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Use of Fungal Pathogens for Insect Control in Greenhouses

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Introduction

With an annual wholesale value of > $7 billion, the greenhouse and nursery industries are a vital, and expanding, part of the national economy. The greenhouse has an optimal environment for plant production that also, unfortunately, favors insect survival and reproduction. Outbreaks of major pests such as thrips, whiteflies, aphids and fungus gnats can precipitate major losses of revenue through direct feeding damage, disease vectoring, and the costs associated with their control.

Management strategies have largely been based on the routine use of agrochemicals, but numerous factors are today leading to a reevaluation of current control practices, and a move to develop and adopt a more diverse approach to insect pest management. Probably one of the major driving forces behind this move is the recognition that we must reduce our reliance on chemical plant protectants. There are many reasons for this, the main ones being: 1. the widespread appearance of pesticide-resistant insects (Dittrich et al. 1990, Immaraju et al. 1992); and 2. concerns over environmental contamination and applicator safety. These have in turn created a public that generally has a negative perception of pesticides; and has brought increasingly restrictive legislation on pesticide use so that many standard insecticides that growers have relied upon for years are no longer registered for greenhouse use. Replacements are more expensive or not available, and the need to develop and integrate alternative control options has become paramount. Integrated pest management (IPM) represents a change of philosophy over pest control, away from total pest eradication and a 'spray and pray' approach. IPM brings with it the need to investigate and utilize a variety of pest suppression tactics, each contributing in part to the overall reduction of pest numbers (Parella 1993).

> At a recent House committee hearing, federal EPA administrator Carol Browner announced plans to develop specific pesticide-use reduction goals for various segments of production agriculture by the year 2000 and implement Integrated Pest Management programs for 75% of total crop acreage within the next 7 years.
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Various components may be considered in an IPM strategy. Certain elements may only be feasible in the protected greenhouse environment; other approaches may only be used in long-term crops. Considering the range of insects attacking greenhouse crops and their uncanny ability to develop resistance - even to biocontrol agents (Abbot 1993) - an arsenal of management tools is needed. Once discounted as being ineffectual, biological controls are becoming vital components of IPM. There is increasing interest in the exploitation of biocontrol agents such as predators, parasitoids and pathogens.

A wide variety of predators and parasitoids are available, or are under development, for control of thrips, whiteflies, aphids and fungus gnats (Osborne et al. 1991, Higgins 1992, USDA 1992, Harris 1993, Parrella 1993). Such beneficials have a significant role to play in greenhouse IPM but are not the answer to all pest problems and situations. Establishing a breeding population for mass-production of many promising beneficials has proved to be very difficult or uneconomical. The need to have quality controls on those which are produced has only recently become an issue with the recognition that sub-standard batches of beneficials have failed to control pest populations (Steiner 1993). Predators or parasitoids alone are often unable to reduce heavy or expanding pest populations rapidly and timing of releases is critical if pest numbers are to be maintained at low levels. Rearing and maintaining breeding populations of natural enemies in a crop at times of low host density is difficult, so repeated releases may have to be made. Certain beneficials such as *Orius* are less effective, or may hibernate, at lower temperatures, and their reproductive rates can be greatly reduced at times of short day length (Parrella 1993). In addition their high cost may preclude their use in certain crop types.

What of the pathogens - viruses, protozoa, nematodes, bacteria and fungi? Nematodes, bacteria and fungi are probably the only feasible candidates for greenhouse use. Commercial preparations of nematodes are available for control of fungus gnats, *Bradysia* sp. (Harris 1993); and nematodes have shown potential for control of western flower thrips, *Frankliniella occidentalis* (Greene & Parrella 1993). However, there are problems in commercialization of these organisms; mass rearing, formulation and application technologies need to be considerably improved. Furthermore, their ability to infect and survive over a range of environmental conditions is limited. Of the bacteria, *Bacillus thuringiensis* var. *israelensis* is successfully used for control of fungus gnats, but repeated applications are required over the growing season (Harris 1993). Such pathogenic bacteria have to be ingested, which makes them ineffective against sap feeding insects such as thrips, whiteflies and aphids, and the open, underleaf environment where many of these pests are found may be too hostile for nematodes to survive and be effective. Entomopathogenic fungi are probably the most promising and versatile microbes for use in a management program for these insect pests.

Entomopathogenic Fungi

How do the fungi kill insects? The fungi essentially act as contact insecticides. First, the insect must come into direct contact with viable fungal conidia, or spores. The spores attach themselves to the insect's cuticle in a variety of ways, depending on the fungal species, but the process of infection is basically the same for them all. Once attached to the exoskeleton, the spore germinates and produces a germ tube. On receipt of the correct cues from the insect cuticle, the germ tube differentiates to form a specialized infection structure which initiates penetration of the cuticle. If the insect molts before penetration occurs, then it can escape infection. Once inside the insect, the fungus must overcome the insect's immune system in order to initiate a lethal infection. Death generally follows in one of two ways: certain fungi produce toxins that kill the insect, followed by growth of the pathogen throughout the body cavity; or, as a result of fungal growth within the insect's body and destruction of the inner organs. Some fungi appear to cause death by asphyxiation following growth of the fungus over the body of the live insect, plugging the breathing pores; invasion and growth within the body of the host then occurs.

Once an individual dies of a fungal disease, it can act as a source of infection for other, healthy insects. If external conditions are favorable - high humidity levels are normally required - the fungus will grow back through the cuticle and sporulate on the surface of the dead insect. These spores may infect other insects that come into contact with them, and the disease can thus spread within a population.

There have been many attempts to use fungi to control insect populations. Inconsistent results were obtained in early trials, largely due to an insufficient understanding of the biology of both the host and the pathogen. A greater understanding of the intricate relationships between the two, and advances in mass production and formulation technologies now allow us to re-examine the role mycopathogens may play in pest suppression programs.

The fungi possess a number of desirable characteristics which favor their development and use: 1. they have acceptable host specificity and are safe to humans and livestock; 2. they can be cheaply mass produced on artificial media; 3. they can effectively challenge the target insect over a range of environmental conditions; and 4. they can rapidly kill a high proportion of the pest population. Several strategies may be proposed for their use, including: 1. establishment of the disease within a pest population to provide self-replicating, long-term control; 2. application as mycoinsecticides, where the fungi are applied with the aim of maximizing mortality rates from a single application in the same way as a chemical pesticide. The greenhouse provides a particularly attractive environment to target with fungi, being relatively protected and, in some instances, the environment may be manipulated to enhance performance.

Use of fungi as mycoinsecticides requires a cheap and rapid means of mass production on inexpensive artificial media, essentially limiting the pathogens which can be utilized to the Hyphomycetes. For the purposes of this article, therefore, information on this type of mycopathogen only will be reported.

Fungi Tested Against Greenhouse Pests - A Brief Review

Thrips

A diverse range of fungal pathogens has been recovered from thrips, but surprisingly few attempts made to specifically use these agents in a thrips suppression program. Western flower thrips and *Thrips tabaci*, the onion thrips, are susceptible to *Beauveria bassiana, Metarhizium anisopliae, Paecilomyces fumosoroseus* and *Verticillium lecanii* (Fransen 1990). Onion thrips and western flower thrips have been satisfactorily controlled on cucumber with *V. lecanii* (Binns et al. 1982, Van der Schaaf et al. 1991). An experimental product based on a thrips isolate of *V. lecanii*, Thriptal, was developed in the United Kingdom, but sadly never marketed due to inconsistencies in product efficacy. Fungal pathogens have been recovered from *Thrips palmi*, a new and potentially devastating intruder to the continental USA, but so far little work has been done to evaluate additional strains against this pest.

Whiteflies

V. lecanii has been extensively studied in experimental trials for control of the greenhouse whitefly, *Trialeurodes vaporariorum*. Successful control was achieved using a whitefly isolate of this fungus in glasshouse trials done in the UK between 1980 and 1985, and a product was developed for use against this pest. Unfortunately, consistent product efficacy could not be guaranteed and it was withdrawn from use. Improvements in production and formulation of the fungus by Koppert BV of the Netherlands resulted in production of a wettable powder, Mycotal[R], which is recommended for control of whitefly in cucumber, tomatoes, and other greenhouse-grown vegetables and ornamentals. Spraying at weekly intervals can reduce infestations by over 90%, even when the ambient humidity levels are as low as 75%, while standard production operations can be maintained (Van der Schaaf et al. 1991). ***Aschersonia aleyrodis, P. fumosoroseus*** and ***B. bassiana*** have been used to successfully control greenhouse whitefly in laboratory and experimental greenhouse trials (Osborne & Landa 1992, Fransen 1990). Each of the species tested has different merits which could warrant their development and use.

Sweet potato whitefly, *Bemisia tabaci*, is a relative newcomer to the scene, but has rapidly become established as a pest of major economic significance in greenhouse, winter vegetable, cotton and melon crops nationwide. Many of the fungi active against greenhouse whitefly are also effective against sweet potato whitefly. Strains of *P. fumosoroseus* and *B. bassiana* appear to be particularly promising (Osborne & Landa 1991, USDA 1992). Development of selected isolates is being pursued by a number of commercial and research organizations for control of *Bemisia* on field, greenhouse and ornamental crops.

Aphids

V. lecanii is effective against a number of aphid species in greenhouse conditions, including: the green peach aphid, *Myzus persicae*; the chrysanthemum aphid, *Macrosiphoniella sanborni*; the cotton aphid, *Aphis gossypii*; and the black bean aphid, *A. fabae* (Hall & Burges 1979). A commercial formulation, Vertalec[R], is available in Europe for aphid control on greenhouse chrysanthemum and vegetable crops. Gardner et al. (1984) evaluated this product for control of green peach and chrysanthemum aphids in U.S. greenhouses, and were able to integrate applications of this bioaphicide with benomyl treatments to consistently control green peach aphid. Presently though, no products are available in the U.S. for aphid control. High humidity levels may be required to obtain satisfactory levels of control with *V. lecanii*, however, a potential drawback to the use of this fungus in drier environments; although the work of Van der Schaaf (1991) suggests that such requirements may be overcome if dose levels are increased.

The work of Hall (1980) suggested that other fungal species were not as effective as *V. lecanii* against greenhouse aphids. Feng et al. (1990) demonstrated that *B. bassiana* was more pathogenic for a variety of cereal-infesting aphids though, suggesting that testing of a broader range of fungal isolates and species would provide additional effective strains for use against green peach aphids.

Fungus Gnats

Entomopathogenic fungi might also be used to control fungus gnats in potting soils (Harris 1993). Strains of *B. bassiana* and *M. anisopliae* are particularly promising in this type of environment, although *P. fumosoroseus* may have a role to play in the regulation of this pest.

Development of Fungi for Greenhouse Pest Management at the University of Vermont, Entomology Research Lab

The fungi show great variation in their: 1. pathogenicity for a target host; 2. host range; 3. ability to infect a targeted host independently of high relative humidity; 4. performance and survival over a range of temperatures; 5. epizootic potential; and 6. performance in distinctively different environments, e.g. foliage vs. soil. It is therefore important that the fungal strain, the biology of the target pest, and the target site and conditions all be considered when making strain selections. We have acquired an impressive bank of fungi, isolated from a wide variety of insect pests and source materials. Having access to a broad base of strain types, our rationale has been that we would be able to identify pathogenic strains for use in a variety of management approaches. In 1991, we began testing native insect-killing fungi against three major greenhouse pests: western flower thrips, sweet potato whitefly and green peach aphid. As there are generally several pests in any greenhouse crop, by taking a more holistic view to insect pest management, it has been our goal to identify virulent fungal strains for individual pest species, and ones with a broader spectrum of activity.

Western Flower Thrips

Initially, a single dose screening bioassay was performed to identify effective strains. Dose-mortality tests followed and comparison of the calculated LD_{50} and LD_{90} values allowed us to select the most potent strains for further testing. As western flower thrips are found in two distinctive habitats at different stages of their life cycle (adults, eggs, 1st and early 2nd instars on foliage and flowers; late 2nd instars and pupae in the soil) trials are being conducted against thrips infestations in flowers and potting soils. From a bank of 190 isolates, 16 have been selected for these trials, including potent strains of *B. bassiana, M. anisopliae, V. lecanii, P. farinosus* and *P. fumosoroseus*.

Flower treatment. Thrips-infested flowers are sprayed with fungal conidia suspended in 0.05% Tween using a modified air brush. Control flowers are sprayed with 0.05% Tween only. Flowers are then placed in small vials in pots containing potting soil. Larvae surviving the fungal treatment drop to the soil to pupate, and emerging adults are trapped on sticky lids placed over the pots. Comparison of the number of adults on the sticky lids, from treated and untreated flowers, indicates the efficacy of the fungal treatment on the pest population.

Soil treatment. Infested flowers are placed in small vials over potting soils treated by application of a drench containing fungal conidia. By monitoring adult emergence as in the flower treatments, the survival rate of the larval/pupal stages in the treated vs. untreated soil can be determined.

	MEAN NO. ADULTS/STICKY LID*			
	Flower Treatment		Soil Treatment	
Fungus	Treated	Control	Treated	Control
B. bassiana B33	29.3 (53%)	54.7 (100%)	37.9 (69%)	54.8 (100%)
M. anisopliae 1080	52.5 (78%)	67.2 (100%)	37.9 (37%)	54.8 (100%)

*mean from 4 replicate experiments, 4 pots/replicate

Table 1. Sticky lid counts of western flower thrips adults following flower or soil treatment with fungal conidia.

Table 1 illustrates the performance of two isolates against western flower thrips in flowers or soil. The surviving thrips population from flowers treated with *B. bassiana* B33 was approximately 47% lower than that of the control. When applied as a soil treatment, B33 was less effective and the surviving population was around 31% lower than the control. *M. anisopliae* 1080 performed better against western flower thrips in the soil, and the surviving population was about 72% lower than the control. This highlights the importance of strain selection according to the targeted habitat and developmental stage.

As we are evaluating efficacy against the soil-inhabiting stage of western flower thrips, knowledge on the persistence of fungal inoculum in different potting media would be beneficial. To this end, we have done some preliminary assays to monitor persistence of selected fungal strains, applied by drenching, in four types of potting soil. *M. anisopliae* strains maintained higher soil populations than the other species tested. These results have significant implications for maintenance of a long-term source of infection for thrips larvae entering the soil to pupate. The persistence of *B. bassiana* and *M. anisopliae* strains showing superior performance against western flower thrips in soil assays is presently being evaluated. This will allow us to correlate soil persistence with efficacy over time, and to identify the best strains for use in a thrips control strategy directed at the soil-stages.

Sweet Potato Whitefly

Screening assays have been carried out on 107 fungal isolates against first instar whiteflies. Strains of *B. bassiana, P. farinosus, P. fumosoroseus* and *V. lecanii* were the most pathogenic. Some *B. bassiana* and *P. fumosoroseus* isolates also show high levels of pathogenicity to western flower thrips. Fifteen virulent strains are currently being assayed in dose-mortality tests against first and fourth instar whiteflies to determine the relative virulence of the isolates to these larval stages. First instars are generally more susceptible to an infection but the fourth instar represents the longest larval developmental stage in the whitefly life cycle. This potentially provides a large 'window of opportunity' for targeting with a fungal preparation.

Thirty five fungal strains, identified in the screening phase of this project, have also been tested against 24 h old whitefly eggs. Eggs are also cited as being highly resistant to insecticide treatments. Efficacy was measured by recording the number of larvae hatching from the treated eggs. None of the fungi appeared to infect the eggs as the hatch rate was not significantly affected. However, some strains induced high levels of infection in the newly hatched larval population, indicating that conidia persisted on the leaf surface long enough to be picked up by, and infect, the hatching 1st instars.

Experiments to determine the susceptibility of newly eclosed adults to potent fungal strains, and the influence of the treatment on fecundity, will soon be initiated.

These experiments will ultimately allow us to select strains that have the greatest potential for practical use, and we will evaluate these in the next phase of our research. In an established greenhouse infestation, whitefly generations overlap and are continuous. With the information generated, it will be possible to: identify susceptible developmental stages which can be effectively targeted with a fungal preparation; determine the spore concentration required to be effective against one or more of these stages; and ascertain whether a single fungal application, or repeated applications, would be more effective in suppressing an infestation.

Green Peach Aphid

Fungal isolates exhibiting toxicity to western flower thrips, and isolated from balsam fir twig aphid, green peach aphid and melon aphid, have been included in assays against adult green peach aphid. Forty strains have been tested, each isolate being critically evaluated using three criteria: i. the rate and level of adult mortality caused by the treatment; ii. reduction in the number of offspring produced by the treated adults; iii. spread of the disease within the offspring population. A standard conidial dose in 0.05% Tween was used in all assays. Control treatments consisted of 0.05% Tween only.

Results of these assays for eight of the most effective isolates are presented in Table 2. Six isolates: *B. bassiana* 3216, A59 and B33; *M. anisopliae* 1080 and B10; and *V. lecanii* FR24, killed 100% of the treated aphids within 4 d of treatment. A total of 10 isolates killed all of the treated aphids after 5 d. Adults treated with *B. bassiana* A59 and B33, and *V. lecanii* FR24, produced fewer offspring than aphids infected with all other isolates and the controls. Isolates A59 and FR24 initiated the highest levels of infection in the offspring population, with 24% and 23%, respectively, of the population showing symptoms of infection 5 d after the initial treatment was applied.

Fungal Isolate	%Adult Mortality After 4 d[1]	No. Offspring Control/Treatment[2]	%Infected Offspring
B. bassiana A59	100	1.23	24
V. lecanii FR24	100	1.30	23
M. anisopliae 1080	100	1.21	2
B. bassiana 3216	100	1.20	10
B. bassiana B33	100	1.29	5
M. anisopliae B10	100	1.42	1

[1]control treatment mortality in all assays did not exceed 5%.

[2]ratio calculated by dividing mean no. offspring produced/aphid in the controls by the mean no. produced/aphid in the respective fungus-treatment; the higher the value, the more effective the treatment.

Table 2. Fungal isolates tested against the green peach aphid.

Isolates A59 and FR24 performed well in all three criteria. Because of the high reproductive rate of aphids, it is important to consider such performance for selection of strains for further testing. Ideally, an effective isolate would rapidly kill adults, lower the reproductive rate, and develop an epizootic within a developing population following a single treatment.

Tests are currently underway to: i. obtain dose-mortality data on the most effective isolates identified from the screening assays; ii. determine the relative efficacy of selected isolates when applied to plants prior to infestation with aphids; and iii. evaluate their effectiveness against established aphid colonies on plants under greenhouse conditions.

Research Plans

For all three pest species, we are in the final stages of selecting fungal strains for small-scale greenhouse trials. A significant amount of research must be done to develop these agents further for ultimate incorporation into a comprehensive IPM package. We are beginning work to identify appropriate formulation and application procedures for control of western flower thrips on mums, and sweet potato whitefly on poinsettia.

It is impossible to address all greenhouse pest problems at one time, or work on all crops at the same time. Nevertheless, the information and technologies we develop have ramifications for a diverse range of pest and crop types.

Product Development and Future Prospects

Good candidate strains have been identified for control of thrips, whiteflies, aphids and fungus gnats, but considerable work must be done before a product becomes available for general use. Ultimately, the fungi have to be mass produced and formulated into products that are easy to use, stable in shipment and storage, reliable, and economically viable to ensure and retain grower confidence.

Mass Production

Fungal strains which grow and sporulate readily on artificial media are best suited to mass production. Recent developments in mass production processes now make it possible to cost-effectively produce large quantities of viable material (Goettel & Roberts 1992).

Formulation

Development of suitable formulations that maintain fungal virulence in storage and application, and enhance performance and persistence, is essential if these agents are to be used successfully. Stable wettable powder formulations of *V. lecanii* have been developed for control of aphids, whiteflies and thrips (Van der Schaaf et al. 1991).

Oil-based formulations may be more appropriate for other fungal species such as *B. bassiana, P. fumosoroseus* and *Metarhizium* spp. Formulation in oil appears to enhance infectivity if direct contact with the host is obtained, and rapid pest mortality can result even under conditions of low humidity (Bateman et al. 1993). Some oils can improve storage characteristics and persistence. Oil formulations of *B. bassiana* and *P. fumosoroseus* are currently under evaluation for use against sweet potato whitefly in cotton, melon and winter vegetable crops.

For pest such as western flower thrips and fungus gnats which have soil-inhabiting stages in their life cycle, development and use of formulations for soil application may be considered. *M. anisopliae* appears to persist particularly well in potting soils (Brownbridge unpubl. data, Moorehouse et al. 1993), potentially providing a reservoir of inoculum for insect control over an extended period. Fungi could be formulated in oil or water and applied by drench (Moorehouse et al. 1993); or produced as granules which could be seeded into the soils (Pereira & Roberts 1991). Inclusion of nutrients in granular formulations could enhance the establishment of such fungi in a potting soil medium.

Application

Application of fungi for whitefly and aphid control has largely been carried out using high-volume hydraulic sprayers (Fransen 1990, Van der Schaaf et al. 1991). Low-volume electrostatic sprayers have been used with some success (Sopp et al. 1989). Further research into the use of electrostatic applicators may be especially rewarding, particularly for pests like sweet potato whitefly which colonize the undersides of leaves and are difficult to contact using conventional sprayers. The influence of droplet size and dosage rates must also be evaluated, as these critically affect efficacy. Host behavior, motility, and age must be considered as well in the development of application protocols.

Compatibility and Integration With Other Control Strategies

Monitoring. Use of good monitoring practices will indicate the most appropriate time to apply and, if needed, re-apply a fungal treatment. Monitoring is essential as an indicator of the success of the control method and is the cornerstone of any management approach.

Host plant resistance. While market forces, or location, may not always allow insect-resistant plant varieties to be grown, host-plant resistance may enhance the performance of a mycopathogen. If the growth rate and development of the pest is affected, not only is the rate of population growth retarded, but the chances of the insect developing a lethal infection are improved (Hare & Andreadis 1983). If the inter-molt period is extended, then the fungus has longer to penetrate the insect cuticle.

Beneficials. Can fungi be used in conjunction with other biocontrol agents such as predators and parasitoids? Very little published work is available on the influence of fungi on non-target beneficials. It seems that, in a true field setting, non-targets are relatively unaffected by some entomopathogenic fungi (Magalhaes et al. 1988, Bethke & Parrella 1989, Fransen 1990, Prior 1990). Investigations to confirm this are badly needed. Such information is critical in deciding which beneficials may be used and in scheduling their release.

Chemical protectants. Although the goal of developing and using alternative control approaches is to reduce chemical pesticide usage, it is unreasonable to expect modern agriculture to survive at current production and pricing levels without some chemical inputs. Information on the compatibility of entomogenous fungi with commonly used insecticides and fungicides is important in the selection and scheduling of chemical treatments. Fungi are much less likely to be adversely affected by insecticides than other biocontrol agents, and the tolerance of some fungi to pesticides has been demonstrated (Gardner et al. 1984, Osborne et al. 1990). There is a need to constantly evaluate compatibility as new pesticides are introduced onto the market, and new fungal pathogens considered.

Fungi and biotechnology. Advances in molecular biology, genetic engineering and biotechnology can contribute to the development of improved mycopathogens. With an improved understanding of the genetic processes behind spore formation and production, insect infection, etc. it may ultimately be possible to select for, or introduce beneficial genes to enhance such desirable traits (Heale et al. 1989). Transgenic strains may also be developed for other purposes. For example, strains of *M. anisopliae* have been stably transformed to exhibit resistance to benomyl without impairing virulence for the target insect pest (Goettel et al. 1990). However, biotechnology will not preclude selection of effective strains, and development of appropriate mass production, formulation and application techniques. It is merely a way of enhancing field performance.

The Role of Fungi in Insect Pest Management

Thrips, whiteflies, fungus gnats and aphids account for major losses in the greenhouse industry. Chemical controls are failing, and there is an urgent need to develop alternative, effective, biological management strategies. Insect-pathogenic fungi represent a viable alternative to chemical pesticides for incorporation into greenhouse IPM. Fungi alone may not be able to effect the high levels of control desired for greenhouse floral and vegetable crops, but true IPM does not rely on any one component to achieve this. Rather, several control techniques need to be utilized that, together, provide the control levels required on a particular crop (Parrella 1993). The fungi represent one of the additive components of the IPM program of the future. Research results to date have been encouraging, and past successes and new developments give great cause for optimism. Use of more virulent strains and more effective formulation and application techniques will promote entomogenous fungi as realistic and practical pest management options. Their use will not totally replace chemical insecticides, rather they will provide effective alternatives in a biorational IPM program where chemical inputs are minimized. This contributes to reducing the pesticide loadings produced by the greenhouse industry, and provides for a pest management system which, although more complex to implement, is ultimately more sustainable, cost-effective, and safer for humans and the environment.

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