Artificial Rearing and Application of Natural Enemies for Citrus Pests

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Abstract Citrus is the highest-yielding fruit crop globally, with China ranking first in both cultivation area and production worldwide. During citrus growth, the crop is often damaged by various pests such as *Diaphorina citri*, scale insects, and aphids. Among these, *D. citri*, the vector of Huanglongbing (citrus greening disease), is particularly severe and has caused substantial economic losses globally. Currently, chemical pesticides remain the primary method for controlling citrus pests. However, their overuse can lead to pest resistance and excessive pesticide residues, posing threats to human health and the environment. Therefore, utilizing natural enemy insects for biological control is of significant importance. This paper systematically reviewed the research progress in artificial rearing of natural enemy insects for citrus pests, aiming to provide references for green pest management in citrus cultivation and promote the healthy and sustainable development of the citrus industry.

Key words Citrus pests; *Diaphorina citri*; Natural enemy insects; Artificial rearing; Feed; Biological control

DOI: 10.19759/j.cnki.2164 – 4993.2025.06.009

As the world's largest producer and consumer of citrus, China's citrus industry holds a significant position in its agricultural economy[1]. However, the growth and development of citrus are often severely threatened by pests and diseases. Taking Guangdong Province as an example, although its warm and humid climate is suitable for citrus growth, it also provides conditions for the occurrence of diseases and pests. Pests and diseases not only inhibit citrus growth and reduce yield, but also cause external defects such as fruit scarring, deformation, and discoloration, significantly diminishing their commercial value and affecting the economic returns and market stability of production areas[2].

With the increasingly prominent issues of environmental residue, declining biodiversity, and heightened pest resistance caused by the long-term and excessive use of chemical pesticides, the development of environmentally-friendly and sustainable pest management strategies has become an urgent need in the agricultural field[3]. Against this backdrop, biological control technologies centered on the use of natural enemy insects have gained widespread attention in academia and industry due to their high ecological safety, strong target specificity, and low risk of inducing resistance. Natural enemy insects have demonstrated effective control effects against agricultural pests. For instance, *Propylaea quatuordecimpunctata* shows significant predatory effects on cotton aphids (*Aphis gossypii* Glover) and spider mites (Tetranychidae), establishing itself as a dominant species in Xinjiang's farmlands for controlling these pests. The application of natural enemy insects in citrus pest management has a long-standing history. Among the natural enemy insects, *Oecophylla smaragdina* represents one of the earliest documented insects used in biological control[4–5]. It effectively preys on various citrus pests such as aphids and scale insects, demonstrating significant potential for biological control[6]. However, the application of *O. smaragdina* in citrus pest management lacks systematization and standardization, and artificial rearing techniques remain underdeveloped, leading to challenges like high costs and significant losses in practical applications[7–8].

Natural enemy insects play an indispensable role in Integrated Pest Management systems by continuously suppressing pests within natural ecosystems. However, traditional reliance on field collection is not only inefficient but also susceptible to seasonal and climatic constraints, making it difficult to meet the modern agricultural demand for large-scale and standardized releases of natural enemy populations. Therefore, establishing efficient and stable artificial rearing technology systems has become crucial for advancing the industrialization and scaled application of natural enemy insects.

Artificial rearing technology for natural enemy insects refers to the efficient large-scale reproduction of these insects in laboratories or factories under controlled environmental conditions. It is achieved by simulating their natural habitats, providing scientifically formulated diets and nutrition, and implementing systematic

Received: September 12, 2025 Accepted: November 16, 2025

Supported by National Innovation and Entrepreneurship Training Program for College Students (202410580010); Construction Project of the Zhaoqing Citrus Comprehensive Experimental Station Platform under the National Modern Agricultural Industry Technology System (202413004); Key Projects of the Second Round Project of High-quality Development in Hundred Counties, Thousands Towns and Ten Thousand Villages for Rural Science and Technology Special Commissioners Dispatched by the Guangdong Provincial Department of Science and Technology (KTP20240684); Doctoral Scientific Research Initiation Fund Project of Zhaoqing University (611/230009).

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population management and genetic regulation. This technology not only ensures on-demand supply and consistent quality of natural enemy products, but also creates opportunities for enhancing their environmental adaptability and pest control performance through targeted breeding. Therefore, this paper systematically reviewed research progress in various technical aspects of artificial rearing of natural enemy insects, with the goal of providing theoretical references and technical support for in-depth research and industrial applications in this field. It also seeked to advance the development of semi-artificial diets by investigating the predatory preferences of natural enemy insects, so as to ultimately promote the upgrading of green pest control technologies and the healthy development of the citrus industry.

Overview of Artificial Rearing Technologies for Natural Enemy Insects

Artificial rearing of natural enemy insects serves as the foundation for their successful application in biological control. This technological system primarily consists of the following core components:

Rearing facilities and equipment

Establishing specialized facilities that align with the biological characteristics of natural enemy insects is a primary requirement. This includes environmentally controlled rearing chambers and systems capable of precisely regulating temperature, humidity, and light. For instance, many parasitic natural enemies require specific photoperiods to synchronize their development and reproductive behaviors, making facility design crucial in this regard[9].

Feed and nutrition

Feed constitutes the core challenge and cost factor in large-scale rearing. Depending on the feeding habits of natural enemy insects (parasitic or predatory), feeds can be categorized into fully-artificial diets (formulated with specific nutrient ratios) and semi-artificial diets (based on natural hosts or substitutes). A successful feed formula must not only provide balanced proteins, carbohydrates, lipids, vitamins, and minerals, but also possess appropriate physical properties and palatability to ensure recognition and consumption by the natural enemy insects[10].

Environmental condition control

Precise environmental control is crucial for ensuring the normal development, reproduction, and sustained vitality of natural enemy insects. Parameters such as temperature, relative humidity, photoperiod, and light intensity directly influence insect development rates, survival rates, fecundity, and diapause induction/termination. For example, manipulating photoperiod can precisely induce diapause in parasitic wasps such as *Trichogramma*, enabling long-term storage and on-demand release of products. This is essential for achieving year-round production and application[11].

Population management and genetic improvement

To prevent population degradation (such as inbreeding depression and reduced adaptability) caused by long-term artificial rearing, scientific population management strategies must be implemented. These include maintaining a sufficiently large effective population size and periodically introducing fresh genes from wild populations. Meanwhile, targeted genetic improvement (*e.g.*, breeding strains with stronger resistance to specific environmental stressors or enhanced searching capability) can proactively enhance the field performance and pest control efficacy of natural enemy insects. This represents a cutting-edge direction for improving their market competitiveness[12].

General Techniques for Artificial Rearing of Natural Enemy Insects

Relatively Mature Artificial Rearing Techniques for Natural Enemy Insects

(1) *Trichogramma*, as an important egg parasitoid, has relatively well-established artificial rearing techniques. Researchers have developed a rearing system using *Sitotroga cerealella* eggs as alternative hosts and optimized temperature, humidity, and photoperiod conditions[13]. Recent research has focused on developing host-free artificial diets and improving large-scale production efficiency[14]. The main challenges in the artificial rearing of ladybugs include larval cannibalism and adult reproductive diapause. Providing sufficient space and food can reduce cannibalism, while photoperiod and temperature regulation can break diapause[15]. In terms of artificial diets, formulations based on bee larval powder or tussah pupal powder have achieved favorable results[16].

(2) The artificial rearing of green lacewings requires special attention to larval rearing density and food supply. Researchers have developed a rearing system combining artificial eggs and aphids, and have optimized oviposition substrates and nutritional supplementation for the adult stage[17]. In terms of temperature and humidity control, maintaining high humidity is crucial for larval survival.

(3) The artificial rearing technology for predatory true bugs (such as *Orius sauteri*) is relatively complex, requiring the provision of live prey or specially designed artificial diets. In recent years, semi-artificial diets based on pork liver or fly larvae have achieved success with certain true bug species[18]. In terms of environmental control, precise regulation of temperature and light conditions is necessary according to different species[19].

Rearing natural enemy insects using pest-based diets

Three species of aphids, two types of insect eggs, and six formulations of self-prepared artificial diets were used to rear *Propylaea quatuordecimpunctata*. Life table techniques were employed to study the effects of different food sources on larval development and survival, adult reproduction and longevity, as well as various population parameters. Meanwhile, the impact of diets on the predatory capacity of *P. quatuordecimpunctata* was evaluated. This approach enhanced the ladybird's ability to prey on pests, making it more effective for application in agricultural production.

Experimental results indicate that among aphids, cotton aphids and peach aphids (*Myzus persicae* Sulzer) are the most suitable natural prey for the development and reproductive fitness of *P. quatuordecimpunctata*. They facilitate population growth. Among insect eggs, both *Ephestia kuehniella* eggs and *Sitotroga cerealella* eggs were conducive to the establishment and expansion of *P. quatuordecimpunctata* populations. Regarding self-prepared diets, feeds primarily composed of beef and pork liver could serve as temporary alternative foods for maintaining *P. quatuordecimpunctata* populations. Therefore, the artificial rearing of *P. quatuordecimpunctata* holds practical significance[20]. Additionally, recent studies on the natural enemy insect *Coccinella transversoguttata* have also demonstrated that artificial rearing significantly enhances pest control efficacy. The host insects of *C. transversoguttata* include wheat aphids and cotton aphids, which primarily infest crops such as wheat, cotton, and rice. As a dominant predatory ladybird in the Tibet region, *C. transversoguttata* exhibits a long overlapping period with field aphid populations and strong feeding capacity, playing a substantial role in the natural control of aphids in Tibet. In Ren's study[21], aphids were used as feed to promote the growth and development of *C. transversoguttata*. The research experimentally investigated the effects of feeding different aphid species on *C. transversoguttata* from five aspects: developmental duration and survival rate, pupal weight, body length and width, and egg production. Furthermore, by examining the predatory efficiency of *C. transversoguttata* under varying aphid densities, intraspecific interference effects, foraging efficiency and the impact of different nutritional formula feeds on its growth and reproduction, the study demonstrated that artificial rearing of *C. transversoguttata* plays a significant role in controlling aphid populations.

In China, the artificial rearing of natural enemy insects is still in the development stage. Relevant literature indicates that by observing the predatory behavior and prey preferences of insects, semi-artificial or artificial feeds can be independently developed to rear these insects. This approach enhances the reproduction rate and population growth of predatory insects, thereby achieving better biological control effects. Successful cases of artificially rearing predatory insects were documented over a decade ago. For example, the medicinal insect *Myrmeleon formicarius* has been successfully reared artificially using feed insects such as *Musca domestica* Linnaeus larvae, yellow mealworms (*Tenebrio molitor*), and termites (*Macrotermes carbonarius*). By identifying their feeding preferences, artificial diets were developed to rear *M. formicarius*, addressing the shortage of medicinal insect sources[22]. Additionally, recent studies have shown that *Eocanthecona furcellata*, an important predatory natural enemy insect of agricultural and forestry pests, preys on over 40 pest species in Lepidoptera, Hymenoptera, Coleoptera, Hemiptera, and other orders. In the study by Gong *et al.*[23], to evaluate the predatory capacity of artificially reared *E. furcellata*, laboratory experiments were conducted using predatory functional response methods. With *E. furcellata* reared using *T. molitor* as the control group, Gong *et al.*[23] assessed the predatory efficacy of artificially reared *E. furcellata* at different developmental stages on both *T. molitor* larvae and *Spodoptera litura*. The final results demonstrated that artificially reared *E. furcellata* exhibited strong predatory effects on the target field pest *S. litura* larvae.

In summary, by referencing the aforementioned materials, we can achieve artificial rearing of *O. smaragdina* and develop artificial or semi-artificial diets that optimally support their reproduction. This approach can enhance the production efficiency of *O. smaragdina*, thereby facilitating biological control of citrus pests and diseases.

Challenges and Prospects in Artificial Rearing

Challenges

Artificial rearing of natural enemy insects has been widely applied, and in recent years, increasing attention have been paid to this field, indicating the practical relevance and feasibility of artificial rearing technology for predatory natural enemy insects. The shift from using live prey insects to artificial diets for feeding has made the management and breeding of predatory insects more convenient and efficient, thereby supporting subsequent applications in biological control within agricultural production and the development of economic insects. Despite the flourishing development of the natural enemy insect industry, their artificial rearing still faces numerous challenges that constrain production costs, scale, and efficiency.

Technical bottlenecks Lack of low-cost and efficient artificial diets: Currently, many important natural enemy insects (particularly predatory species such as ladybugs and lacewings) still require live prey (*e.g.*, aphids, rice moth eggs) for rearing, which is costly and unstable. The development of alternative artificial diets faces significant challenges, including: (1) incomplete nutritional composition, leading to prolonged development cycles and reduced reproductive capacity in natural enemies, (2) lack of effective feeding stimulants, resulting in feeding refusal by natural enemies, and (3) susceptibility of feed to mold, making preservation difficult. These issues severely constrain the large-scale, industrial production of predatory natural enemies[24]. Shortage of automated equipment for mass production: The rearing of natural enemy insects involves multiple steps such as oviposition, collection, separation, and counting, most of which currently rely on manual operations, leading to low efficiency and a high error rate[25]. Specialized automated equipment designed for handling small and fragile live insects such as precision counting and filling machines, non-destructive separation sieves, and similar tools is extremely scarce. This shortage has become a critical bottleneck hindering industrial upgrading. Risk of genetic degradation from long-term rearing: After multiple generations of breeding in confined artificial environments, natural enemy populations are prone to inbreeding, genetic drift, and adaptive selection (adaptation to artificial conditions but not to field environments). These issues lead to reduced genetic diversity, diminished vitality, and decreased predatory or parasitic capacity—a phenomenon known as "domestication syndrome"[24].

Industrial chain shortcomings Storage and transportation of live products: Natural enemy insects are living organisms with extremely short shelf lives, imposing strict requirements on temperature, humidity, ventilation, and time during transportation[26]. Currently, there is a lack of efficient, low-cost long-term storage technologies (such as reliable diapause induction and termination techniques) and remote logistics solutions that ensure viability and density, limiting market radius. Additionally, Field precision release technology requires optimization. Determining the optimal timing, locations, and quantities for releasing natural enemies based on field pest monitoring data to achieve the highest cost-effectiveness remains a challenge at the application level. Imprecise release leads to unstable control outcomes, undermining user confidence.

Prospects

Looking ahead, the development of this field will be characterized by the integration of multiple disciplines, advancing toward precision, intelligence, and systematization.

Breakthroughs in fundamental research: from "black box" to "white box" Genomics and synthetic biology: Loci controlling important economic traits (such as feeding habits, fecundity, and stress resistance) of natural enemy insects can be elucidated by sequencing their genomes. Based on engineering principles, synthetic biology can design microorganisms or artificial cells capable of efficiently producing nutrients required by natural enemies (*e. g.*, specific unsaturated fatty acids). This provides a foundational solution for developing entirely new artificial diets. Meanwhile, technologies such as gene editing offer the potential for precise genetic improvements and prevention of population degeneration[27]. Metabolomics and nutrition: Artificial diet formulations can be designed more precisely by analyzing the metabolite profiles and nutritional requirements of natural enemy insects at different developmental stages, thereby achieving a transition from "edible" to "optimal" nutrition.

Production technology upgrades: from "manual" to "intelligent" Automation and artificial intelligence (AI): Machine vision technology can be utilized for automatic identification and counting of natural enemy insects. Robotic arms can perform delicate transfer operations. AI algorithms can dynamically optimize rearing environment parameters by analyzing environmental data (temperature, humidity, light) and insect growth data, achieving fully intelligent and standardized production[28]. Quality control and traceability system: A big data and Internet of Things (IoT)-based quality control system can be established to monitor and record key indicators (such as emergence rate, sex ratio, and activity) for each batch of natural enemy products, and generate unique traceability codes. This not only ensures product quality, but also enables cause tracing when control effectiveness is suboptimal, forming a data closed loop.

Deepening applied ecology: from "individual soldiers" to "coordinated corps" Research on synergistic enhancement technologies: Future biological control will no longer rely on the release of a single natural enemy species, but will emphasize "multi-force joint operations". For example, the synergistic effects between predatory natural enemies (*e. g.*, ladybugs) and parasitic natural enemies (*e. g.*, *Trichogramma*), entomopathogenic fungi (*e. g.*, *Beauveria bassiana*), or entomopathogenic nematodes will be studied to avoid mutual interference. This could even will lead to the design of sequential control strategies, such as "parasitic wasps clearing foliar surfaces, followed by ladybugs preying on remaining pests". Building a multi-dimensional biological control network: Natural enemy insects can be integrated with plant-derived attractants, crop-resistant varieties, and ecological landscape management technologies to establish a stable and self-sustaining biological control ecosystem at the farmland or even regional scale. This approach aims to achieve sustainable pest control[29].

Cross-innovation with multiple cutting-edge technologies Future advancements can leverage the frontier cross-technologies to drive specific innovations and address core challenges. Novel feeds can be developed based on microencapsulation or 3D printing technologies. (1) Microencapsulation technology: This method encapsulates essential nutrients, feeding stimulants, and preservatives required by natural enemies within micron-scale capsules. It mimics the morphology of natural prey, protects easily oxidized components, and enables controlled nutrient release, significantly improving acceptance rates and feeding efficacy of natural enemies[30]. (2) 3D printing technology: It enables the creation of "customized" feeds with complex three-dimensional structures, varying textures, and gradient nutrients. This approach not only meets the needs of natural enemies at different developmental stages, but also integrates feeding stimulants, representing a key direction for personalized artificial diets in the future[31]. (3) Application of IoT technology: The use of IoT technology enables precise and dynamic regulation of the rearing environment. By deploying numerous sensors for temperature, humidity, light, carbon dioxide, and other parameters within the rearing facility, real-time environmental data can be collected and uploaded to the cloud. The control system, based on preset models and AI algorithms, dynamically adjusts actuators such as air conditioning, humidifiers, and lighting systems to create an optimal and minimally fluctuating rearing environment for natural enemy insects, thereby maximizing their production efficiency and vitality. (4) Diapause regulation technology: Research on diapause regulation technology can significantly extend the shelf life of products. Diapause is a developmental arrest state in insects that helps them cope with adverse environmental conditions. By precisely controlling photoperiod, temperature, and nutrition, natural enemy insects can be artificially induced and maintained in a diapause state, extending the effective shelf life of products from a few weeks to several months. This effectively acts as a "pause button" for live products, greatly addressing storage and logistics challenges and enabling natural enemies to be managed in inventory and distributed globally like chemical pesticides[32].

These innovative technologies, ranging from microscopic feed design to macroscopic production system management, collectively outline the future landscape of artificial rearing technology for natural enemy insects. Their deep integration and synergistic development will powerfully drive the industry to overcome existing bottlenecks, advancing toward greater efficiency (high yield, high vitality), economy (low cost, long shelf life), and intelligence (data-driven, automated). Ultimately, this will provide indispensable core support for global green agriculture and food security.

Quality Control and Evaluation System for Artificially-reared Natural Enemy Insects

A comprehensive quality control system is implemented throughout both the production and end stages. Production process control focuses on continuous monitoring of feed quality, environmental parameters (temperature, humidity, light), and population health. Product inspection, on the other hand, involves final evaluation of the quality of natural enemy insects prior to release, commonly assessed through methods such as flight capability tests and parasitism/predation capacity bioassays[33].

The quality of artificially reared natural enemy insects is a decisive factor for the success of field control. A comprehensive quality evaluation system typically includes morphological indicators (such as body size, weight, wing development), physiological indicators (such as lifespan, fecundity, fat content), and behavioral indicators (such as searching ability, aggressiveness, flight capacity)[34]. To ensure objectivity in evaluation, these indicators must be regularly assessed using standardized methods.

To further enhance the field performance of natural enemy insects, various quality enhancement technologies have been developed and applied. Nutritional enhancement involves adding specific nutrients (such as carbohydrates and polyunsaturated fatty acids) to their diets to directly improve their vitality and longevity. Behavioral training (*e.g.*, rearing in environments with plant volatiles) can enhance their ability to locate pests or hosts. Meanwhile, long-term genetic selection can directionally improve key traits in populations (such as stress resistance and fecundity)[35]. The integrated application of these technologies can effectively mitigate the individual degradation issues that may arise from artificial rearing.

Conclusions

Artificially reared natural enemy insects have become a key technology for sustainable agriculture worldwide, with applications spanning from highly intensive protected agriculture to complex open-field ecosystems. In controlled environments such as greenhouses and polytunnels, the high degree of environmental controllability provides an ideal setting for the deployment of natural enemy insects. The use of *Trichogramma* to control diamondback moths and cotton bollworms on tomatoes and cucumbers, along with the application of ladybugs and predatory thrips to manage melon aphids and whiteflies, has established a complete commercial chain from production to release. Users can directly purchase products from biological control companies and access technical services[34]. This "product + service" model has significantly promoted the adoption of biological control in protected agriculture.

In open-field crops (such as cotton, corn, and sugarcane), the application of natural enemy insects emphasizes the concept of ecological engineering. Planting nectar-producing plants and establishing ecological islands provides habitats and supplementary nutrition for natural enemies, and their colonization and pest control capabilities can be enhanced. For example, releasing lacewings and ladybugs in cotton fields can effectively control cotton bollworms and aphids, while the use of predatory bugs (such as *O. sauteri*) demonstrates exceptional control efficacy against small pests like mirids and thrips[36]. These practices demonstrate that integrating natural enemy releases with farmland ecological optimization can establish a more resilient and stable pest management system on a large scale.

Compared with chemical control, the core advantage of natural enemy insect-based control lies in its sustainability. In terms of environmental benefits, it leaves no chemical residues, is safe for non-target organisms, and helps protect biodiversity while restoring ecosystem services in farmland. Economic analyses show that although the initial investment in natural enemy products may be higher than that of conventional pesticides, the long-term returns are significant. On one hand, it directly reduces production and labor costs by decreasing pesticide use. On the other hand, the produced "green" or "organic" agricultural products are of higher quality, possess stronger market premium capabilities, and can generate direct economic benefits[37]. Additionally, the reduction of health risks for pesticide applicators and the protection of water sources and soil also yield significant social benefits.

Substantial scientific research and field trial data confirm that the scientific release of natural enemy insects can suppress target pest populations below economic thresholds, typically achieving 60% – 80% control efficacy. In enclosed and low – interference environments such as greenhouses, the combined application of multiple natural enemy species can even enable 100% replacement of chemical pesticides[38]. China's successful cases are particularly notable. In the northern cotton regions, large-scale releases of artificially reared *Trichogramma dendrolimi* to control cotton bollworms have successfully reduced chemical pesticide use by over 70%, achieving a win-win scenario for both economic and ecological benefits. In southern citrus orchards, systematic releases of predatory mites such as *Neoseiulus cucumeris*, combined with other ecological measures, effectively control pests such as red spiders and rust mites. This significantly improves the surface smoothness of the fruits, laying a foundation for the development of high-end fruit brands[39].

In summary, natural enemy insects have evolved from a supplementary method into an irreplaceable core component of the modern Integrated Pest Management system. Their role is no longer confined to emergency control after pest outbreaks, but has shifted to a proactive and routine ecological regulation tool. Against the backdrop of agricultural ecological environment management and green plant protection, the prevention and control of crop diseases and pests, reduction of chemical pesticide use and enhancement of biological control measures have become inevitable trends in agricultural development.

In the future, with further breakthroughs in artificial rearing technologies, continuous cost reduction, and increased awareness among farmers, natural enemy insects are destined to play an even more critical role in promoting the green transformation of agriculture, ensuring the quality and safety of agricultural products, and safeguarding ecological and environmental security.

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protection of aquatic ecosystems.

Conclusions

In this study, an LC-MS/MS synchronous detection method was established for fluxapyroxad and pyraclostrobin (with LOD reaching ng/L level), and the toxic effects of these two fungicides on zebrafish were systematically evaluated. The results showed that the toxicity of pyraclostrobin (96 h-LC_{50} = 0.052 mg/L) was approximately 25.8 times that of fluxapyroxad (1.34 mg/L). This significant difference stems from their specific inhibition of mitochondrial complex III and complex II, respectively. The joint toxicity assessment revealed that most mixture ratios exhibited additive effects (AI = 0.62 – 1.47), while a high fluxapyroxad ratio showed antagonism. The analysis of toxicity mechanisms demonstrated that both fungicides induced oxidative stress and cellular damage through mitochondrial dysfunction, with pyraclostrobin exhibiting stronger effects. Ecological risk assessment indicated that fluxapyroxad posed a moderate risk, while pyraclostrobin posed moderate to high risks, with crustaceans being the most sensitive species. This study provides a scientific basis for the differentiated management of mitochondrial inhibitor fungicides and the protection of aquatic ecosystems.

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