TERMITE FEEDING STIMULANT AND METHOD FOR USING SAME

FIELD OF THE INVENTION

The present invention is directed to a termite feeding stimulant and a method for using the same, and more particularly, is directed to sitosterol containing formulations which are useful for increasing feeding or inducing phagostimulatory responses by all termite species. Termite species known to show increased feeding on cellulosic baits containing sitosterol include *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus*. Other important pest species of termites not yet tested but which may also show feeding stimulation by sitosterol are *Coptotermes formosanus*, *Reticulitermes hesperus, Reticulitermes hageni,* and *Heterotermes* species.

BACKGROUND OF THE INVENTION

The damage caused by various insects, and in particular, wood boring and eating inspects, such as termites, is extensive around the world, totaling in the billions of dollars. Various methods and devices have been used in the past in an attempt to alleviate or at least ameliorate the significant destruction caused by such insects. For example, so called "baits stations" have been utilized in an attempt to attract termites and thereby trap and/or destroy the termites that enter into such bait stations. Bait stations are available in a variety of shapes, sizes and structures, but principally rely upon the attractiveness of a cellulose product, such as paper or wood, to attract termite populations. It is believed that the termites are attracted to the cellulose wood product as a food source, however, prior art investigators have never conclusively determined what particular aspect of the cellulose product used in such bait stations actually is the attractive agent. Such cellulose products are typically treated with a toxin so that when the termites consume the treated cellulose products, such termites are incapacitated and/or killed. A significant problem in termite control, however, is the rejection of baits by termites.

There is presently a long felt but unsolved need for a method and device that overcomes bait rejection by termites, overcomes desertion of bait stations, and causes termites to increase their consumption of baits that contain insecticides, in a fashion superior to prior art methods and devices.

The damages caused by such insects is estimated to be over one billion dollars in the U.S. alone. Although pesticides have been used in the past to remedy such problems, they

have been largely ineffective and have proven to cause environmental problems and to be fairly expensive.

SUMMARY OF THE INVENTION

One aspect of the present invention is directed to behavioral manipulation of termites, and in particular, 3 particular species of termite, *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus*, using sitosterol or sitosterol mimics to increase the feeding of such insects. The use of sitosterol or sitosterol mimics on termite baits and/or on substances useful in detecting the presence of such termites is also encompassed by the scope of the present invention. In addition to sitosterol, the present invention also encompasses the inclusion of other co-feeding stimulants, and further includes the inclusion of various toxic compounds, as well as compounds that interfere with the sexual reproduction of such insects.

In one particular embodiment of the present invention, sitosterol compounds are used in combination with D-aspartic acid, L-glutamic acid, hydroquinone (as well as other termite labial gland secretions) and ergosterol-containing compounds.

One advantage of the present invention is that the use of sitosterol, without short acting toxic compounds avoids problems encountered in the prior art where termites are killed by a toxic agent before such termites spread a toxicant throughout the termite colony, thus leading to a decrease in baiting efficacy. Other problems in the prior art relate to eating deterrent effects evidenced when particular bait materials have pesticides added to such material.

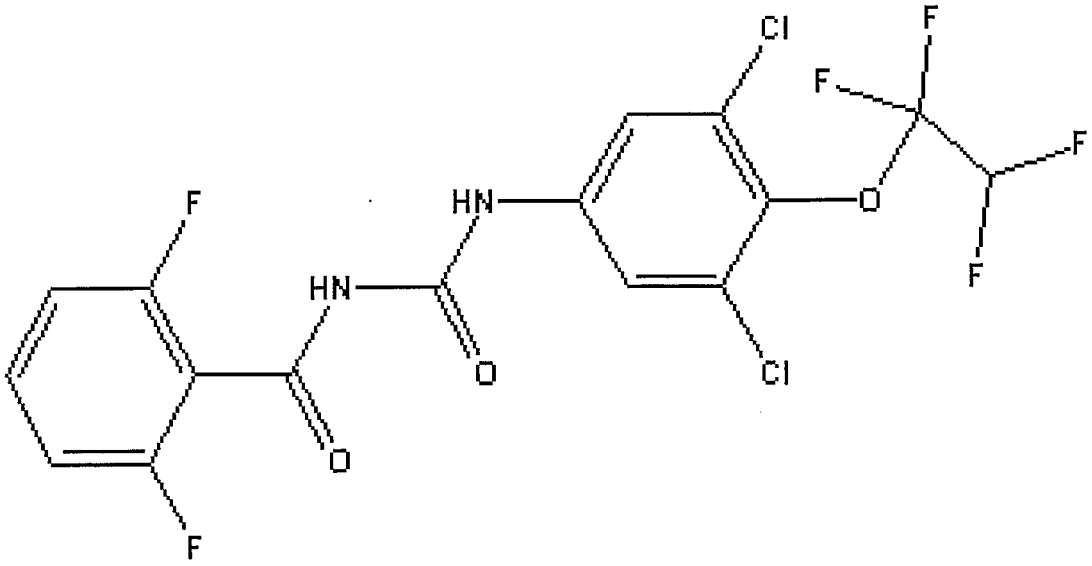
The present invention encompasses the use of any chemical source of sitosterol at various concentrations in order to increase and/or otherwise manipulate the feeding habits of R. tibialis. Moreover, any biological source of sitosterol at any effective concentration to increase or manipulate such feeding is also within the scope of the present invention. For example, bacteria, yeast, etc. that produce sitosterol, particularly genetically engineering microorganisms, can be used in the present invention. Finally, chemical mimics of sitosterol (which one of ordinary skill in the art will appreciate how to make and use given the guidance provided in the present specification as well as that provided in the prior art incorporated herein by this reference) can also be used at a wide range of effective concentrations to increase or manipulate the feeding of the above-referenced termite. .

Thus, in one embodiment, sitosterol containing compounds or formulations are used without any other compounds which may have significant toxic effects on termites. Although sitosterol is a hydrophobic compound which resists being washed off by soil moisture, a further aspect of the present invention relates to providing such formulations with features of increased hydrophobicity, encapsulation, mixture with adhesive compounds, etc. to facilitate effective placement or retention of such sitosterol formulations to achieve the desired goal of attracting and increasing the feeding of particular termites.

Any particular concentrations of sitosterol can be used in a given formulation, e.g., from about .25% (by weight), more preferably at least about .5%, even more preferably, at least about 1% and in various different applications, at or about 5%, although even greater concentrations are also contemplated.

It is further aspect of the present invention to provide a formulation including sitosterol and one or more substances toxic to termites, wherein such substances include:

(a) One or more of: hexaflumuron, a chitin synthesis inhibitor, a substance that prevents molting. In particular, it is within the scope of the present invention to use the product Sentricon having the following chemical formulation:



(b) one or more of the pesticides: lufenuron, thiamethoxam, a pesticide from the neo-nicotinoid family of pesticides;

Still further aspects of the present invention relate to the use of sitosterol formulations in construction and use of termite bait traps and other typical termite controlling and regulating devices.

One feature and benefits of the present invention will become evident from the accompanying figures and the Detailed Description hereinbelow.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a box graph that illustrates the percent eaten in grams of a control substance as compared to a sitosterol substance.

Fig. 2 is a box graph that illustrates TLC feeding plates indicating a control substance as compared to different levels of sitosterol containing substances.

Fig. 3 is a graph illustrating the percent paper bait matrix eaten by comparing a controlled substance with various concentrations of a sitosterol-containing substance.

DETAILED DESCRIPTION

The present invention is generally directed to using sitosterol as a feeding stimulant for the subterranean termite species *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus*. Aspen wood has been documented as a preferred wood by termites (Myles, T. 1994. Forest Products Journal 44: 33-36). Aspen wood (*Populus tremuloides*) was purchased from a local lumber yard (Sears Trostel, Fort Collins, Colorado). The aspen wood was cut into shavings with a drill press (Delta 16.5@ Drill Press) and extracted briefly with hexane (1 gram, 10 minutes). Trimethylsilyl (TMS) derivatives of the compounds in the hexane extract were prepared with bis(trimethylsilyl)trifluoroacetamide (BSTFA), and 1% trimethylchlorosilane by heating at 65°C for 30 minutes. Gas chromatography-mass spectrometric analysis was conducted with a Hewlett-Packard II 5890 GC and 5971 mass selective detector. Sitosterol was chemically identified from the aspen wood extract on the basis of its mass spectrum. An authentic sample of sitosterol (Sigma Chemical Company) was also derivatized with BSTFA , and the sitosterol trimethylsilyl derivative had an identical mass spectrum and gas chromatographic retention time.

Field Tests

Sitosterol was tested in the field as a feeding stimulant for *Reticulitermes tibialis*. Sitosterol (300 mg)was dissolved in 30 ml of acetone and added to 30 gm cellulose powder (Bio-Serv Inc., Frenchtown, New Jersey), and the powder was dried overnight. A hydraulic press (15,000 pounds per square inch) was used to made hard pellets (3 gm, 2.5 cm diameter, 5 mm thickness, 1% sitosterol and 99% cellulose). Control pellets of cellulose powder alone (3 gm) were made similarly.

The pellets were tested in underground traps made from Nalgene jar s (10 cm diameter, 10 cm height)with 3 mm holes drilled at 3 cm intervals to allow termites to enter. The jar traps were filled with soil (20% moisture). Two pellets were placed on the surface of the soil at the top of the jar trap, one pellet containing cellulose (3 gm) plus sitosterol (300 mg) and the other pellet containing only cellulose (3 gm).

A jar trap containing a 1% sitosterol pellet and a cellulose control pellet was placed at the base of a fence post that was previously determined to be infested with termites. The traps were checked once a week for feeding. When termite feeding was apparent, both pellets were removed and weighed to determine the amount of each pellet that had been eaten. A total of 15 jar traps were tested in the field.

Cellulose TLC Plate Feeding Bioassay

The present inventors show in the results of Fig. 2, the laboratory behavioral bioassays that *Reticulitermes tibialis* fed significantly more on cellulose when treated with sitosterol for every dose tested (30 ug, 100 ug, 300 ug). Such behavioral feeding bioassays were conducted using the following protocol.

Termite workers (100) were removed from their storage tubs and placed in a smaller Rubbermaid7 tub (414 ml) that contained soil (20% moisture). These termites were allowed to acclimate to their new surroundings for 24 hours before the feeding bioassay began. Solutions of sitosterol in acetone (1, 3, or 10ug/ul) were applied to the cellulose TLC squares with a Hamilton syringe (100ul). A single cellulose square (13.43 mg of cellulose) was evenly wetted with 30 ul of our acetone solution, thus applying a dose of 30, 100 or 300 ug of sitosterol. Concentrations tested were therefore 2.23, 7.45 and 22.34 ug sitosterol/mg cellulose. Placement of solutions on particular squares was random, and the cellulose TLC

plates were allowed to dry for 24 hours before testing. The bioassay began when an experimental TLC plate was placed into a tub containing termites (100). The plate in a tub was checked daily for 7 days and removed. If the termites had completely consumed any one of the four squares before 7 days, the plate was removed. A scanned image of the TLC plate was used to determine the amount of cellulose eaten by the termites for each particular square (Hewlett-Packard Office Jet R80 and Adobe Photoshop7 5.5 using the "Histogram" command to count pixels). This experiment was replicated 36 times for each dose.

Paper Feeding Bioassay

Fig. 3 shows the results a second collection of tests where termite workers (100) were removed from their storage tubs and placed in a smaller plastic tub (Rubbermaid , 414 ml) that contained soil (20% moisture). These termites were allowed to acclimate to their new surroundings for 24 hours before the feeding bioassay began. Solutions of sitosterol in acetone (1, 3, or 10ug/ul) were applied to the bait matrix with a Hamilton syringe (100ul). A single bait square (50.06 mg bait substrate) was evenly wetted with 30ul of our acetone solution, thus applying a dose of 30, 100 or 300 ug of sitosterol. Concentrations tested were therefore 0.60, 2.00, and 5.99 ug sitosterol/mg bait substrate. Placement of solutions on particular sides (left or right) of the bait matrix was random, and the bait matrix was allowed to dry for 24 hours before testing. The bioassay began when the bait matrix was placed into a tub containing termites (100).

The present invention is therefore useful in several different applications relating to termite pest management. Without being all inclusive, the present invention is directed to the following:

(A) The use of any biological or chemical source of sitosterol for increasing feeding or inducing phagostimulatory responses by the termite species *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus*.

(B) The use of any chemical mimics of sitosterol for increasing *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus* feeding or inducing phagostimulatory responses.

(C) Behavioral manipulation of *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus*, including but not limited to the following:

a. Use of sitosterol or sitosterol mimics to increase feeding of *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus* on baits and/or substances used to detect the presence of *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus*.

b. Use of sitosterol or sitosterol mimics to increase feeding of *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus* on an insecticide.

c. Use of sitosterol or sitosterol mimics to affect the feeding behavior of *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus*.

d. Use of sitosterol or sitosterol mimics as co-feeding stimulants for *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus* along with other materials that may have fundamentally different chemistry.

e. Use of various methods, materials, devices for performing the function of the present invention, as set forth herein

Other termite feeding stimulants (reported by several researchers) can also be used in conjunction with the present sitosterol formulations, including those mentioned in the following references:.

1. D-aspartic acid and L-glutamic acid were fed upon more than a water control by *Coptotermes formosanus* Shiraki. Chen, J., and G. Henderson (1996) J. Chem. Ecol. 22: 2359-2369.

2. Hydroquinone, a termite labial gland secretion, stimulated feeding in *Mastotermes darviniensis* and *Coptotermes lacteus*). PCT patent WO 00/36914 (29 June 2000).

3. An ergosterol-containing bait matrix was fed upon preferentially by *C. formosanus* in a two-choice test with southern yellow pine. Rojas, G., and J. Morales-Ramos (2001) J. Econ. Entomol. 94:506-510

The disadvantages of any of these used alone, however, is as follows:

1. D-aspartic acid has shown toxicity to termites in extended feeding tests (> 30 days). If termites are killed before they can spread a toxicant throughout the colony, a decrease in baiting efficacy could result.

2. The phagostimulatory effects of hydroquinone lasted only one month due to the volatility of this compound.

3. Chitin synthesis inhibitors, such as hexaflumuron, were added to this bait matrix resulting in less feeding on the treated bait matrix compared to its control. This suggest a possible feeding deterrent effect when this bait had a pesticide added to it.

The component parts or steps of the present invention include:

- The use of any chemical source of sitosterol at any concentration to increase or manipulate *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus* feeding.
- The use of any biological source of sitosterol at any concentration to increase or manipulate *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus* feeding.
- The use of any chemical mimics of sitosterol at any concentration to increase or manipulate *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus* feeding.

Several aspects of sitosterol make it a promising compound for enhancing *Reticulitermes tibialis* control. Sitosterol is a stable, nonvolatile compound which has shown no toxic effects to termites. Termite baits are most frequently placed in the soil where moisture conditions are a factor. Sitosterol is a hydrophobic compound which resists being washed off by soil moisture. Sitosterol has little toxic risk, and it has been tested in humans as an alternative method to lower plasma cholesterol levels. Sitosterol is an economically rational additive to a bait system. The cost of the sitosterol needed to treat one bait roll of the size and proportion of paper is about one cent to reach the concentration of our highest tested dose (300 ug).

The present invention is further directed to the use of a particular plant sterol or stanol, such material selected from the group consisting of beta-sitosterol, alpha-sitosterol and gamma-sitosterol, or a combination thereof, and including any mimics of such chemicals which have similar chemical functionality and/or structure.

Also incorporated herein in its entirety is pending U.S. Patent Application No. 09/573,795 filed May 16, 2000 by BERNKLAU et al.. Moreover, to supplement the written

description provided herein, the following issued United States patents are incorporated herein by this reference in their entireties, such references providing additional information with respect to the manufacture of compounds, in particular sitosterol, useful in the present invention: U.S. Patent Nos. 6,162,483 filed April 22, 1998 by Wester; 4,265,824 filed December 5, 1979 by Koskenniska, et al.; 3,879,431 filed October 5, 1993 by Clark et. al.; 6,136,349 filed June 29, 1998 by Karppanen, et al.;and 6,068,867 filed July 14, 1997 by Nussinovitch, et al..

While various embodiments of the present invention have been described in detail, it will be apparent that further modifications and adaptations of the invention will occur to those skilled in the art. It is to be expressly understood that such modifications and adaptations are within the spirit and scope of the present invention.

What is claimed is:

1. A method for increasing the intake of food by a termite of the genus and species *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus* comprising:

providing said termite a formulation containing sitosterol so that the termite will feed on the formulation, wherein said formulation is obtained by adding sitosterol to the formulation.

2. The method of Claim 1, wherein said formulation includes approximately 1% of sitosterol.

3. The method of Claim 1, wherein said formulation includes approximately 2.23 ug sitosterol/mg of cellulose.

4. The method of Claim 1, wherein said formulation is provided in a termite trap.

5. A termite trap comprising an enclosure containing a concentration of sitosterol, wherein said concentration is effective for inducing an enhanced phagostimulatory response in a termite over a termite feeding source having a lesser or substantially no concentration of sitosterol.

6. The termite trap of Claim 5, wherein said concentration is approximately 1% of sitosterol.

7. The termite trap of Claim 5, wherein said concentration is approximately at least 0.60 ug sitosterol/mg of a termite bait.

8. The termite trap of Claim 5, wherein said concentration is in pellet form.

9. The termite trap of Claim 5 further including soil therein.

10. A formulation for increasing the intake of food by a termite of the genus and species *Reticulitermes tibialis*, *Reticulitermes flavipes,* and *Reticulitermes virginicus*, wherein sitosterol or a mimic thereof is present in an amount of at least 300 micrograms at a termite trap.

11. The method of Claim 10, wherein said formulation includes approximately 1% of sitosterol.

12. The method of Claim 10, wherein said formulation includes at least one of: beta-sitosterol, alpha-sitosterol and gamma-sitosterol.

13. The method of Claim 10, wherein said formulation includes one of: D-aspartic acid, L-glutamic acid, hydroquinone, and ergosterol.

14. The method of Claim 10, wherein said formulation includes one or more of the following substances toxic to termites hexaflumuron, a chitin synthesis inhibitor, a substance that prevents molting, lufenuron, thiamethoxam, a pesticide from the neo-nicotinoid family of pesticides.

15. A formulation for use at termite traps comprising an effective amount of sitosterol, or mimics thereof, to increase a feeding habit of *Reticulitermes tibialis* at said trap as compared to feeding habits thereof when feeding on other than said formulation.

16. The formulation of Claim 15, wherein includes at least 1% by weight is sitosterol, or mimics thereof.