source. The production of mycelium can also be intensified by the constant supply

(fed batch addition) of molasses during the fermentation process. The fermentation can be carried out in batch, continuous or in fed batch mode.

5 In order to increase the yield of filamentous mycelium in the fermenter, it can be advantageous to add stimulant nutrients to the cultivation media. Such stimulant nutrients include liquid fat, oils, surfactants, and polyacids such as linoleic acid, silicon oils, water emulsion, etc.

10 A fed batch fermentation process can be advantageous to use, especially when the pH of the fermentation is shifting toward acid conditions. By adding molasses or other suitable complex carbon sources, it is possible to maintain constant conditions in the fermentation without using large amounts of base for pH adjustment. Fed batch
15 addition of molasses, for example, can be used as buffer control for the fermentation. Preferably, the addition of molasses during fermentation is at a rate of about 1% molasses per 24 hours. Preferably, molasses addition
20 begins after about 48 hours or when the pH shifts toward acidic conditions (below about pH 5.0).

The fermentation should be carried out in such a manner that agitation and aeration will be maximized. Any convenient means of aeration and agitation can be
25 employed. If agitation is low, aggregation of mycelium occurs. Therefore a minimum agitation rate of about 400 rpm to 600 rpm is preferred with aeration in the order of about 0.8 to 1 vvm. The fermentation process preferably should be carried out in the temperature range
30 of about 28-30°C.

The adjustment of the pH value is necessary in order to achieve formation of mycelium. Most favorable is pH 4.0 to 6.0, preferably about 5.0. Adjustment and control

of the pH value can be achieved by the addition of an organic or inorganic base, preferably sodium hydroxide, ammonium hydroxide or any solution of triethyl amines.

5 In order to prevent developing undesirable amounts of foam during fermentation, standard chemical defoaming agents can be added. Standard chemical defoaming agents include silicon oil, polypropylene or glycol compounds, or other synthetic antifoams. The end of the cultivation can be easily determined by the standard method of
10 biomass determination (e.g., dry mass determination). A 96-hour cultivation typically will be sufficient to yield 30-50 g/liter of dry mycelium. This yield is sufficient for use in the formulation stage of this invention.

15 The separation of the filamentous mycelium biomass from the fermentation media can be accomplished by standard procedures such as filtration, centrifugation or other convenient means of separation. To avoid
contaminating the mycelium with undesirable
microorganisms, harvesting the mycelium under sterile
20 conditions is recommended.

Formulation

In order to prepare the biopesticide formulation carrier of this invention, carrier materials are used
25 which are capable of supporting fungal growth and promote rapid sporulation. Suitable carriers include inert filling compounds such as clay, bentonite, talcum, perlite, peatmoss, diatomaceous earth, kaolin, vermiculite, bran, dry milk, and minerals. Vermiculite
30 is preferred because it results in a stable biopesticide product and also because it lowers the density of the product, which allows the mycelium to conidiate faster. The vermiculite (or other carrier material) most

preferably is pretreated to reduce the level of
contaminating microorganisms. This preferably is by
heating (i.e., at 100°C or higher for at least up to
about 1 hour) or may be by irradiation. Microbial
5 decontamination may be also accomplished chemically,
provided that there is no retained chemical interference
with fungal viability and growth. Returning to the
preferred embodiment, the filamentous mycelium biomass is
then added to the vermiculite or other carrier component
10 by mixing.

Nutrients can be included in the mycelium
formulation. Such nutrient sources may include carbon
and nitrogen sources such as molasses, whey, milk powder,
cotton seed flour, different autolyzed peptone, bran,
15 wheat, malt extract or yeast extracts. It may be desired
to add stabilization and/or protective agents to the
formulation, such as polyalchols, glycerin or sugars.
Antioxidants compounds such as ascorbic acid or propyl
gallate may be added, if desired.

20 The formulated mycelium mixture is then prilled by
methods known to one skilled in the art. The prilling
process can be conducted by adding a prilling agent such
as sodium alginate or potassium alginate to the
biomass/carrier mixture and by dropwise adding the
25 mixture to a coagulant bath containing calcium chloride
or calcium gluconate. The sodium alginate concentration
in the propagule mixture can vary from about 0.2 to about
3% depending on which formulation is used and degree of
propagation needed. Calcium chloride or calcium
30 gluconate concentration for coagulating can vary from
about 1 to about 15% w/v as needed for suitable prill
formation. Coagulation proceeds faster when the
concentration of salts in the coagulation bath is

increased. The prill formed by this method can be dried immediately by using any convenient drying method, such as air drying or oven drying. However, a fluidized bed process is preferred to obtain flowable prill with uniform, physical characteristics (shape, mechanical strength, size, and density).

In order to increase the conidiation potential of the prill, it may be desired to add nutrients which will stimulate conidiation to the prill. Such stimulating material can contain natural food ingredients such as molasses, peptone, cotton seed flour, glucose solution, etc. The addition of the nutrient can be before drying or in the drying process. Addition of nutrients before drying can be achieved by submerging the prill in a concentrated nutrient solution until diffusion is complete and then drying the treated prill. Addition of nutrients in the drying process can be achieved by coating the prill during their movement in the fluidized bed. Such coating procedures are well known. The prill should be dried to a moisture or total volatiles content of about 2 to about 12% (w/w). However, a total volatiles content of about 6-8% is preferred. The moisture content can be easily determined according to standard methods using moisture balances. To avoid contamination with undesirable microorganisms, the drying process should be done in sterile air, for example by using air filters.

Prill that have been produced according to the invention can be stored under dry conditions at temperatures between about 4°C to about 25°C. To preserve the viability of the prill beyond 12 months, it is recommended to store the prill in a vacuum or in an

inert atmosphere such as under argon, nitrogen or other inert gases.

Reactivation and Harvest

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The formulated prill can optionally be reactivated prior to use. The stable prill formulation can be reactivated prior to use to provide a concentrate of highly active, fresh conidia spores. The formulated prill contain optimum nutrients which stimulate sporulation and production of a large number of spores in a short time upon activation. Each prill (less than 1mm diameter) can produce as much as 10^7 - 10^8 conidia spores per prill and the conidia spores have high insecticidal effect on controlling pests. Reactivation is easily achieved by addition of moisture, preferably water, to the prill and allowing the growth of mycelia and production of conidia. Optimal growth conditions are incubation at about 20-28°C, preferably about 25°C for less than about seven days, preferably about 3 to about 5 days. Reactivation can take place in any suitable closed, sterile container, such as a petri dish or tray. The prill preferably should be placed as a monolayer in the container in order to achieve maximum surface area for the sporulation and germination process. The reactivated prill can be stored up to about 6 months, preferably between 0 and about 3 months under cool conditions of about 4°C.

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The conidia spores can be easily harvested from the reactivated prill by washing with water which contains a surfactant. Any suitable surfactant can be used including Tween 80® or Tween 20® surfactants in the range of 0.01 - 0.1%. The harvested conidia can be used immediately or stored until application preferably, under

cool conditions of about 4°C. The harvested conidia can be stored for up to about 4 months, preferably about 2 weeks at 4°C. The amount of conidia spores obtained by the cultivation of the prill is about 10^7 - 10^8 spore per prill.

It is possible to add nutrients to the conidia spores which are harvested from the reactivated prill before application on the target. Such nutrients can enhance the germination of the conidia on the pests. Alternatively, it can be useful to pregerminate the conidia prior to application. Such pregermination can be easily established by stirring the harvested conidia for about 4-8 hours in a water aerated container or any other suitable means.

Use

The application of the prill, the reactivated prill, the harvested spores or the pregerminated spores prepared according to the invention is dependent on the nature of the pest to be controlled and the particular application (agriculture, horticulture, forest or mosquito control). If the pest target is a plant pest, the prill, reactivated prill or spores can be applied near the plant or on the plant. If the target is a soil pest, the prill, reactivated prill or spores can be applied to or mixed with the soil. If the target is mosquitos, the prill, reactivated prill or spores can be applied to the water surface.

Different entomogenic fungi can be used in the biopesticide prill of the invention to control pests. Pests that can be controlled by the biopesticides of this invention include arthropods and nematodes. Particularly preferred pests include insects such as mosquitoes and

blackflies and pests belonging to the acarina and arachnid family. The biopesticides are effective against pests which are normally sensitive and also those which are resistant to conventional pesticides. They are effective against all or individual pest development stages. The biopesticides can be used effectively against pests from the following: *Isopoda*, *Oniscus asellus*, *Armadillidium*, *Diplopoda*, *Chilopoda*, *Symphyla*, *Thysanura*, *Collembola*, *Orthoptera*, *Dermaptera*, *Isoptera*, *Anoplura*, *Mallophaga*, *Thysanoptera*, *Heteroptera*, *Homoptera*, *Lepidoptera*, *Coleoptera*, *Hymenoptera*, *Diptera*, *Siphonaptera*, *Arachnidia*, and *Acarina*.

Also, the biopesticides can be used against plant parasitic nematodes including *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus similis*, *Ditylanchus dipsaci*, *Heterodera* spp., *Xiphenema* spp., *Globodera* spp. and *Hoplolaemus* spp.

The principle target insect groups which are preferred for the biopesticides of this invention are: *Culicidae* (mosquitoes) and other *Diptera*, *Aphidae* (aphids), *Dalmanella* (planthoppers), *Cicadellidae* (leafhoppers), *Cercopidae* (spittlebugs), *Aleyrodidae* (white fly), *Coccoidea* (scales), *Thysanoptera* (thrips), *Coleoptera* (beetles), and *Lepidoptera* (caterpillars).

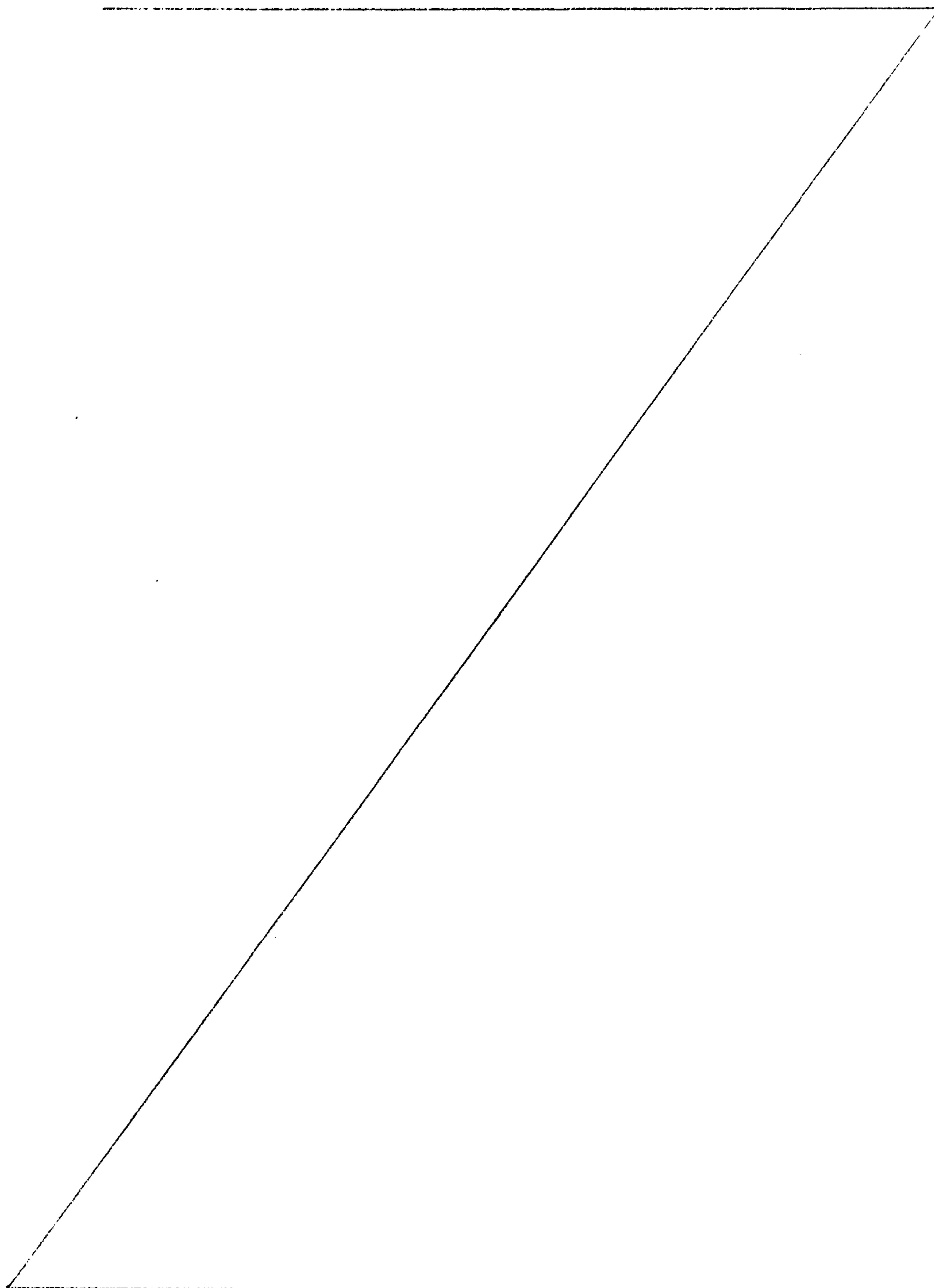
The prill dosage will vary greatly depending on the application. Factors to consider include the kind of prill formulation used (e.g., vermiculite prills are more efficient in controlling mosquito larvae than bran prills because of floating properties of the vermiculite prills), the kind of pest, the state of crop infested with the pest, the prevailing weather conditions, and the kind of the agriculture area (e.g., agriculture, horticulture, forestry or other conditions). In general,

for controlling plant insects, an application dose of about 10^7 conidia spores/ml is recommended. Such dose can be obtained based on the following ratio: one gram of prill yields a suspension of 10^7 conidia spores/ml in liter. For controlling soil insects, an application dose of about 1-10 kg, preferably about 5 kg of inactivated and/or activated prill per hectare is recommended. The biopesticides can be applied by any convenient application method including, broad cast spreading on the soil or plant, or mixture with the soil.

The examples which follow are given for illustrative purposes and are not meant to limit the invention described herein. The following abbreviations have been used throughout in describing the invention.

15	CFU	-	Colony forming unit
	CPU	-	Conidia spores per prill unit
	°C	-	degree(s) Centigrade
	g	-	gram(s)
	hr	-	hour(s)
20	l	-	liter(s)
	μ	-	micro
	%	-	percent
	lb	-	pound(s)
	ft ²	-	square foot (feet)
25	M	-	molar
	ml	-	milliliter
	N	-	normal
	PDA	-	peptone dextrose agar
	RH	-	relative humidity
30	rpm	-	rotation per minute
	w	-	weight
	vvm	-	volume per volume per minute

AIS - Assessing Index Scale
GI - Growth Index



Example 1Production of Mycelium of *Paecilomyces*
fumosoroseous in 20-Liter Batch Fermenter

5 The fungus *Paecilomyces fumosoroseous* (ATCC 20874)
was maintained on a slant agar containing 20 g/l malt,
20 g/l glucose, 1 g/l peptone, and 10 g/l agar. The
slant was stored at 4°C.

10 Slants were transferred under sterile conditions to
a shake flask medium of 30 g/l glucose, 20 g/l yeast
extract, and 20 g/l corn steep liquor. The solution was
adjusted to pH 6 before sterilization. After
inoculation with the slant fungi, the shake flask
containing the fungus was maintained at 30°C for 24 hours
on a round shaker at 300 rpm. The shake flask product,
15 mainly blastospores, was used to inoculate a 20-liter
fermenter containing 16 liters of production media
composed of 60-80 g/l molasses, 20 g/l cotton seed flour,
and 20 g/l corn steep liquor. The fermentation pH was
controlled to pH 5.3 by adding base (2M NaOH). Aeration
20 was maintained at between
0.8-1.0 vvm, and agitation was maintained at
400-600 rpm. In order to avoid formation of foam, 1.5 ml
of Macol® P-2000 antifoam agent (Mazer® chemicals) was
added to the fermentation solution. The fermentation
25 was completed after 96-100 hours, and the filamentous
mycelium was harvested by centrifugation. The yield of
filamentous mycelium obtained was 30 g/l (dry weight).

Example 230 Preparation of Biopesticide Formulations

The mycelium of *Paecilomyces fumosoroseous* obtained
in Example 1 was used to prepare the formulations
described in Table I. Briefly, the *Paecilomyces* mycelium

(300 g at 25% moisture content) was blended and mixed with the described amounts of carriers. The carriers had been previously autoclaved for 1 hour at 121°C with 1 liter of water. Sodium alginate was added and each of the blended mixtures was brought to a total volume of 3 liters and 1N NaOH was added to obtain the indicated pH. For prill formation of each mixture, a bath containing 5 liters of a calcium chloride solution at concentration range of 13-27% (pH 6.35-7.00) was used. The blended mycelium/porous carrier mixture was loaded onto a prilling column, and the mixture was added dropwise to the coagulation bath to form alginate prill. The prill were submerged in the coagulation bath for 1 hour or longer. The prill were easily removed from the bath by screening them through a metallic screen. The wet prill, which contain as much as 80-85% moisture, were loaded onto a fluidized bed dryer and dried at an air temperature below 30°C. The prill were dried to a water content of 6% to 10% w/w.

TABLE I

Formulation	Porous Carrier	Amount	Alginate Amount	pH
A	Bran Milk Powder	300 g 80 g	60 g	6.0
B	Bentonite	500 g	35 g	8.5
C	Vermiculite	400 g	70 g	5.9
D	Peat Moss	350 g	35 g	8.5
E	Vermiculite Bran Cotton Seed Flour	200 g 200 g 100 g	60 g	8.5
F	Vermiculite Bran	300 g 100 g	45 g	5.3
G	Vermiculite Bran	300 g 100 g	45 g	9.0

Example 3Evaluation in vitro of Prill and Conidia Viability

Batches of biopesticide prill prepared according to Example 2 were evaluated and assessed for viability, germination and conidiation after storage.

Prill Mycelium Viability

Thirty-six prill were rehydrated by submerging in water for 30 minutes. The reactivated prill were placed on the surface of an agar plate or empty plastic well and incubated for 48 hours at room temperature. The percent viability of the prill was evaluated by observing and tracking the mycelium developed on the surface of the prill. Table II represents percent prill viability of different batches which were stored at different temperatures for up to 12 months.

Conidiation In Vitro Test

Conidiation of the different prill formulations which sporulated on agar plates or empty plastic wells for 48 hours as above were examined by transferring three sporulated prill to a sterile screw cap tube containing 10 ml of a 0.01% Tween™ 80 surfactant solution. The tube was shaken vigorously for 20 seconds and the conidia spores were counted using a hemacytometer. Table III represents spore counts for prill which were stored at different temperatures for as up to 1 year. Figure 1 presents spore counts for different prill formulations stored at different temperatures for as long as two months. All conidia germinated to 100%.

TABLE II

Formulation	Storage Temperature	Storage Time (months)	Prill Viability
D	4°C	6	100%
D	25°C	6	99%
A	4°C	6	100%
A	25°C	6	99%
A	4°C	5	98%
A	25°C	9	95%
A	25°C	12	95%
A	4°C	12	95%
B	4°C	11	100%
C	4°C	12	100%
F	4°C	6	100%
G	4°C	6	100%

TABLE III

Percent of Dead Whitefly:			
Formulation	Storage Temperature	Storage Time (months)	Conidiation (Spore per Prill)
A	4°C	10	1.95 x 10 ⁷
A	25°C	10	1.07 x 10 ⁷
A	25°C	11	1.33 x 10 ⁷
C	25°C	6	5.0 x 10 ⁷
F	25°C	6	1.87 x 10 ⁷

Conidia Germination In Vitro Test

The conidia spores which were produced in the preceding section by the sporulated prill were collected and a 1 x 10⁵ spore per ml solution was prepared. Sterile

sabourads dextrose broth (100 μ l) was added to 2 rows of a 96 well plate (Corning 25860 polystyrene). The spore suspension (100 μ l) was added to a total of 16 wells of each plate. The spores were incubated at 28°C for 24 hours and were examined for germination (forming germ tube). Table II lists the results, indicating that all the prill formulations tested were able to form germinating conidia, under various storage conditions and times.

Example 4

Efficacy Study

The efficacy of the bran/milk biopesticide prill formulations prepared in Example 2 was evaluated in a greenhouse for control of whiteflies. Each test was conducted by infesting a blue salvia plant with whiteflies. The insects were allowed to oviposit for 24 hours, then all adults were removed. The salvia were grown under greenhouse conditions allowing the immature whiteflies to develop. Several early to mid-fourth instar scales were removed and incubated in 100% relative humidity to demonstrate that no viable *Paecilomyces fumosoroseus* fungi were present in the greenhouse. The infested plants were then subjected to the following treatments:

Treatment A - 1 g of prill per plant was scattered directly on the moistened surface of the soil.

Treatment B - 1 g of prill per plant was soaked for 1 hour in deionized water. The prill were placed in a petri dish atop 1 piece of moistened Whatman™ 5-filter paper and incubated for 24 hours. The prills were

then scattered directly on the soil surface.

Treatment C - 10 prill were incubated on a PDA agar plate for 7 days at 25°C under a 12-hour photo period. The plate was scraped with a sterile instrument. Five ml of this solution were pipetted into 500 ml of a 2% sucrose solution. Plants were dipped in this solution.

Treatment D - Plants were dipped in 500 ml of deionized water (no prill added).

Eight plants were used per treatment. Living, dead, and infected scales were counted on 3 leaves per plant weekly. Each week, 24 instar scales were removed from leaves in each treatment and incubated in 100% RH to measure mortality. Table IV represents the mortality and efficacy results.

TABLE IV

Percent of Infected Whitefly:		
Treatment Type	Day 3	Day 7
Treatment A	60%	94%
Treatment B	48%	58%
Treatment C	40%	60%
Treatment D	18%	18%

Example 5

In Vivo Bio Assay (Reactivation of Prill)

A standard in vivo bio assay to assess the viability and infectivity of the conidia spores produced by the prills has been developed. The procedure consists of harvesting conidia from activated prills by soaking 200

prill in 0.05% Tween™80 solution and then diluting the conidia harvested to a concentration of 1.0×10^7 conidia per 1 ml.

5 Germination (Viability) Assays

10 The conidia suspension was spread over a sterile microscope slide coated with a thin layer of water agar (2.0% Difco agar) by means of a sterile inoculating loop. Slides were then placed into wet chambers (petri dishes with filter paper) and 0.2 ml of sterile water was added to the filter paper. Conidia were incubated for 16 hours in an incubator (25°C) and assessed for germination under a light microscope. All conidia with a germ-tube(s) of any size were counted as being germinated.

15

Infectivity Assay

20 The sweet potato whitefly B. tabaci was the host insect used for measuring infectivity. Early 4th instar nymphs of B. tabaci (un-synchronized population reared on Hibiscus sp) were collected from leaves with a flattened needle and placed on the surface of a sterile microscope slide. Conidia suspension which was prepared as described above was used by placing a drop with known concentration on the surface of a sterile microscope slide (20 drops per slide, 10 in each of two rows running the length of each slide). Previously collected nymphs of B. tabaci were placed on each droplet and into a wet chamber and 0.2 ml of sterile water were added to the filter paper. Samples were kept in an incubator (25°C) for a period of 7 days. Two chambers (total of 40 nymphs) were used for each sample tested. As a control, slides with nymphs only were prepared by the same manner as previously described but without conidia added to the

25

30

Tween 80 solution. To evaluate the growth of the fungus without nutrient source, slides with drops of conidial suspension only (without nymphs) were also prepared.

Each nymph or drop of conidia was observed under a light microscope (100 x magnification) and rated according to the following index (**Assessing Index Scale = "AIS"**):

- 0.0 No changes (conidia do not germinate, no visible changes anywhere in the drop area)
- 0.5 Germination of conidia (beginning of germination, conidia with one or two germ-tubes are present anywhere in the drop area or un-branched hyphae are present)
- 1.0 Beginning of mycelial growth (anywhere in the drop area presence of branched hyphae is visible, no growth of fungus on host is noted)
- 1.5 First occurrence of the fungus hyphae on the host (anywhere on the host surface hyphae of fungus are present)
- 2.0 Regular growth of mycelium on the host (on most of the surface or alongside of host body overgrowth by mycelium is visible)
- 2.5 First occurrence of newly formed conidia (anywhere on the host surface or on a mycelium alongside the host body first conidiophores and newly formed conidia in chains are present)
- 3.0 Regular sporulation (fully sporulated mycelium covers most of the surface of infected host or takes place alongside of host)

All samples were rated on days 1,3,5, and 7. The results were expressed as a daily average of "AIS" from each sample (40 nymphs). Control nymphs were assessed with the developmental stage of the nymph and presence of any infection being recorded. Control drops (no nymphs of B. tabaci placed into the drop) were observed and the developmental phase of fungus noted (germination of conidia, mycelial growth, or sporulation).

Basic physical characteristics of different types of

alginate prill used in the experiment are presented in the following table.

Table V.

Formulation	Avg Wt of 1 prill (g)	Avg Amt of prills per 1 g	Shape of prills
G	0.0027	370	homogenous
D	0.0025	400	homogenous
C	0.0035	286	diverse
E	0.0037	270	homogenous
E	0.0030	333	homogenous
E	0.0017	590	homogenous
E	0.0014	714	homogenous

* The E formulations differ by date of manufacture.

Visual and physical weight changes which occur during activation are shown in Table VI.

Table VI.

Formulation	Initial weight (g)	Day 1 wt change (g) (%)	Day 2 wt change (g) (%)	Day 7 wt change (g) (%)
F	0.0027	0.0039 +44.4	0.0042 + 55.6	0.0046 + 70.4
G	0.0025	0.0040 +60.0	0.0044 + 76.0	0.0050 +100.0
D	0.0035	0.0048 +37.1	0.0056 + 60.0	0.0065 + 85.7
C	0.0037	0.0053 +43.2	0.0055 + 48.6	0.0046 + 24.3
E	0.0030	0.0047 +56.7	0.0050 + 66.7	0.0063 +110.0
E	0.0017	0.0033 +94.1	0.0035 +105.9	0.0032 + 88.2
E	0.0014	0.0027 +92.9	0.0029 +107.1	0.0026 + 85.7

Table VII Compares different types of alginate pellets with incorporated mycelium of entomogenous fungus

Paecilomyces fumosoroseus isolate PFR 97 - an average growth index during activation of prills in laboratory conditions (counted as an average GI from 20 prills*).

Table VII.

Formu- lation	GI - Day 1	GI - Day 3	GI - Day 5	GI - Day 7
F	1.8	3.4	4.6	5.0
G	1.1	3.3	4.4	5.0
D	1.4	3.6	4.1	5.0
C	0.8	1.8	3.8	5.0
E	2.0	3.6	4.4	5.0
E	1.8	3.0	4.2	5.0
E	1.7	3.2	4.4	5.0

* Growth index scale:

- 0 - no visual changes on prills
- 1 - swelling phase (change of size, shape and color, no growth of fungus noted)
- 2 - first hyphae growth on surface of prills
- 3 - regular overgrowth, mycelium covers most of the prill's surface
- 4 - beginning of sporulation (first conidiophores and conidial chains)
- 5 - full sporulation (most of the prill's surface covered with sporulated mycelium)

Production of conidia and germination of Formulation E over 7 day period after activation is described in Table VIII.

Table VIII.

Time of Activation	An Average Amount of Conidia per 1 Prill	Germination Test	
		% Germination	Shape of Germinated Conidia
Day 2	1.42×10^6	93.7	2 germ - tubes
Day 3	1.29×10^7	98.8	2 germ - tubes
Day 4	1.50×10^7	100	2 germ - tubes
Day 5	2.97×10^7	100	2 germ - tubes
Day 7	3.31×10^7	100	2 germ - tubes
Day 10	3.37×10^7	99.2	2 germ - tubes

Comparison of different types of prills for germination is shown in Table IX.

Table IX.

Formulation	% Germination	Dominant Shape of Germinating Conidia
F	99.8	2 long germ-tubes (*)
G	99.2	1 long germ-tube
D	93.3	2 short germ-tubes
C	81.6	1 short germ-tube
E	99.6	2 long germ-tubes (*)
E	99.7	2 long germ-tubes (*)
E	99.0	2 long germ-tubes (*)

(*) - first secondary branches presents

Infectivity of the conidia produced from Formulation E after different periods of activation is described in Table X.

Table X.

Period of Prills Activation	Average "AIS" Day 1	Average "AIS" Day 3	Average "AIS" Day 5	Average "AIS" Day 7
2 days	0.75	0.90	1.20	1.85
3 days	0.80	1.35	1.75	2.15
4 days	0.85	2.15	3.00	3.00
5 days	0.90	2.30	3.00	3.00
7 days	0.80	2.15	3.00	3.00
10 days	0.65	1.90	2.70	3.00

* Assessing Index Scale - "AIS

- 0.0 No changes (conidia do not germinate, no any visible changes anywhere in a drop area)
- 0.5 Germination of conidia (beginning of germination, conidia with one or two germ-tubes are present anywhere in a drop area or unbranched hyphae are present)
- 1.0 Beginning of mycelial growth (anywhere in a drop area presence of branched hyphae is visible, no growth of fungus on host is noted)
- 1.5 First occurrence of the fungus hyphae on the host (anywhere on the host surface a hyphae of fungus are present)
- 2.0 Regular growth of mycelium on the host (on most of the surface or alongside of host body overgrowth by mycelium is visible)
- 2.5 First occurrence of newly formed conidia (anywhere on the host surface or on a mycelium alongside the host body first conidiophores and newly formed conidia in chains are present)
- 3.0 Regular sporulation (fully sporulated mycelium covers most of the surface of infected host or takes place alongside of host)

Comparison of different type of prill for infectivity is described in Table XI.

Table XI.

Formu- lation	"AIS"-Day 1	"AIS"-Day 3	"AIS"-Day 5	"AIS"-Day 7
F	0.95	2.30	3.00	3.00
G	0.85	2.00	2.60	3.00
D	0.70	1.90	2.30	2.80
C	0.45	1.60	2.20	2.70
E	0.80	2.15	2.55	3.00
E	0.85	2.35	3.00	3.00
E	0.90	2.40	3.00	3.00

Example 6

In order to compare the germination and infectivity of the conidia produced by the prill to standard conventional methods of growing conidia on different substrate, the following experiment was conducted. Three (3) samples of conidia were obtained from 3 different sources:

- 1) from prills (formulation E),
- 2) from agar petri dishes (PDA - Potato Dextrose Agar Difco), and
- 3) from whitefly nymphs infected with PFR.

In all experiments, conidia of strain PFR ATCC 20874 were used.

The conidia from prills were harvested as previously described. The PDA-conidia were obtained from surface cultures on a solid artificial media. PDA plates were inoculated with 1 ml of conidial suspension (1.0×10^7 per ml), spread over the entire surface of the plate and kept an incubator (25°C). When harvested on day 20, conidia from the surface of the culture were washed with sterile

0.05% Tween 80 surfactant and this suspension was diluted to a concentration of 1.0×10^7 conidia per 1 ml. The total amount of conidia produced on a surface cultures (PDA plates) was stated by the similar procedure as from activated prills. For the same experiment, conidia harvested from infected hosts (B. tabaci, early 4th instar nymphs) were used. The same method as for standard bioassay (described below) was used to obtain infected nymphs. Infected nymphs on which the fungus was sporulating were collected and placed into plastic ampules and soaked into 1 ml of sterile 0.05% Tween 80 solution. The suspension of conidia obtained in this manner was diluted to a concentration of 1.0×10^7 conidia per 1 ml. The total length (from tip of germ tube to the tip of the opposite one, if present) of at least 250 conidia was measured using an ocular micrometer. The following table presents the viability and virulency of conidia obtained from PFR strain ATCC 20874 which was obtained from different nutrient sources.

As can be seen from Table XII, the conidia obtained from the prill have a higher quality than those produced on the surface of PDA culture. The time to obtain conidia on PDA culture is twice as long as obtaining conidia from prills.

Table XII.

Nutrient Source	% Germination	Dominant Shape and Length of Germinating Conidia	"AIS" Day 1	"AIS" Day 3	"AIS" Day 5	"AIS" 7 day
Formula-ion E	99.3	2 germ-tubes (4.4-5.8 um	0.90	2.10	3.00	3.00
PDA	93.8	1 germ-tube (3.0-3.8 um)	0.75	1.85	2.60	3.00
B.tabaci E4 nymphs	99.8	2 germ-tubes (8.2-11.2 um)	0.95	2.20	2.90	3.00

Example 7

In order to evaluate the ability of PFR prill to sporulate and germinate in soil, the following experiment was conducted. Eight 250 ml Erlenmyer glass flasks were filled with 15 g of Redi lite soil mix (Terra-Lite®). The flask and the soil were autoclaved for 20 minutes at 121°C. After the soil was cooled to room temperature, 20 vermiculite prill (as prepared and described in Example 2) were added to the soil mixture in the flask. The soil and the prill were shaken well, and 50 ml of sterile deionized water was added to enhance humidity. The soil prill mixture was incubated at 25° and 30°C. After a month the number of spores in the soil were evaluated. The spore count was determined by the following method:

CFU Determination

1. Aseptically mix thoroughly the flask contents using a sterile spoonula.
2. Carefully remove 1 g of wet soil mixture.

3.
- Make 1:10 serial dilutions using 50 mM phosphate buffer pH 7.0.
4.
- Plate the sample by adding 100 μ l of sample onto a Rose Bengal plus 100 mg chloramphenicol plate and make a spread plate. Prepare triplicate plates.
5.
- Incubate the plates at 28°C.
6.
- After 5 to 6 days count the fungal colonies present.

Table XIII represents the results of this study.

TABLE XIII

Flasks	CFU/g after 30 Days Incubation Temperature	
	25°C	30°C
Flask 1,2	2.48 x 10 ⁶	2.2 x 10 ⁶
Flask 3,4	2.0 x 10 ⁵	2.6 x 10 ⁶
Flask 5	2.6 x 10 ⁶	

Example 8

Large Scale Protection of Hibiscus Against White Fly

The PFR conidia harvested from reactivated prills were tested to evaluate the ability to protect hibiscus sp. plants against white fly (*Bemisia tabaci*). The reactivated prill described in Example 5 were used to protect hibiscus plants grown in commercial greenhouses in Apopka, Florida. Large quantities (100 g) of dried prill were activated in large plastic boxes (450 x 250 x 100 mm). The same procedures for evaluating and cultivating the prill as described in Example 5 were used. Briefly, after 7 days incubation at 25°C, the conidia spores produced were stored at 4°C. About 3-6

hours prior to application, 10 g of the spores were harvested from the reactivated prill into 2 liters of water plus 0.05% Tween 20® surfactant. The concentration of conidia in this concentrate was counted using a

5 Neubaer hemocytometer. Prior to application, the conidial concentrate was diluted with water to a final concentration of $1.0 - 1.5 \times 10^7$ conidia/ml. Fifty liters of the final conidia suspension were applied in one

10 treatment to 800 hibiscus plants using a backpack sprayer (Birchmeier) with special attention to cover the underside of the leaves. The biopesticide was applied weekly for a period of one month. As a control, Hibiscus plants were treated with different chemical agents (see

15 Table for chemical application). Plants were checked once a week, and leaves were examined randomly from the chemical control and from the PFR treatment. Figures 2-4 show the comparison of the treated plant with PFR to a

20 control area which was treated with chemicals. As can be seen from the figures, PFR works better than the chemical treatment. It is important to note that parasitized scales were found mainly in the chemical treatment and not at the PFR site. However, no parasites infected with

25 PFR were found. Therefore, mortality of whitefly at the PFR treated area is mainly because of the fungus establishment.

Example 9

Pregermination

30 Conidia spores harvested from reactivated prill described in Example 5 (Formulation E) were evaluated to determine whether pregermination of conidia or addition of stimulating nutrient to the conidia can enhance the

infectivity of the conidia. The conida were applied to plants infected with whiteflies.

The results shown in Table XIV indicate that pregerminated conidia have the greatest effectiveness in controlling whitefly within the shortest time (12-24 hours).

Table XIV

Percent of Dead Whitefly:			
Time at 100% humidity directly after Application*	Formulation E in Water	Formulation E with Nutrient	Formulation E Pregerminated
12	64	72	84
24	56	77	82
48	47	91	89
72	68	94	90

*Subsequently switched to 75% humidity

Example 10

Induced resistance

The conidia of the invention were tested to determine the influence of the entomogenous fungus *Paecilomyces fumosoroseus* on the colonization and induced resistance of host plants. A total of 60 uninfested poinsettia plants of the same size and shape (an average of 6 leaves/plant) grown in small pots were used. Adult sweetpotato whiteflies were caught into plastic tubes and released onto the treated plants. The plants were treated with *Paecilomyces fumosoroseus* PFR 97 harvested from alginate pellets (Formulation E activated as described in Example 5). The conidial suspension used

was a 0.05% Tween solution adjusted to a titre of 1.0×10^7 conidia per ml.

The treatments used were:

5 A - preventive treatment (plants were treated with conidial suspension one week before exposure to sweetpotato whitefly adults. Before whitefly adults were released, the treated plants were deposited into a nylon cage to prevent any undesirable infestation);

10 B - treatment before release of whitefly adults (plants were treated with conidial suspension and then exposed to the whitefly adults when the surface of treated plants dried);

15 C - Control plants (plants were treated with 0.05% Tween solution and then exposed to the whitefly adults when the surface of treated plants dried).

20 All treated plants were placed in a greenhouse in a randomized square fashion. Twelve plants from each of the variants (A-B-C) were placed in a randomized square and adults of whitefly were released at 5 points (center of the square and each of the corners in the second row).

25 The number of adults per plant was determined every 24 hrs for a period of 1 week. When counted, the following data were noted:

- 30 a) alive adults (total amount per all plants in each group)
b) dead adults (total amount per all plants in each group)
c) infected adults (total amount per all plants in each group)

35 Treatment of plants with *Paecilomyces fumosoroseus* ATCC 20874 conidia harvested from prills resulted in significantly poorer establishment of pest populations when treated 1 week before exposure than when treated just prior or not at all. Treatment just prior to exposure was better than not at all. These results shown
40 in Table XV indicate that conidia of *Paecilomyces*

fumosoroseus ATCC 20874 harvested from prills illicit an immune response (induced resistance) by the plant.

Table XV

Day	Total number of alive adults	Variant A number / %	Variant B number / %	Variant C number / %
1.	1157	238 / 20.6	336 / 29.0	583 / 50.4
2.	1146	170 / 14.8	379 / 33.1	597 / 52.1
3.	967	130 / 13.4	298 / 30.8	539 / 55.8
4.	697	71 / 10.2	186 / 26.7	440 / 63.1
5.	447	34 / 7.6	93 / 20.8	320 / 71.6
6.	315	19 / 6.0	70 / 22.2	226 / 71.8
7.	270	23 / 8.5	63 / 23.3	184 / 68.2
14.	68	10 / 14.7	22 / 32.4	36 / 52.9

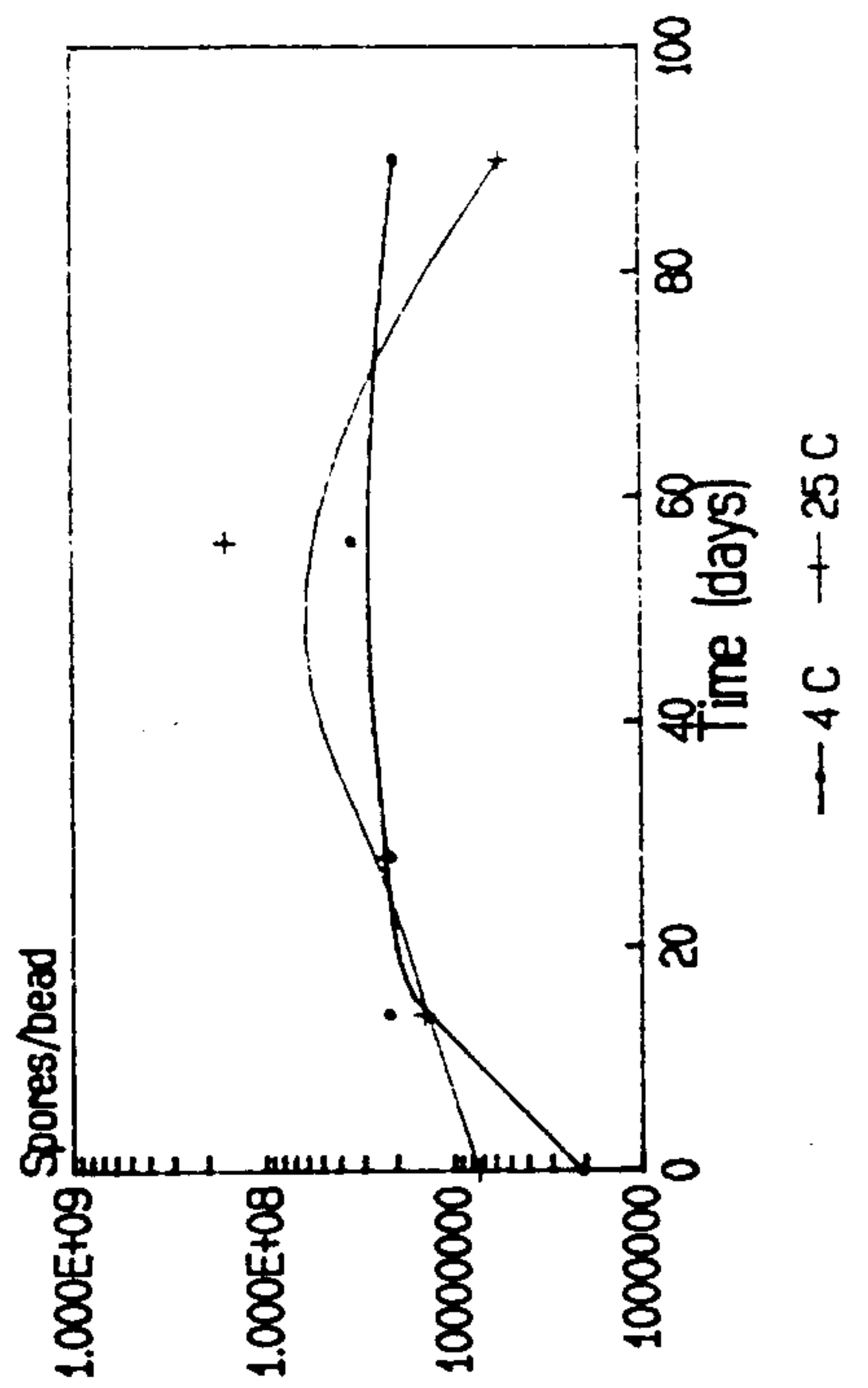
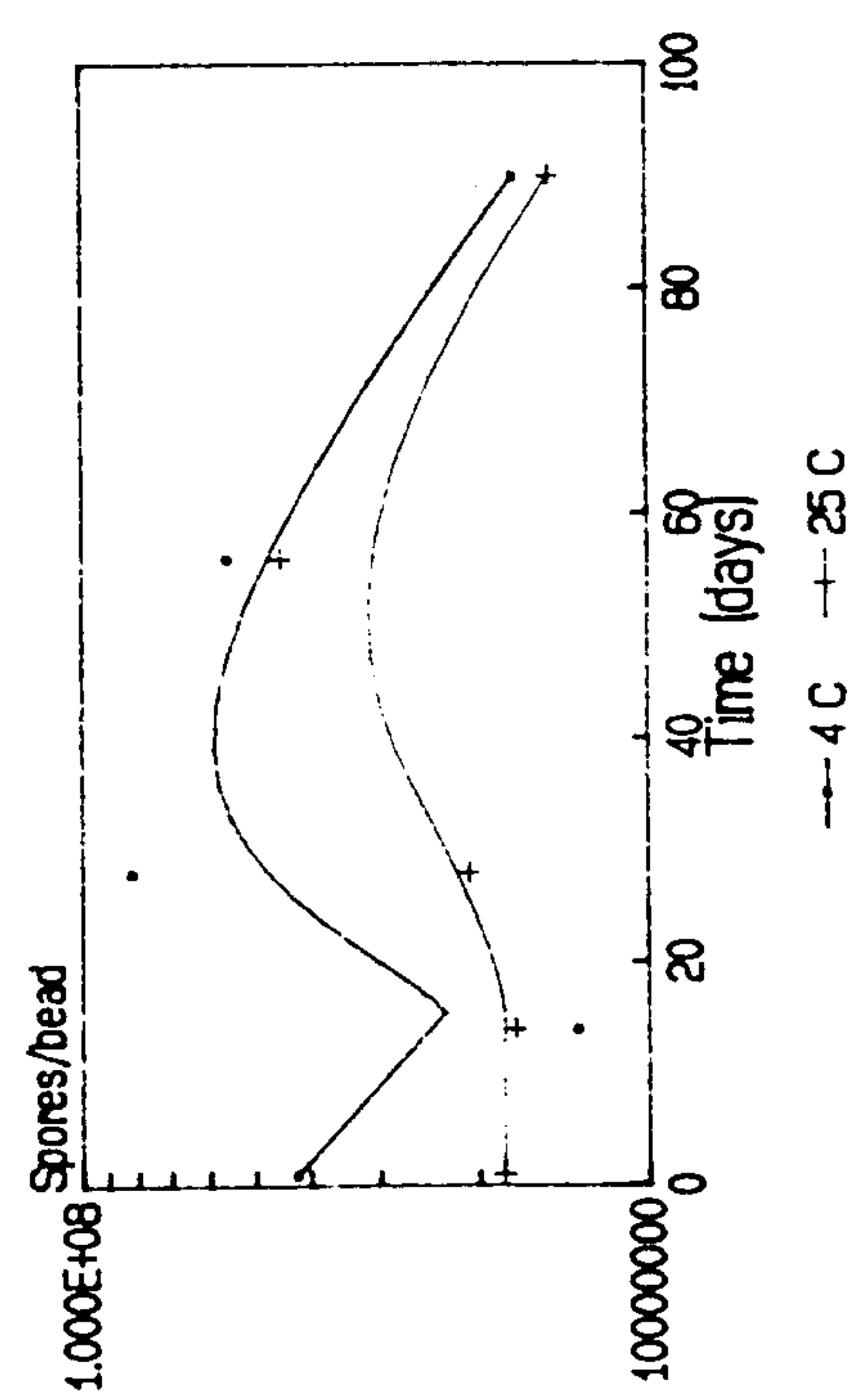
WE CLAIM :

1. A process for preparing an improved fungal biopesticide for control of insect infestation comprising
 - a) fermenting a fungus *Paecilomyces fumosoroseus* isolate ATCC no. 20874 in culture medium by submerged fermentation to produce biomass such that at least about 80% of the biomass is in the form of mycelium;
 - b) harvesting the biomass;
 - c) mixing the biomass with a carrier;
 - d) forming prill from the biomass carrier mixture;
 - e) optionally, drying the prill;
 - f) treating the prill to produce pathogenic conidia spores; and
 - g) harvesting the conidia spores from the treated prill.
2. The process of claim 1 which further comprises the following steps:
 - h) pregerminating the harvested conidia spores.
3. The process of claim 1 wherein at least about 90% of the biomass is in the form of mycelium.
4. The process of claim 1 wherein the mycelium is filamentous in form.
5. The process of claim 1 wherein the conidia spores are harvested by washing the treated prill with water comprising a surfactant.
6. A fungal biopesticide prepared by the process of claim 1, 2, or 5.
7. The biopesticide of claim 6 which is effective for the control of insect infestation.
8. The biopesticide of claim 7 which is effective for the control of infestation by whiteflies, mosquitos, aphids, planthoppers, leafhoppers, spittlebugs, scales, thrips, beetles or caterpillars.
9. A method for controlling insect infestation of a treatment area comprising applying a pesticidally effective amount of the biopesticide of claim 6 to the area
10. An improved stable, dried, prilled biopesticidal composition comprising an inert carrier which is capable of supporting fungal growth and promoting conidia sporulation, and an entomogenous fungal biomass at least about 80% of which is in the form of mycelium and is prepared by submerged fermentation of the fungus *Paecilomyces fumosoroseus* isolate ATCC No. 20874.
11. The biopesticidal composition of claim 10 wherein the fungus has been treated to produce conidial spores.

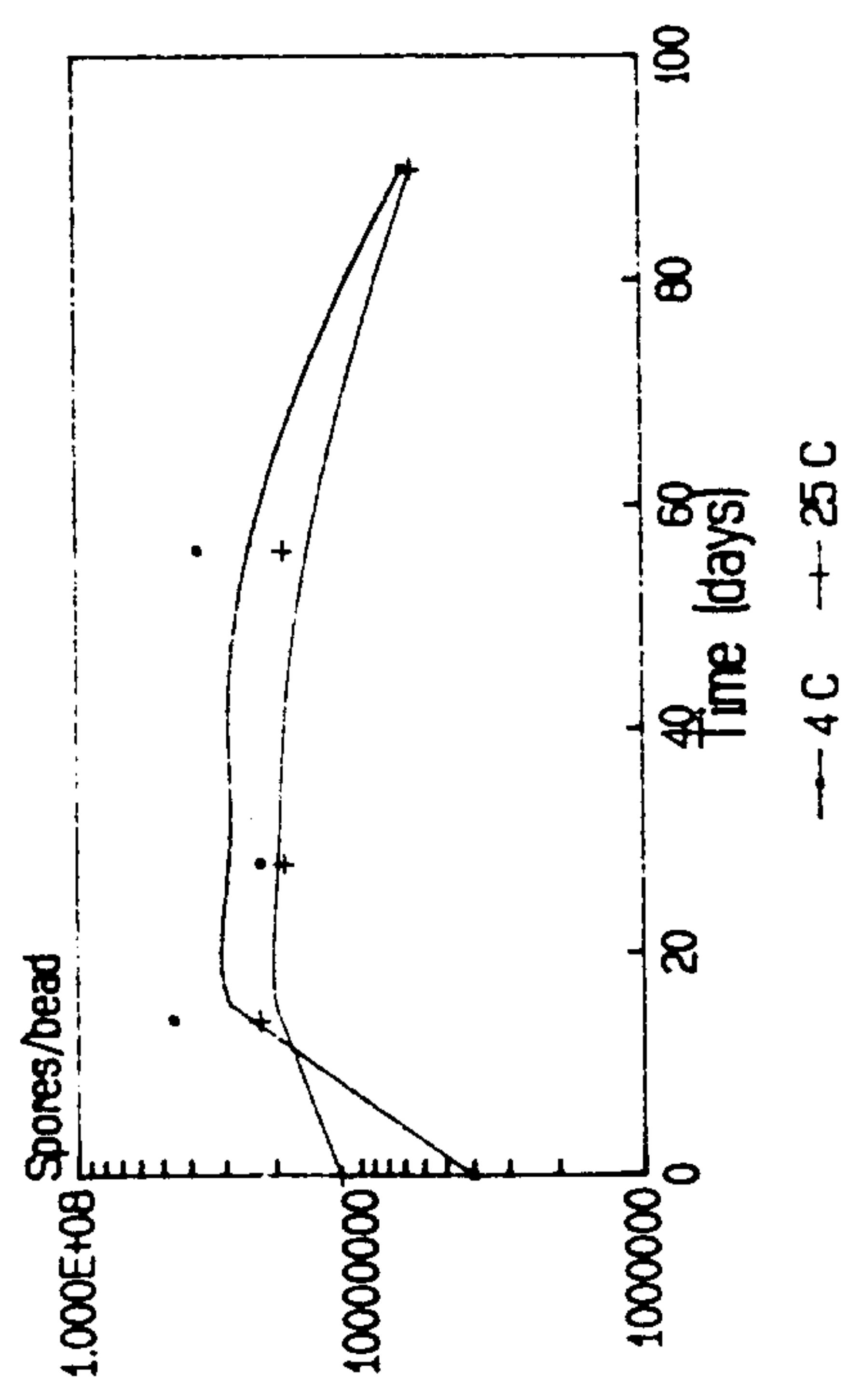
12. The biopesticidal composition of claim 10 or 11 wherein at least about 90% of the biomass is in the form of mycelium.
13. The biopesticidal composition of claim 10 or 11 wherein the mycelium is filamentous in form.
14. The biopesticidal composition of claim 10 or 11 which further comprises a nutrient.
15. An improved biopesticidal formulation for controlling insect pests comprising conidial spores of the fungus *Paecilomyces fumosoroseus* isolate ATCC No. 20874 wherein said conidial spores have been prepared from a stable, dried, prilled biopesticidal composition comprising an inert carrier which is capable of supporting and promoting growth of the fungus and a biomass of the fungus, at least 80% of which biomass is in the form of mycelium, wherein the fungal biomass is prepared by submerged fermentation.
16. The biopesticidal formulation of claim 15 wherein the conidial spores have been pregerminated to produce germ tubes.
17. A method of assaying the infectivity and viability of a fungal bioinsecticide to control insect infestation comprising:
 - a) activating prill to produce conidia spores, wherein said prill comprises an inert carrier which is capable of supporting fungal growth and promoting conidia sporulation, and a fungal biomass at least about 80% of which is in the form of mycelium, prepared by submerged fermentation of *Paecilomyces fumosoroseus* isolate ATCC No. 20874;
 - b) harvesting the conidia spores from the prill;
 - c) contacting the harvested conidia spores with an aqueous suspension of fungal conidia spores;
 - d) contacting a nymph of an insect to be assayed with an amount of the aqueous fungal suspension sufficient to infect the insects;
 - e) incubating the treated nymph to germinated the conidia spores; and
 - f) determining the growth of the fungus on the insect nymph.
18. The method of claim 17 wherein the aqueous suspension contains 1.0×10^7 conidia spores per ml of aqueous solution.

FIG. 1

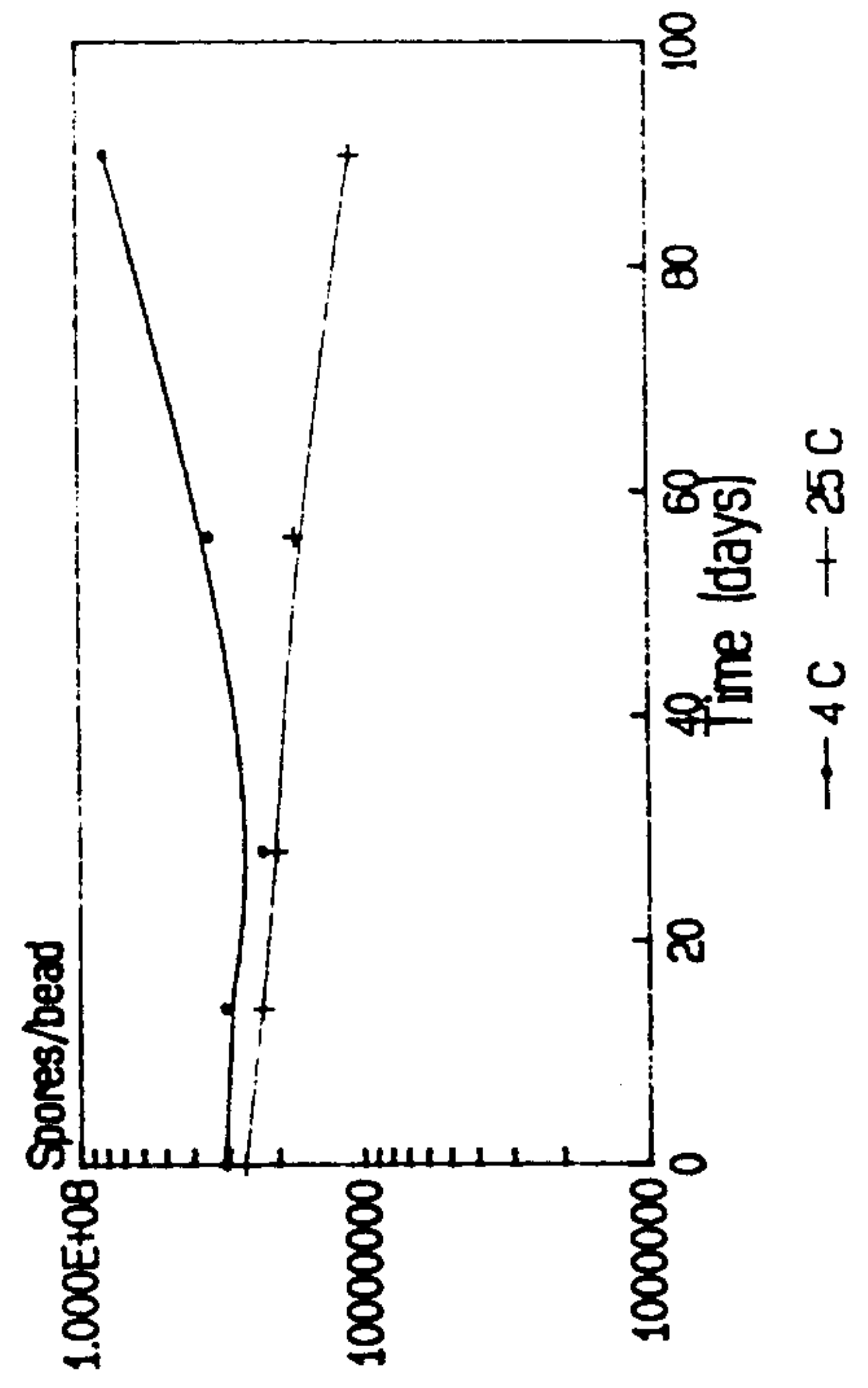
Prill Sporulation on Agar Plates at Different Temperatures
PA102 Peat Moss
PA103 Vermiculite



PA102 Bran



PA104 Bentonite



.2059057

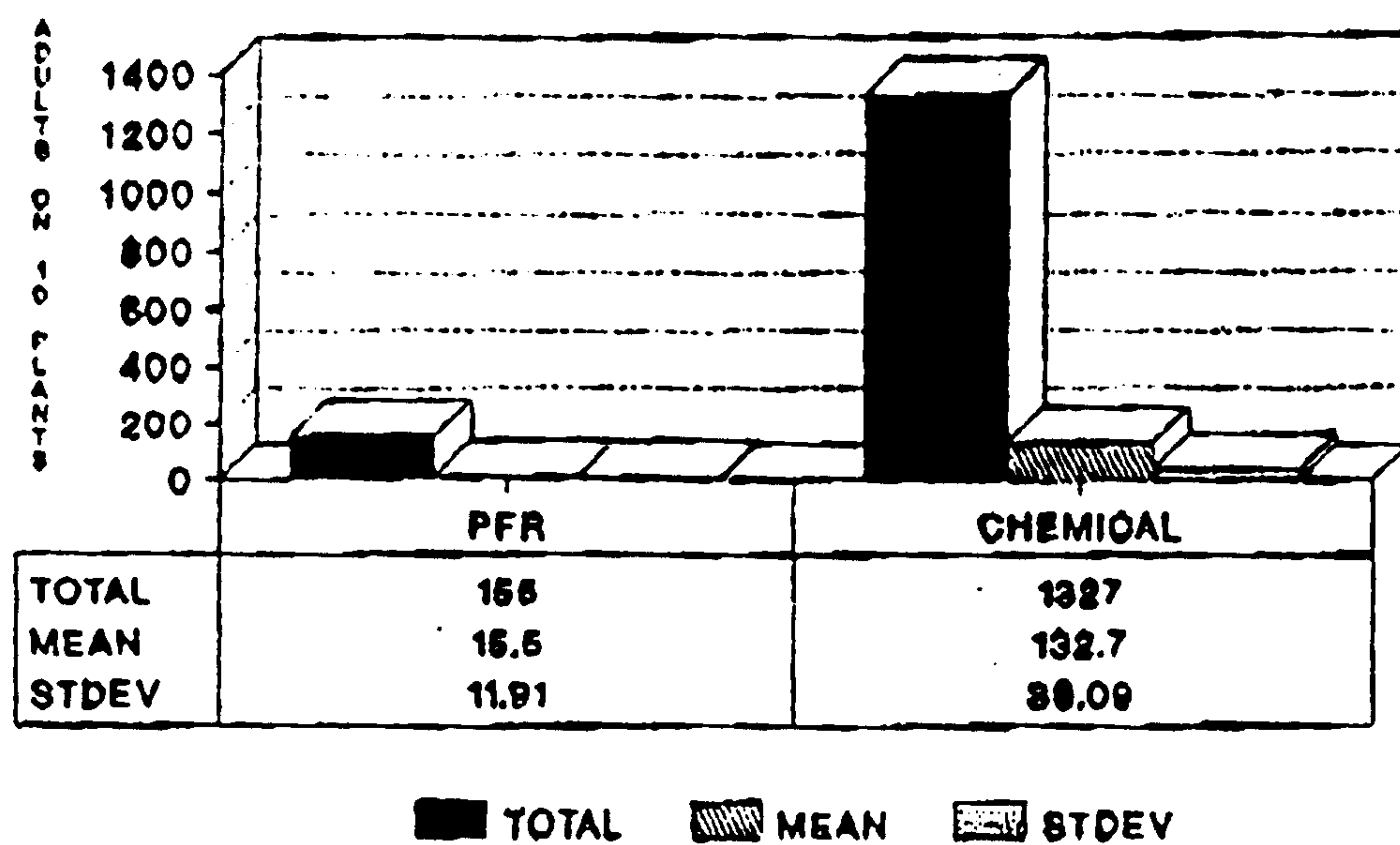


FIG. 2

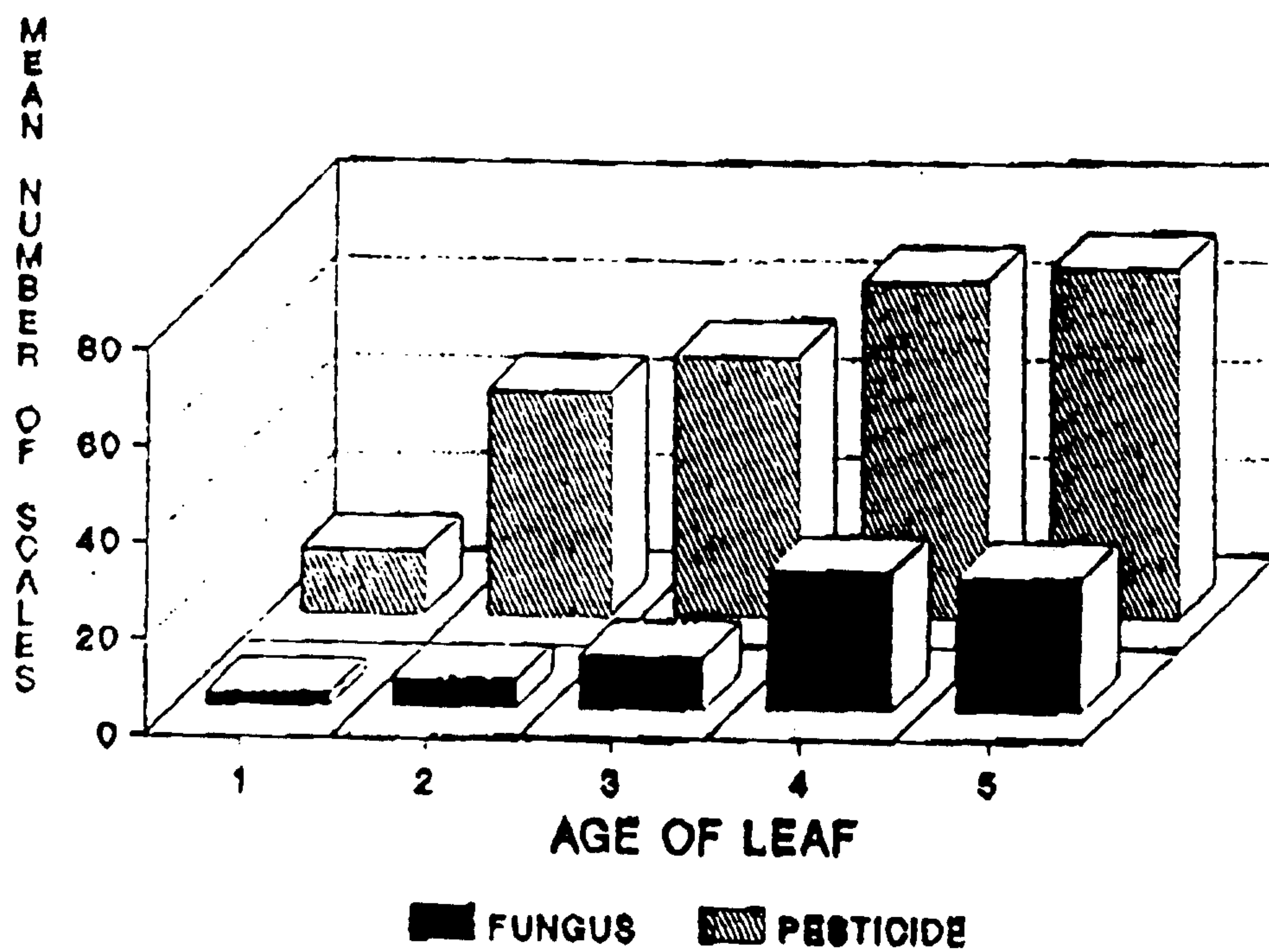


FIG. 3

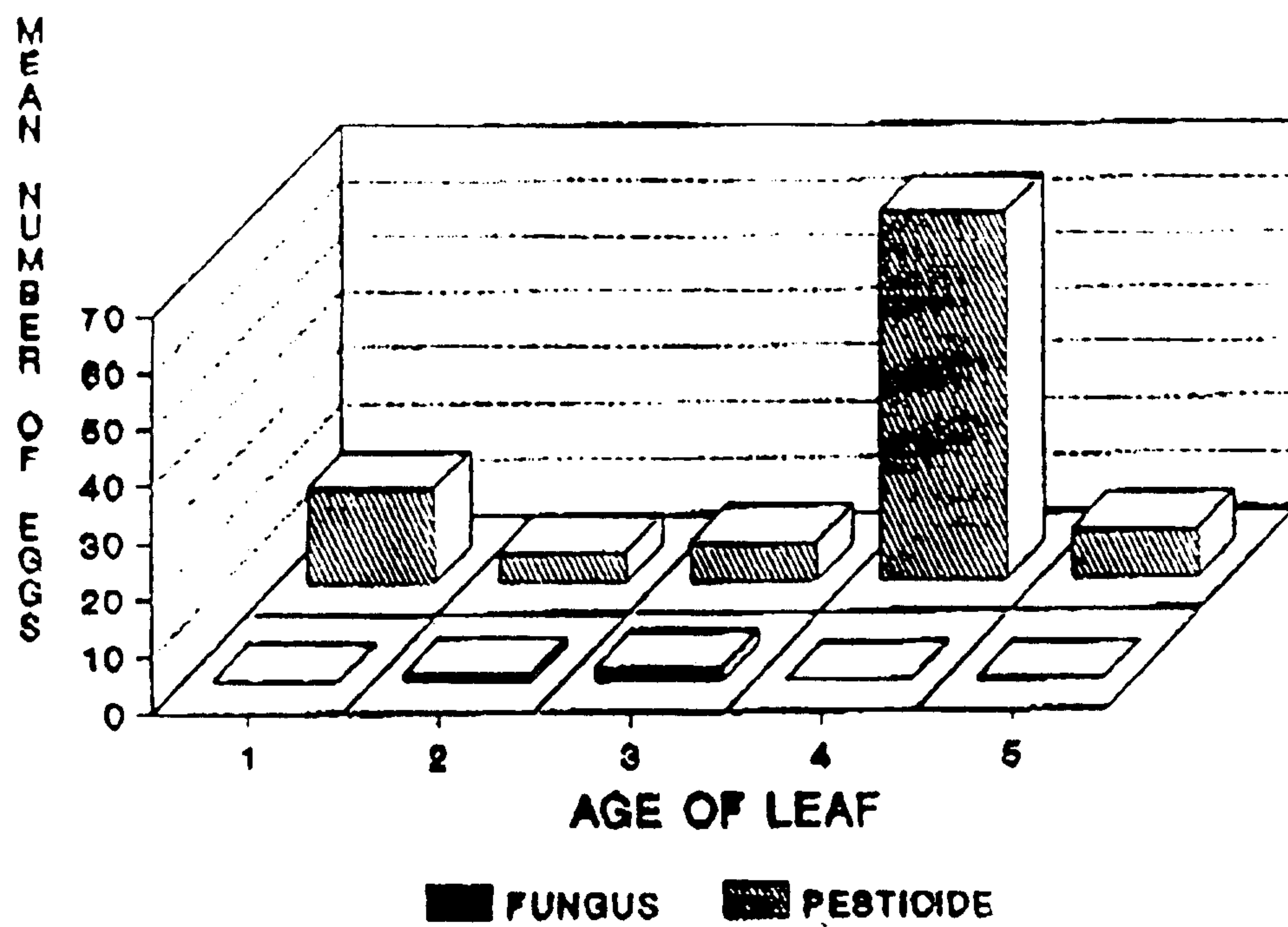
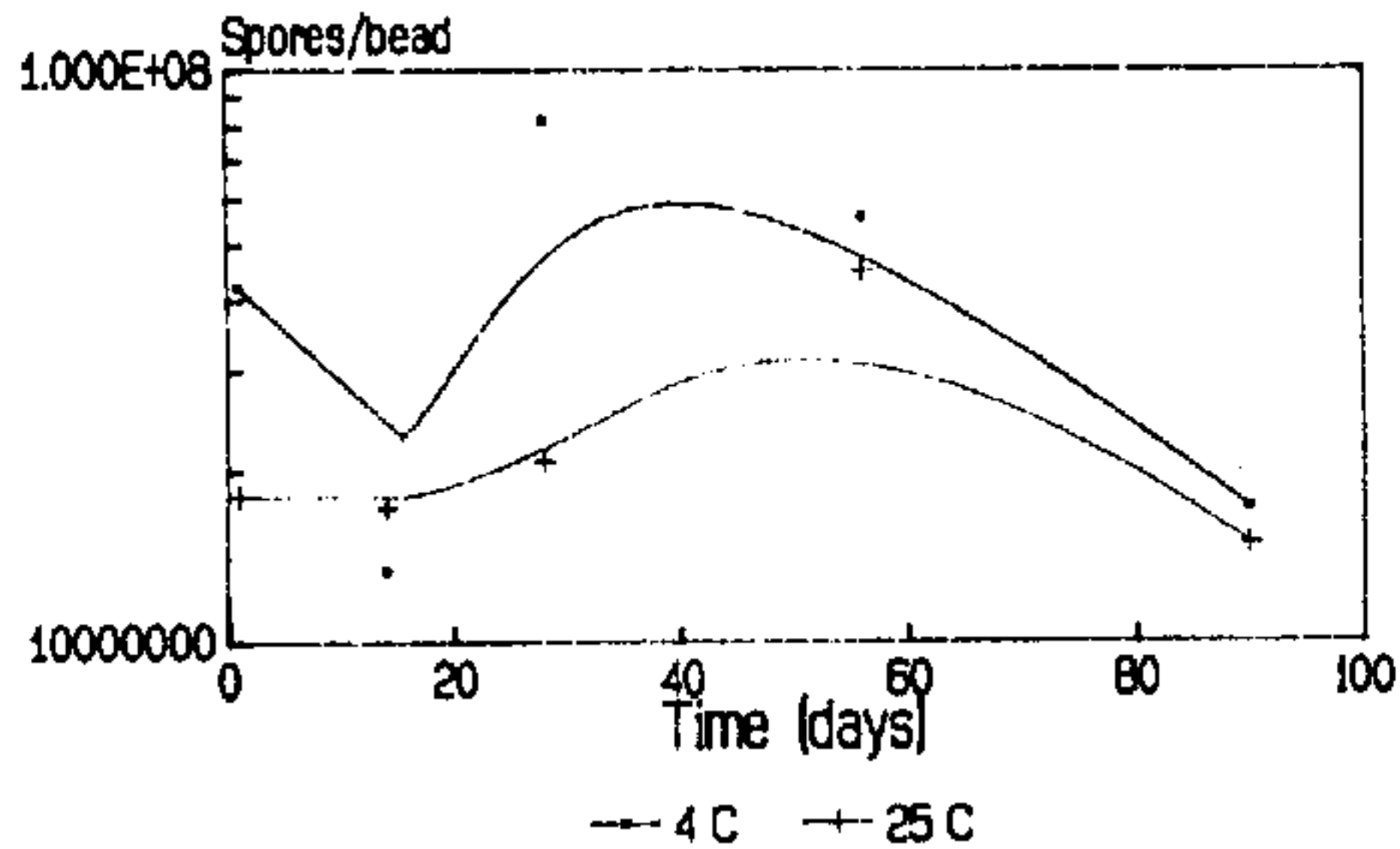


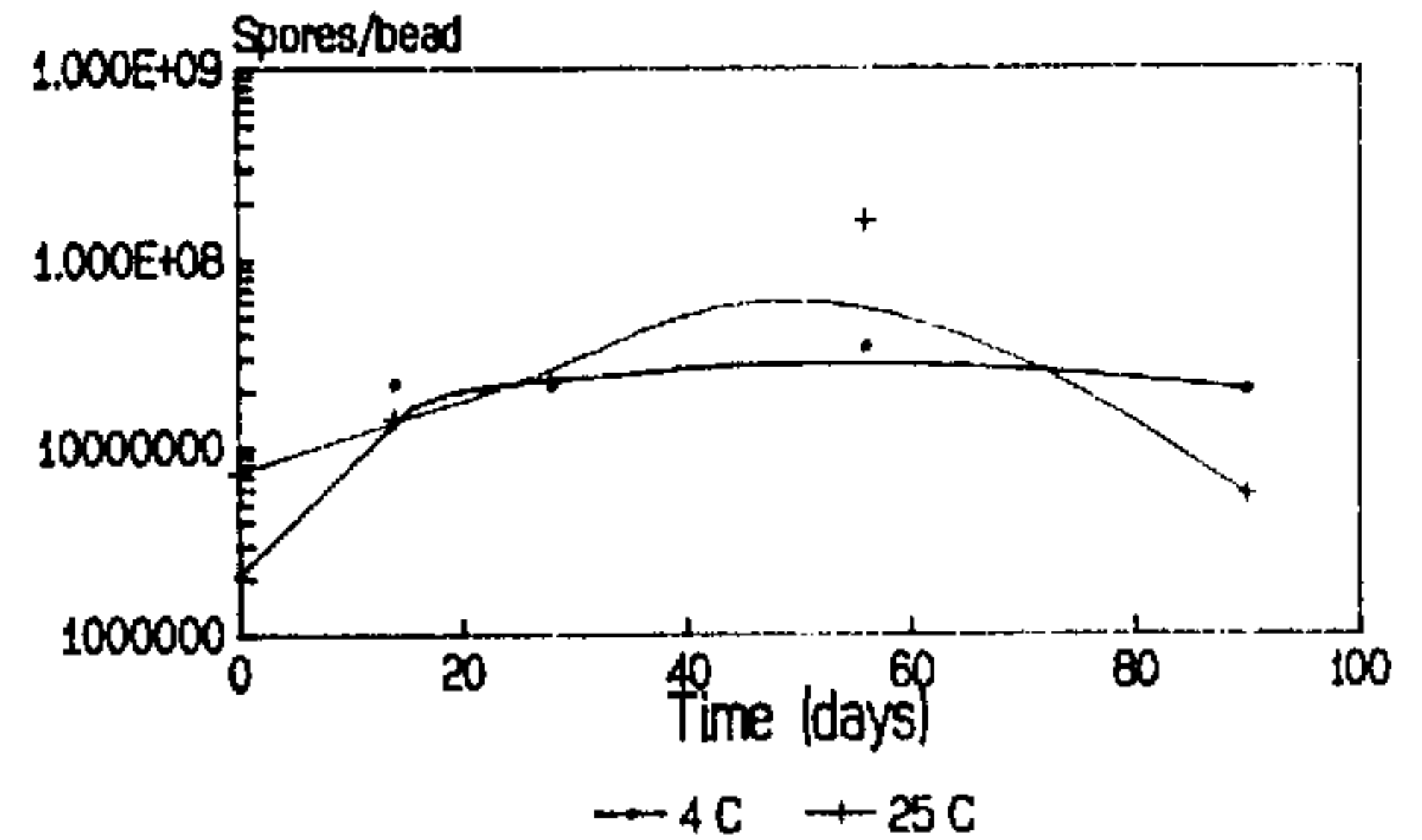
FIG. 4

Prill Sporulation on Agar Plates at Different Temperatures

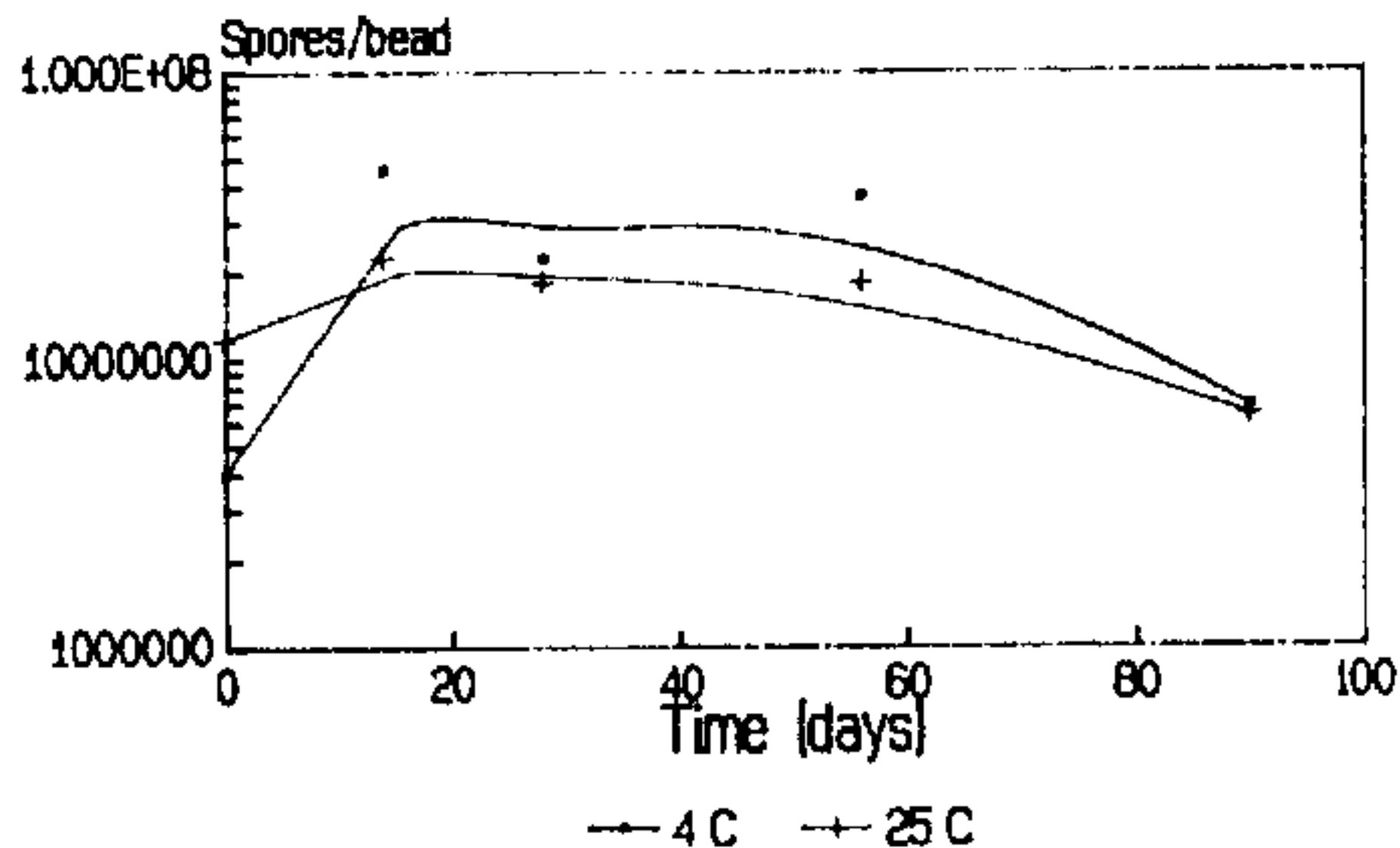
PA102 Peat Moss



PA103 Vermiculite



PA102 Bran



PA104 Bentonite

