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一、论文（首页）



Pectinase-responsive carriers based on mesoporous silica nanoparticles for improving the translocation and fungicidal activity of prochloraz in rice plants

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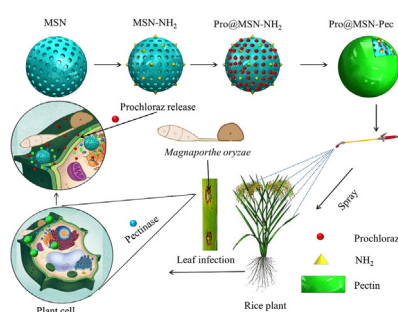
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HIGHLIGHTS

- Stimuli-responsive carrier was prepared employing pectin as the gatekeeper of MSNs.
- Pro@MSN-Pec improved the translocation of prochloraz in rice.
- Prochloraz release of Pro@MSN-Pec depended on pectinase of rice blast disease.
- Pro@MSN-Pec had a longer duration and excellent antifungal activity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Mesoporous silica nanoparticles
Controlled release
Stimuli-responsive
Translocation and fungicidal activity
Rice blast disease
Prochloraz

ABSTRACT

Stimuli-responsive carriers as delivery systems of agrochemicals into plants can increase the utilization efficiency of pesticides against target pests and reduce hazards on nontarget living organisms and the environment. In the present work, prochloraz was loaded into mesoporous silica nanoparticles (MSNs) and coated by pectin on the particle surface as a gatekeeper to study the translocation and distribution of prochloraz@MSN-pectin (Pro@MSN-Pec) in rice plants. The results showed that Pro@MSN-Pec was successfully fabricated and had an average size of 70.89 nm and a good loading efficiency (30% w/w). The MSNs were labeled using fluorescein isothiocyanate (FITC) to track the distribution of the carriers in the rice plants. Confocal scanning analysis showed that MSN-FITC could be transported to all rice parts from the treated leaves or roots. The release of prochloraz (Pro) from Pro@MSN-Pec was pectinase-dependent. Compared with the commercial emulsifiable concentrate (EC formulation) of Pro, the Pro@MSN-Pec formula had a better uptake and translocation in rice plants as well as a longer duration and better antifungal activity against rice blast disease (*Magnaporthe oryzae*). The final residue level of Pro in the rice plants was lower than the maximum residue limits. These results suggest that the use of stimuli-responsive carriers as pesticide-delivery systems in plants can be potentially implemented in applied agriculture.

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Thermoresponsive polymer-encapsulated hollow mesoporous silica nanoparticles and their application in insecticide delivery

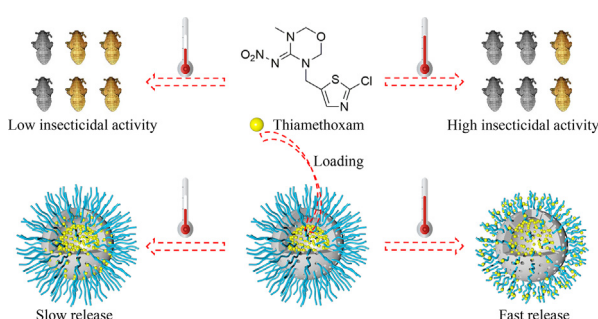
Yunhao Gao¹, Yanan Xiao¹, Kaikai Mao, Xueying Qin, Yuan Zhang, Donglin Li, Yanhui Zhang, Jianhong Li, Hu Wan*, Shun He*

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HIGHLIGHTS

- An HMS hybrid employing P(NIPAM-MAA) as the outer shell of HMS with temperature-responsiveness.
- THI-loaded HMS hybrid could utilize the relationship between pesticide efficacy and temperature.
- HMS hybrid possessed good biocompatibility and UV-shielding properties.
- THI-loaded HMS hybrid provided sustained protection in rice plant against *N. lugens*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Thiamethoxam
Temperature-responsive release
Hybrid material
Nilaparvata lugens (Stål)
Bioactivity

ABSTRACT

Improving pesticide efficacy, which depends on the smart delivery of pesticides in the field, has been the basis for reducing the use of agricultural chemicals to an optimal level. Herein, a temperature-responsive release formulation (THI@HMS@P(NIPAM-MAA)) that can regulate pesticide release based on the relationship between pesticide efficacy and ambient temperature is proposed. The THI@HMS@P(NIPAM-MAA) was prepared by seeded precipitation polymerization, wherein hollow mesoporous silica (HMS) was employed as the core; a commonly used thermoresponsive copolymer, poly(*N*-isopropylacrylamide-co-methacrylic acid) (P(NIPAM-MAA)), was used as the outer shell; and a type of positive temperature coefficient insecticide, thiamethoxam (THI), was selected as the model pesticide. The prepared THI@HMS@P(NIPAM-MAA) can effectively protect THI against degradation under UV irradiation and showed strong adhesion to rice leaves. The bioactivity results showed that the mortality of THI@HMS@P(NIPAM-MAA) against *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) was positively correlated with temperature and mainly benefitted from the temperature-induced variation in the release rate. Furthermore, the THI@HMS@P(NIPAM-MAA) possessed long-term bioactivity (14 days) and showed negligible effects on rice seedling growth. This temperature-responsive release formulation may be extended to other positive temperature coefficient pesticides in the future, thus greatly advancing smart pesticide formulation development.

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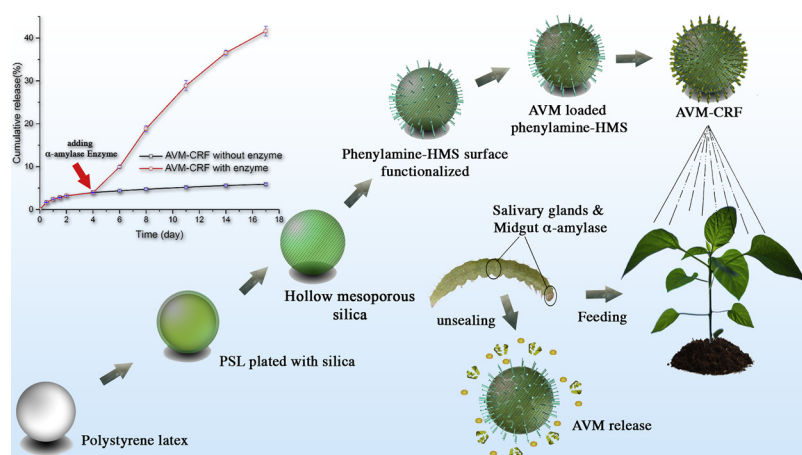
α -Amylase triggered carriers based on cyclodextrin anchored hollow mesoporous silica for enhancing insecticidal activity of avermectin against *Plutella xylostella*

Amir. E. Kaziem^{a,b}, Yunhao Gao^a, Yuan Zhang^a, Xueying Qin^a, Yanan Xiao^a, Yanhui Zhang^a, Hong You^a, Jianhong Li^a, Shun He^{a,*}

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GRAPHICAL ABSTRACT



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ABSTRACT

α -Amylase-responsive carrier for controlled release of avermectin (AVM) was prepared based on α -cyclodextrin (α -CD) anchored hollow mesoporous silica (HMS) using α -CD as a capping molecule. The release of AVM was studied at different temperatures, pH values and in the presence or absence of α -amylase. The results revealed that the AVM-encapsulated controlled release formulation (AVM-CRF) has a drastic enzymatic dependence, an excellent encapsulation efficacy reaching 38%, and outstanding UV and thermal shielding ability. The AVM-CRF biological activity survey shows excellent toxicological properties against *Plutella xylostella* larvae, which confirms that α -CD caps could be uncapped enzymatically *in vivo* and release AVM, inducing *P. xylostella* larval death. AVM-CRF has a notable capability to keep 0.6 mg L^{-1} AVM biologically active until 14th day with 83.33% mortality of the target insect, which was 40% higher than that of treated with AVM commercial formulation. The study provides a theoretical basis for the application of pesticide reduction.

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A hollow mesoporous silica and poly(diacetone acrylamide) composite with sustained-release and adhesion properties

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ABSTRACT

Controlled release formulation of pesticides is a highly desirable way to increase the efficiency of the pesticide as well as help reduce environmental pollution issues. In the present study, a novel adhesive organic-inorganic hybrid material with a uniform size and morphology was fabricated employing hollow mesoporous silica (HMS) as an inlayer material and poly(diacetone acrylamide) (PDAAM) as an outer layer material. HMS was fabricated using polystyrene (PS) spheres as a hard template. Then, HMS was functionalized with 3-aminopropyltriethoxysilane and 2-bromoisobutyl bromide after removal of PS. Finally, PDAAM was grafted onto HMS using surface-initiated atom transfer radical polymerization. Cyantraniliprole (CNAP) utilized as a model pesticide was incorporated into hybrid material to prepare controlled release formulation. The results showed that both CNAP-loaded HMS and HMS-PDAAM had extraordinary loading efficiencies (approximately 50% w/w). Kinetic studies of CNAP release demonstrated that CNAP-loaded HMS-PDAAM exhibited a sustained release property for at least 25 days. The stability test identified that CNAP-loaded HMS and HMS-PDAAM were much more stable under UV irradiation and thermal conditions than CNAP technical. Tests of the adhesive properties showed that the adhesive property of HMS-PDAAM was far stronger than that of HMS on rice leaves. Given the advantages of HMS-PDAAM, this hybrid material may be applied to other photosensitive pesticides, especially those used for foliar spraying.

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1. Introduction

Pesticides play an irreplaceable role in integrated pest management due to their efficient, fast, economical, and easily implementation. However, more than 90% of the active ingredients of conventional pesticide formulations applied in the field are lost to the environment and cannot reach the target organisms due to wind, UV light, washing away, evaporation, precipitation and other factors [1]. This not only increases the application amount of pesticides for pest control, but also leads to environmental pollution [2–4]. Controlled release technology defined as a system in which the concentration of an active substance is preset and has a

sustained release, thus achieving the desired results [5]. It has been remarked as a seed of new technology and has been widely used in the fields of food, medicine, environmental engineering, coatings, cosmetics and pesticides [6–11]. Controlled release formulation of pesticides can remarkably protect active ingredients against degradation, can prolong the duration, and regulate the release of active ingredients required for effective pest control, and it is highly desirable for attaining the most effective utilization of the pesticide as well as for reducing environmental pollution [12–16].

Organic-inorganic nanocomposites, commonly achieved by locating the inorganic particles on the surface of polymer grains or by adding modified nanoparticles into the polymer matrices, are used in a wide range of applications in various fields, such as sorption of toxic ions and controlled drug-delivery systems [17,18]. Mesoporous silica nanoparticles, due to their porous structure, large surface area, good drug loading capacity relying on large pore volumes, excellent biocompatibility, and modifiable surface

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Synthesis and Insecticidal Activity of Enzyme-Triggered Functionalized Hollow Mesoporous Silica for Controlled Release

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S Supporting Information

ABSTRACT: In the present study, enzymatic responsive controlled release formulations (CRFs) were fabricated. The CRFs were achieved by anchoring mechanically interlocked molecules using α -cyclodextrin onto the surface pore rims of hollow mesoporous silica (HMS). The CRFs were characterized using Fourier transform infrared spectroscopy, scanning electron microscopy, transmission electron microscopy, and thermogravimetric analysis. The results showed that the CRFs had extraordinary loading ability for chlorantraniliprole (42% w/w) and could effectively preserve chlorantraniliprole against degradation under thermal conditions and UV radiation. The CRFs have been proven to be enzyme-sensitive. The release ratio of chlorantraniliprole from CRFs can be accelerated observably when external α -amylase was introduced. The persistence of CRFs was evaluated by regular sampling feeding experiment using *Plutella xylostella* as the target insect. The results showed that the larval mortality of *P. xylostella* was much higher than that of Coragen under all concentrations after 14 days, which proved that CRFs had remarkable persistence.

KEYWORDS: chlorantraniliprole, hollow mesoporous silica, controlled release, α -amylase sensitivity, biological activity survey

INTRODUCTION

Chewing insects are considered the most injurious enemy for crops, not only for their feeding consequences on shoots and roots but also leaving plants vulnerable to infections with virus, fungi, and bacteria.¹ Over the last several decades, pesticide preparation has focused on extending the active ingredient validity period in the ecosystem as much as possible² but did not consider the environmental exposure period.³ This dilemma can be overcome by using nanoformulation technologies, a process that involves a polymeric particle matrix that carries pesticide molecules inside.⁴ The loaded pesticide is then released in a controlled manner in response to external stimuli. The synthesis of hollow mesoporous silica (HMS) with scalable structures and different synthesis mechanisms^{5,6} and properties^{7,8} has stimulated significant attention in the last several decades. HMS containers have multilateral intrinsic characteristics, such as excellent biocompatibility,^{9,10} high stability,¹¹ high loading capacity, and non-immunogenicity. In addition to its capability of encompassing a gatekeeper on the outer surface,^{12–16} being nontoxic to living cells¹⁷ is its most environmentally valuable feature. Since the first gated silica mesoporous particle was developed,¹⁸ a variety of such systems of silica nanoparticles with stimuli-responsive gatekeepers have been presented to control the release of drug molecules in restraint of outward stimulators, such as light,^{19–21} redox potential,^{22,23} pH,²⁴ and enzymes.^{25–28} Further systems, including pseudorotaxanes,²⁹ carboxylates,³⁰ and complexes such as cyclodextrin,³¹ cucurbit[6]uril,³² and cucurbit[7]uril,³³ have also been prepared. In addition, additional preliminary studies that use alternate exterior stimuli, such as temperature³⁴ and the presence of specific anions,³⁵ have also been reported. Countless nanocarriers had been synthesized for

the delivery of pesticides, such as polymeric nanospheres,^{36–38} microcapsules,^{39–43} solid lipid nanoparticles,^{44,45} layered double hydroxides and clays,^{46–49} nanosized metals and metal oxides,⁵⁰ and carbon nanotubes.⁵¹ Nevertheless, programmed silica nanocontainers that are capable of pH and enzyme responsive release of pesticide molecules are currently under intensive investigation.^{52,53} This not only can strengthen some active ingredients (AI) to withstand deterioration factors that may adversely affect the active substances performance but can also prolong the AI bioavailability.⁵⁴ Chlorantraniliprole (CLAP) is a new compound that belongs to the family of anthranilic diamides as selective insecticides and presents a novel mode of action by stimulating the insect ryanodine receptors (RyRs). CLAP in water can be deteriorated by both chemical and photochemical procedures.⁵⁵ One goal is to utilize the advantage of carbohydrate digestion and the digesting technique in pesticide formulation technology. α -Cyclodextrin (α -CD) is a cyclic oligosaccharide composed of α -(1,4) linked glucopyranose subunits that can be enzymatically hydrolyzed by α -amylase. Whereas enzymes are assimilation keys and exist in any digestive system, α -amylase is our liberation key that exists in the salivary glands and midgut of larvae with chewing mouthparts.^{56,57}

Orienting the pesticide particles to be released inside a specific group of insects is a new promising research field in pesticide formulation science that depends on the insect's feeding type. In particular, insects that eat plant leaves may be specifically

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Short communication

Efficacy of an adhesive nanopesticide on insect pests of rice in field trials

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Keywords:

Nanopesticide

Cnaphalocrocis medinalis (Guénee)

Chilo suppressalis (Walker)

Field trial

Biosafety

ABSTRACT

Nanopesticides with antiwashing capacity on leaves are the most promising new approaches for sustainable pest management and have been fully evaluated in the laboratory. However, few studies have tested these nanopesticides on pests, and their efficacy under field conditions has not been investigated. In this study, an adhesive hollow mesoporous silica hybrid with well-defined spherical shape and good monodispersity was used as a nanocarrier of cyantraniliprole (CNAP) to fabricate an adhesive nanopesticide (CNAP-HMS-PDAAM). The control efficacy of CNAP-HMS-PDAAM was tested under field conditions. The results indicated that the efficacy of four doses of CNAP-HMS-PDAAM (30.0–69.0 g a.i./ha) against *Cnaphalocrocis medinalis* (Guénee) 3, 7, and 14 days after spraying did not significantly differ from that of Benevia (34.5 g a.i./ha). Twenty-eight days after spraying, the efficacy of all four doses of CNAP-HMS-PDAAM was significantly better than that of Benevia. Additionally, the efficacy of CNAP-HMS-PDAAM at doses of 34.5, 39.0 and 69.0 g a.i./ha against *Chilo suppressalis* (Walker) were significantly higher than that of Benevia (34.5 g a.i./ha). Thus, CNAP-HMS-PDAAM showed long-term control efficacies against *C. medinalis* (Guénee) and *C. suppressalis* (Walker), mainly due to its strong adhesive property on rice leaves and its sustained release properties. In addition, the nanocarriers showed good biocompatibility and had no obvious influence on the growth of rice.

Introduction

Rice (*Oryza sativa*) is the leading cereal crop grown worldwide because of its great importance to global food security (Khuhro et al., 2017; Wing et al., 2018). *Cnaphalocrocis medinalis* (Guénee) and *Chilo suppressalis* (Walker) are two important insect pests of rice; their larval leaf folding and stem boring behaviours greatly reduce the photosynthetic ability and vigor of rice plants, resulting in enormous reductions in rice yield (Padmavathi et al., 2013; Sun et al., 2018). To control these pests, many effective insecticides are applied annually in paddy fields worldwide, and the long-term use of which can lead to insect resistance and groundwater pollution (Chagnon et al., 2015; Mao et al., 2019). Therefore, there is a need to develop novel technologies to reduce pesticide use and protect rice from pests via sustainable means.

In recent years, research into nanotechnology applications in pesticide delivery has received much attention (White and Gardea-Torresdey, 2018; Lombi et al., 2019; Kah et al., 2019). By utilizing the

benefits of nanomaterials, nanopesticides achieve smart release, improve the stability of active ingredients (AIs) in the environment, and prolong the effect duration (Nuruzzaman et al., 2016; Zhao et al., 2018; Lowry et al., 2019; Gao et al., 2020). Among them, nanopesticides with strong adhesion force on leaves have been widely studied. For example, Xiang et al. modified natural nanoclay with a high-energy electron beam to fabricate an effective matrix with nanonetworks, which possessed high antiwashing capacity and low chlorpyrifos amount loss on the peanut leaf surface (Xiang et al., 2014). Sharma et al. prepared anti-drift nanostickers made of graphene oxide for chlorpyrifos delivery, which effectively controlled the drift loss of chlorpyrifos on the cauliflower leaf via the piercing effect and the 2-D structure of graphene oxide (Sharma et al., 2017). In another case, Cui et al. reported on catechol group-modified avermectin nanoparticles, which showed good adhesion properties and long retention time on cucumber and broccoli foliage surfaces (Liang et al., 2018).



Taken together, these results indicate that nanopesticides with

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ORIGINAL ARTICLE

Carboxylesterase genes in nitenpyram-resistant brown planthoppers, *Nilaparvata lugens*Kaikai Mao¹, Zhijie Ren¹, Wenhao Li¹, Tingwei Cai¹, Xueying Qin¹, Hu Wan¹ , Byung Rae Jin², Shun He¹ and Jianhong Li¹ ¹Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China and ²College of Natural Resources and Life Science, Dong-A University, Busan, Republic of Korea

Abstract Carboxylesterases (CarEs) represent one of the major detoxification enzyme families involved in insecticide resistance. However, the function of specific CarE genes in insecticide resistance is still unclear in the insect *Nilaparvata lugens* (Stål), a notorious rice crop pest in Asia. In this study, a total of 29 putative CarE genes in *N. lugens* were identified, and they were divided into seven clades; further, the β -esterase clade was significantly expanded. Tissue-specific expression analysis found that 17 CarE genes were abundantly distributed in the midgut and fat body, while 12 CarE genes were highly expressed in the head. The expression of most CarE genes was significantly induced in response to the challenge of nitenpyram, triflumezopyrim, chlorpyrifos, isoprocarb and etofenprox. Among these, the expression levels of *NlCarE2*, *NlCarE4*, *NlCarE9*, *NlCarE17* and *NlCarE24* were increased by each insecticide. Real-time quantitative polymerase chain reaction and RNA interference assays revealed the *NlCarE1* gene to be a candidate gene mainly involved in nitenpyram resistance, while simultaneously silencing *NlCarE1* and *NlCarE19* produced a stronger effect than silencing either one individually, suggesting a cooperative relationship in resistance formation. These findings lay the foundation for further clarification of insecticide resistance mediated by CarE in *N. lugens*.

Key words carboxylesterase; expression profiling; insecticide induction; *Nilaparvata lugens*; nitenpyram resistance

Introduction

Carboxylesterases (CarEs) belong to the α/β -hydrolase fold superfamily, and they are widely present in microorganisms, flora and fauna; these enzymes are involved in the hydrolysis of a variety of ester-containing xenobiotics, neural signal transmission, pheromone degradation, and reproductive development (Wheelock *et al.*, 2005;

Satoh & Hosokawa, 2006; Montella *et al.*, 2012). Insect CarE genes have been classified into nine subfamilies (α -esterase, β -esterase, integument esterase, juvenile hormone esterase, acetylcholinesterase, gliotactin, neuroligin, glutactin, and neurotactin) based on homology and function differences (Ranson *et al.*, 2002). With the development of whole genome sequencing and transcriptome sequencing of insects, members of these nine subfamilies have been identified in the following insect species: *Drosophila melanogaster*, *Musca domestica*, *Anopheles sinensis*, *Acyrtosiphon pisum*, and *Bombyx mori* (Oakeshott *et al.*, 1999; Yu *et al.*, 2009; Ramsey *et al.*, 2010; Feng *et al.*, 2018; Wu *et al.*, 2018). Members of the α -esterase clade, β -esterase clade and acetylcholinesterase clade have been implicated in insecticide

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Hormetic Effects of Mixtures of Carbendazim and Iprodione on the Virulence of *Botrytis cinerea*

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Abstract

Hormetic effects of fungicides on mycelial growth and virulence of plant pathogenic fungi have been reported, but the effects of fungicide mixtures on virulence hormesis of plant pathogens remain to be investigated. In this study, hormetic effects of mixtures of carbendazim and iprodione on the virulence of two carbendazim-resistant isolates of *Botrytis cinerea* were determined. Spraying carbendazim alone at 3 to 800 µg/ml exhibited hormetic effects on virulence to cucumber leaves, and carbendazim at 10 µg/ml had the maximum stimulation of 16.7% for isolate HBtom451. Spraying iprodione alone at 0.0001 to 0.0625 µg/ml exhibited hormetic effects on virulence, and iprodione at 0.025 µg/ml had the maximum stimulation of 18.7% for isolate HBtom451. However, spraying simultaneously carbendazim at 800 µg/ml and iprodione at 0.0625 µg/ml showed inhibitory effects on virulence to cucumber leaves. The mixture of carbendazim at 3 µg/ml and iprodione at 0.0001 µg/ml had much higher virulence stimulations than either fungicide at the same concentration alone. The maximum stimulation for the mixtures occurred

at 10 and 0.0005 µg/ml for carbendazim and iprodione, respectively, and these concentrations were much lower than the concentration of their respective fungicide alone eliciting the maximum stimulations. The maximum stimulation amplitude for the mixture was slightly higher than that of each fungicide alone. These results demonstrated that carbendazim and iprodione mainly had dose-additive rather than amplitude-additive interactions when sprayed simultaneously with regard to virulence stimulations. Studies on virulence stimulations for mycelia treated with fungicide in potato dextrose agar showed that the maximum stimulation for the mixtures occurred at concentrations much lower than the concentration of carbendazim alone, indicating a dose-additive interaction when compared with carbendazim hormesis. Studies on potential physiological mechanisms of hormesis showed that increased tolerance to H₂O₂ may be one of the mechanisms for virulence hormesis for the mixtures of iprodione and carbendazim. These studies will advance our understanding of hormesis of fungicide mixtures.

The necrotrophic plant pathogenic fungus *Botrytis cinerea* Pers. Fr. can infect more than 1,400 plant species in 586 genera across the world. The extremely broad host spectrum mainly comprises dicotyledonous plants, many of which are economically important fruit and vegetable crops (Elad et al. 2015; Van Kan et al. 2017). Gray mold caused by *B. cinerea* usually leads to serious economic losses to the production of many fruit and vegetable crops such as grapes and tomatoes. So far, no crop cultivars with complete resistance to this pathogen have been successfully developed. *B. cinerea* can produce huge amounts of conidia in a favorable environment and form highly resistant sclerotia as a survival structure in an adverse environment. *B. cinerea* has evolved exquisite infection tactics to efficiently attack a fairly wide range of plants, making it one of the most successful plant pathogenic fungi on earth (Amselem et al. 2011; Williamson et al. 2007). Therefore, it has been traditionally difficult to control this intractable pathogen. Among the integrated management tactics, fungicides remain a primary approach to controlling *B. cinerea* (Mbengue et al. 2016; Nakajima and Akutsu 2014).

Among the various fungicides registered in China for control of gray mold, the benzimidazole fungicide carbendazim is one of the most widely used compounds, although mainly as mixtures with other fungicides of different modes of action or different chemical groups such as pyrimethanil, iprodione, and diethofencarb (<http://www.chinapesticide.org.cn/hysj/index.jhtml>). Carbendazim is a typical single site of action fungicide, and point mutations of E198A/G/K/V and F200Y in the target β-tubulin gene usually lead to high and

moderate levels of resistance in plant pathogenic fungi (Banno et al. 2008). The Fungicide Resistance Action Committee (FRAC 2018) lists the benzimidazole fungicides as having a high risk for resistance development. The resistance of *B. cinerea* to benzimidazole fungicides is prevalent, and carbendazim resistance has been reported across the world (Konstantinou et al. 2015; Rupp et al. 2016; Saito et al. 2016). In China, although carbendazim has been primarily used as mixtures with other fungicides for control of gray mold in the last several decades, different levels of carbendazim resistance have also been reported (Liu et al. 2014; Zhang et al. 2006; Zhou and Ye 1987).

The development and prevalence of fungicide resistance have serious implications for control of plant pathogens. High levels of resistance will result in control failures for fungicide in the field. More importantly and unexpectedly, fungicides applied in the field may stimulate rather than inhibit plant pathogens in some situations, especially for resistant strains. In fact, some farmers have witnessed more serious disease outbreaks after application of fungicides than the nontreated control. In the fields, pathogens will be exposed to sublethal doses of the applied fungicides because of the drift of sprayed droplets, degradation of fungicides, and different penetrations through crop canopies. Concentrations recommended on the packaging labels are effective to the sensitive strains of the target pathogen and will become sublethal or relatively low dosages for the resistant strains (Garzón and Flores 2013). Low dosages of fungicides may stimulate mycelial growth and virulence of plant pathogenic fungi. Stimulatory effects of carbendazim on the virulence of resistant isolates of *B. cinerea* have been reported recently (Cong et al. 2018). Our previous studies also reported stimulatory effects of low doses of dimethachlone (Zhou et al. 2014) on mycelial growth and virulence, and trifloxystrobin (Di et al. 2016a), carbendazim (Di et al. 2015, 2016b), and flusilazole (Lu et al. 2018a, b) on the virulence of *Sclerotinia sclerotiorum*. In addition, stimulatory effects of low doses of fungicides on a variety of fungi have also been documented, such as stimulatory effects of sublethal doses of thiabendazole on spore germination of *Penicillium expansum* (Baraldi et al. 2003), propanocarb on mycelial growth of *Lyophyllum palustre* (Landry et al. 2011), prothioconazole on the production of the mycotoxin deoxynivalenol in *Fusarium graminearum* (Audenaert et al.

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Hormetic Effects of Flusilazole Preconditioning on Mycelial Growth and Virulence of *Sclerotinia sclerotiorum*

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Abstract

Hormetic effects of fungicides are highly relevant to fungicide applications and management of plant-pathogenic fungi. Preconditioning (i.e., early exposure to relatively low doses of a toxicant) is a special form of hormesis, and fungicide preconditioning of phytopathogenic fungi is inevitable in the field. The present study showed that spraying the demethylation inhibitor (DMI) fungicide flusilazole at 0.1 $\mu\text{g/ml}$ had stimulatory effects on the virulence of *Sclerotinia sclerotiorum* inoculated at 1 and 24 h after spraying. Flusilazole sprayed at 10 $\mu\text{g/ml}$ showed inhibitory effects on the virulence of *S. sclerotiorum* inoculated during the first 3 days after spraying. Inoculations on the 5th, 7th, and 10th day after spraying did not show any significant inhibitory or stimulatory effects on the virulence. After growing for 2 days on potato dextrose agar (PDA) amended with flusilazole at a dose range from 0.0005 to 0.25 $\mu\text{g/ml}$ as preconditioning treatments, mycelia were transferred onto PDA without fungicide and subsequent mycelial growth was slower than the nonpreconditioned control. However, after the preconditioned colonies were transferred onto PDA supplemented with flusilazole

at 0.2 $\mu\text{g/ml}$, percent stimulations of mycelia growth compared with the control had a parabolic shape across the preconditioning flusilazole concentration range. Similarly, the mycelial growth of the preconditioned mycelial plugs on PDA amended with other DMI fungicides (prochloraz or tebuconazole) also showed a typical hormetic response, whereas mycelial growth on PDA amended with carbendazim or dimethachlone was inhibited in a dose-dependent manner. Preconditioning *S. sclerotiorum* with flusilazole on rapeseed plants elicited virulence stimulations in a dose-dependent manner similar to those on mycelial growth on PDA. After disease lesions developed on rapeseed leaves sprayed with flusilazole as the preconditioning treatment were inoculated onto rapeseed plants, virulence was inhibited on leaves without fungicide or sprayed with carbendazim or dimethachlone compared with the nonpreconditioned control, whereas virulence was stimulated on leaves sprayed with flusilazole, prochloraz, or tebuconazole, and the maximum percent stimulation was 10.2%. These results will advance our understanding of hormetic effects of fungicides and of preconditioning hormesis in particular.

Due to different distributions and penetrations through crop canopies, spraying a fungicide in the field will inevitably lead to exposure of plant pathogens to sublethal doses of the applied fungicide (Flores and Garzón 2013). Exposure of pathogens to sublethal doses of a fungicide may result in increased virulence to crops, and this phenomenon belongs to a common biological concept called hormesis (Di et al. 2015). Hormesis is a kind of dose-response relationship characterized by low-dose stimulation and high-dose inhibition (Calabrese 2015a). Hormesis is a fundamental and general biology concept, and hormetic responses have been reported in a wide range of biological models and across a large variety of chemical classes (Calabrese 2015a). Up to 2011, approximately 9,000 cases of hormesis had been included in a relational retrieval hormesis database (Calabrese and Blain 2011). Qualitative and quantitative features of hormesis such as frequency, stimulation amplitude, and dose range of chemicals have been thoroughly reviewed, mainly by Calabrese (2013, 2015a,b, 2016a,b) and Calabrese and Mattson (2017). Stimulatory effects of sublethal doses of fungicides on mycelial growth and virulence of bacteria, oomycetes (Flores and Garzón 2013; Garzón et al. 2011), and fungi (Baraldi et al. 2003; Di et al. 2015, 2016a,b) have been reported. These studies primarily focused on the stimulatory effects of low doses of fungicides on plant pathogens, in vitro or in planta. However, in the field, fungicides are applied at relatively high doses, and the pathogen may arrive at the surface of crops after a period of time. The time profile of the effects of fungicides on the virulence of plant pathogens is not yet clear and, thus, merits studies.

Preconditioning is a specific type of hormesis (Calabrese 2016a,b). Preconditioning hormesis refers to the phenomenon where early exposure to low to moderate doses of a toxicant or stress will lower the damage caused by subsequent relatively high doses of related or unrelated toxicants or stresses. Fungicide preconditioning has significant implications for plant pathogen management strategies. In the field, plant pathogens exposed to moderate doses of a fungicide may be partially inhibited but not completely eradicated. The inhibited (i.e., preconditioned) spores and mycelia will infect crop plants some time later, and the effects of earlier fungicide exposure on later virulence are not clear. In addition, a fungicide is usually sprayed twice with a time interval of about 2 weeks in a growing season. It is highly possible that a pathogen may have been preconditioned by the first application when exposed to the second spraying. Although preconditioning hormesis has been extensively studied and reviewed recently (Calabrese 2016a,b; Calabrese et al. 2007), to the best of our knowledge, no systematic studies have been reported thus far on the quantitative features of fungicide preconditioning. *Sclerotinia sclerotiorum* is a devastating necrotrophic plant-pathogenic fungus with a wide host range and it often causes heavy yield losses to many economically important crops (Boland and Hall 1994; Bolton et al. 2006; USDA 2016; Wang et al. 2014). The sterol demethylation inhibitor (DMI) fungicide flusilazole is a broad-spectrum fungicide with high preventative and curative efficacies against *S. sclerotiorum* (Lu et al. 2015), and stimulatory effects of low doses of flusilazole on the virulence of *S. sclerotiorum* have been reported (Lu et al. 2018). The objectives of the present study were to (i) assess the effects of different concentrations of flusilazole on the virulence of *S. sclerotiorum* mycelial plugs inoculated at different time points after spraying the fungicide, (ii) evaluate preconditioning hormetic effects of flusilazole on mycelial growth of *S. sclerotiorum* on potato dextrose agar (PDA), and (iii) determine preconditioning hormetic effects of flusilazole on the virulence of *S. sclerotiorum* on rapeseed plants.

Materials and Methods

Collection and preservation of *S. sclerotiorum* isolates. Two *S. sclerotiorum* isolates, HN-24 and GS-7, which had been used in previous studies on toxic and hormetic effects of flusilazole, were collected in 2012 from rapeseed fields of Hunan and Gansu provinces, respectively

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Hormetic Effects of Carbendazim on the Virulence of *Botrytis cinerea*

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Abstract

The ascomycete plant-pathogenic fungus *Botrytis cinerea* infects more than 1,400 plant species worldwide. Stimulatory effects of sublethal doses of fungicides on plant pathogens are of close relevance to disease management. In the present study, stimulatory effects of carbendazim on the virulence of *B. cinerea* to cucumber plants were investigated. Spraying carbendazim on cucumber plants at 3 to 200 µg/ml had stimulatory effects on the virulence of carbendazim-resistant isolates of *B. cinerea* and the maximum percent stimulations were 16.7 and 13.5% for isolates HBtom451 and HBstr491, respectively. Preconditioned mycelia (i.e., mycelia grown on potato dextrose agar [PDA] amended with carbendazim at concentrations of 10, 50, or 200 µg/ml) also showed increased virulence, and the maximum percent stimulations

for isolates HBtom451 and HBstr491 were 7.9 and 9.5%, respectively. Compared with mycelia grown on PDA without carbendazim, virulence stimulation magnitudes of spraying carbendazim on leaves increased moderately but the concentrations of carbendazim that elicited the maximum stimulation increased 20- and 8-fold for preconditioned isolates HBtom451 and HBstr491, respectively. The time course of infection indicated that virulence stimulation was mediated by a direct stimulation mechanism. Studies of the physiological mechanism for stimulation demonstrated that carbendazim had no significant effects on tolerance to hydrogen peroxide, or on oxalic acid production in *B. cinerea*. These studies will deepen our understanding of quantitative features of hormetic effects of sublethal doses of fungicides on plant pathogens.

The ascomycete fungus *Botrytis cinerea* Pers. is a necrotrophic plant pathogen notorious for its extremely broad host range. *B. cinerea* can infect 586 genera, including more than 1,400 plant species worldwide (Elad et al. 2015; Van Kan et al. 2017). *B. cinerea* mainly infects dicotyledonous plants, many of which are economically important fruit and vegetable crops. Pre- and postharvest losses caused by this pathogen are usually enormous due to its wide host range, huge amounts of conidia, highly resistant sclerotia as a survival structure, and exquisite infection tactics (Amselem et al. 2011; Williamson et al. 2007). No crop cultivar with complete resistance to this pathogen has been reported to date. Due to limited gene resources for plant resistance, fungicides remain a primary approach to controlling *B. cinerea* (Mbengue et al. 2016; Nakajima and Akutsu 2014). Fungicides used for control of *Botrytis* and related genera such as *Sclerotinia* and *Monilinia* account for about 8% of the global fungicide market (Fillinger and Walker 2015). The benzimidazole fungicide carbendazim has been widely employed in China to control a variety of fungal plant pathogens since the 1970s. Extensive and repeated applications of single-site fungicides such as carbendazim have resulted in the emergence and development of fungicide resistance in *B. cinerea* around the world (Konstantinou et al. 2015; Rupp et al. 2017; Saito et al. 2016). Since the earliest report of carbendazim resistance in *B. cinerea* in 1971 (Bollen 1971), different levels of carbendazim resistance have been reported around the world (Liu et al. 2014; Zhang et al. 2006; Zhou and Ye 1987). In spite of extensive and high levels of resistance, carbendazim is still used in China, mainly as a partner ingredient mixed with other fungicides such as pyrimethanil and diethofencarb, to control *B. cinerea* (<http://www.chinapesticide.gov.cn/hysj/index.jhtml>). Therefore, it is of practical significance to investigate stimulatory effects of sublethal doses of carbendazim on *B. cinerea*.

Plant pathogens will be inevitably exposed to sublethal doses of a fungicide in the field due to drift of spray droplets, the decay of the active ingredient, and differential penetration through crop canopies.

Fungicide applied at label-recommended concentrations for functionally sensitive pathogens will become sublethal to the resistant strains (Garzón and Flores 2013). Sublethal doses of fungicide may increase rather than decrease mycelial growth and pathogenicity of plant pathogens. The high-dose inhibitory and low-dose stimulatory effects of fungicide on plant pathogens conform well to a more general biological concept called hormesis (Di et al. 2015; Zhou et al. 2014). A hormesis database has been established and approximately 9,000 hormetic dose-response cases had been included up to 2011 (Calabrese and Blain 2011). Quantitative features and mechanisms of hormesis have been extensively studied and reviewed (Calabrese 2013, 2015a,b, 2016a,b).

With respect to fungicide hormesis, numerous stimulatory responses to sublethal doses of fungicides have been reported such as stimulation of mycelial growth of *Pythium aphanidermatum* by low doses of propamocarb and cyazofamid (Flores and Garzón 2013), mycelial growth and virulence of *P. aphanidermatum* by mfenoxam (Garzón et al. 2011; Moorman and Kim 2004), mycelial growth of *Phytophthora infestans* by metalaxyl (Zhang et al. 1997), spore germination of *Penicillium expansum* by thiabendazole (Baraldi et al. 2003), and mycelial growth of *Lyophyllum palustre* by propamocarb (Landry et al. 2011). Sublethal doses of prothioconazole increase production of the mycotoxin deoxynivalenol in *Fusarium graminearum* (Audenaert et al. 2010). Our laboratory reported stimulatory effects of dimethachlone (Zhou et al. 2014), carbendazim (Di et al. 2015, 2016b), and trifloxystrobin (Di et al. 2016a) on the pathogenicity of *Sclerotinia sclerotiorum*. Although fungicide hormesis has been reported in many plant-pathogenic fungi and oomycetes involving fungicides with different modes of action, to the best of our knowledge, no hormetic responses have been reported for the agriculturally important phytopathogenic fungus *B. cinerea*. In order to design judicious fungicide application strategies for management of *B. cinerea*, it is necessary to explore hormetic effects of fungicides on the virulence of this notorious pathogen. Tolerance to hydrogen peroxide (H₂O₂) may increase the virulence of a pathogen because host plants release reactive oxygen species as an early defense response to initial pathogen challenges (Williams et al. 2011). *B. cinerea* produces oxalic acid both in vitro and in planta. Oxalic acid is an important cofactor in pathogenesis and can stimulate cell-wall-degrading enzymes such as polygalacturonase (Williamson et al. 2007). Investigating effects of sublethal doses of carbendazim on oxalic acid production and tolerance of *B. cinerea* to H₂O₂ may shed light on mechanisms of virulence stimulation. The objectives of the present study were to (i) assess stimulatory effects of sublethal doses of carbendazim on the virulence of *B. cinerea* and (ii) explore potential hormetic mechanisms for virulence stimulation.

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Baseline sensitivity and toxic actions of boscalid against *Sclerotinia sclerotiorum*

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ABSTRACT

Baseline sensitivity of *Sclerotinia sclerotiorum* to the succinate dehydrogenase inhibitor (SDHI) fungicide boscalid was established by determining the effective concentration causing mycelial growth inhibition by 50% (EC_{50}) values. The mean EC_{50} values of 76 isolates collected in 2008 and 77 isolates collected in 2014 were 0.0383 and 0.0395 $\mu\text{g/mL}$, respectively. There was no significant difference ($P = 0.695$) in boscalid sensitivity between the two years, and the frequency distribution of EC_{50} values was unimodal, but right-skewed. Correlation analysis showed that there was a statistically significant positive correlation ($R^2 = 0.856$, $P < 0.001$) of sensitivity between boscalid and thifluzamide, but no significant correlation ($R^2 = 0.01$, $P = 0.650$) was detected between boscalid and the demethylation inhibitor (DMI) fungicide prochloraz. Both preventive and curative efficacies of boscalid were significantly higher ($P < 0.05$) than those of the reference fungicide carbendazim. Boscalid in potato dextrose agar (PDA) at 0.04–0.16 $\mu\text{g/mL}$ significantly reduced the number of sclerotia per plate, but the weight of sclerotia increased slightly. Light microscopic observations showed that boscalid in PDA caused hyphae to branch more at the tip. Transmission electron microscopy (TEM) observations showed that mitochondria were deformed for the mycelia grown on boscalid-amended PDA. Boscalid at 0.01 or 0.05 $\mu\text{g/mL}$ had no significant effect on mycelial cell membrane permeability of *S. sclerotiorum*. These results will deepen our understanding of the toxic action of boscalid, and the baseline sensitivity can be used as a reference point in future boscalid resistance monitoring programs.

1. Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a worldwide plant pathogenic fungus, which causes disease on more than 400 plant species. The host range includes many economically important crops and vegetables such as oilseed rape, soybean, chickpea, peanut, dry pea, and sunflower (Boland and Hall, 1994; Bolton et al., 2006). *Sclerotinia* stem rot (SSR) caused by *S. sclerotiorum* is a devastating disease of oilseed rape in many countries, including the USA (Bolton et al., 2006), Australia (Letham et al., 1976), Canada (Bardin and Huang, 2001), and China (Ma et al., 2009; Wang et al., 2014). Annual rapeseed yield losses caused by SSR range from 10 to 20% in China, and may be up to 80% during severe SSR outbreaks seasons (Li et al., 2006). Leaves, stems, and pods of rapeseed can be infected. *S. sclerotiorum* produces sclerotia in adverse environments, and sclerotia can remain viable in the soil for up to 8 years (Bolton et al., 2006), thus making crop rotation problematic as a control strategy.

Control of SSR is usually difficult due to the lack of resistant cultivars. Chemical control is still the primary method for managing SSR of

rapeseed (Wang et al., 2014). The benzimidazole fungicide carbendazim has been widely used to control SSR since the early 1980s in China. Due to repeated applications, carbendazim resistance has been frequently reported since the middle 1990s (Pan, 1998; Shi et al., 2000; Zhang et al., 2003; Wang et al., 2014; Zhu et al., 2016). The dicarboximide fungicide dimethachlone was registered in China around the year 2000 to control SSR. After several years of applications, reduced dimethachlone sensitivity in *S. sclerotiorum* was reported in 2009 (Ma et al., 2009) and field resistance was reported in 2014 (Zhou et al., 2014a). Therefore, introducing alternative fungicides with different modes of action is necessary for control of this devastating pathogen.

Boscalid is a carboxamide fungicide, which binds to the ubiquinone reduction site of complex II of the mitochondrial electron transport chain to suppress the activity of succinate dehydrogenase (SDH), thereby inhibiting fungal respiration (Avenot and Michailides, 2010). Owing to its unique mode of action (MoA), boscalid is an effective fungicide for control of a variety of plant diseases such as powdery mildew, leaf spot, grey mold, and stem rot. However, site-specific MoA implies a high risk for resistance development (Brent and Hollomon,

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基于介孔二氧化硅纳米粒子的农药可控释放研究进展

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摘 要: 鉴于介孔二氧化硅纳米粒子 (mesoporous silica nanoparticles, MSNs) 具有比表面积大、孔径可调节、孔道均匀、内外表面易于修饰和生物相容性好等优点, 其在药物控释方面的应用已成为当前国内外研究的热点。本文综述了介孔二氧化硅纳米粒子的制备方法(软模板法、硬模板法和自模板法), 表征技术[扫描电镜分析 (SEM)、透射电镜分析 (TEM)、X 射线衍射分析 (XRD)、物理吸附分析、热重和差热分析 (TGA-DTA) 和傅里叶红外光谱分析 (FT-IR)] 及其在农药领域的研究应用状况, 探讨了以介孔二氧化硅纳米粒子作为农药载体时存在的问题, 并对其应用前景进行了展望。

关键词: 介孔二氧化硅; 纳米粒子; 农药控释; 制备方法; 表征技术

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Recent progress in the application of mesoporous silica nanoparticles to controlled pesticides delivery system

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Abstract: Research and development of mesoporous silica nanoparticles (MSNs) has gained worldwide interest due to its unique properties such as biocompatibility, large surface area, tunable pore size and facile surface functionalization. This review discussed the research progress of the preparation methods of MSNs (soft-template method, hard-template method and self-templating method), characterization techniques (transmission electron microscopy, TEM; scanning electron microscopy, SEM; X-ray diffraction, XRD; physical adsorption analysis; thermal gravimetric analysis and differential thermal analysis, TGA-DTA; fourier-transform infrared spectroscopy, FT-IR) and their application in the field of pesticide science. Potential problems of employing MSNs as pesticides controlled release carriers and its prospective were also discussed.

Keywords: mesoporous silica; nanoparticles; pesticides controlled release; preparation methods; characterization techniques

2014 年, 中国的化学农药生产量达 370 万吨, 使用量达 180 万吨^[1], 其中主要是乳油、可湿性粉剂和水乳剂等常规农药剂型, 存在大量使用有机溶剂、粉尘飘移、分散性差等问题, 并且

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不同温度下白背飞虱对环氧虫啉的敏感性

任志杰, 毛凯凯, 李朋岳, 刘超亚, 王 越, 李建洪, 万 虎, 何 顺*

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摘 要: 为明确不同温度条件下白背飞虱对环氧虫啉的敏感性, 采用稻苗浸渍法测定了在 18~32 °C 下环氧虫啉对白背飞虱的毒力、白背飞虱蜜露量以及酯酶 (EST)、细胞色素 P450 单加氧酶 (P450) 和谷胱甘肽 S-转移酶 (GSTs) 3 种解毒酶的比活力。结果显示: 环氧虫啉对白背飞虱的毒力随温度升高而提高, 32 °C 下对白背飞虱的 LC_{50} 值是 18 °C 下毒力的 15.6 倍, 表明环氧虫啉对白背飞虱表现为正温度系数; 温度影响白背飞虱的取食量, 随温度升高, 其蜜露排泄量显著增加; EST 和 P450 比活力随温度变化而改变, 其中 EST 比活力随温度升高呈先升高后降低的“钟形”变化趋势, P450 随温度升高呈下降趋势, 且处理间具有显著性差异, 而 GSTs 随温度变化各处理间无显著差异。以上结果表明, 取食量和解毒酶 (EST 和 P450) 可能是导致不同温度条件下白背飞虱对环氧虫啉敏感性差异的主要因素。

关键词: 温度; 白背飞虱; 环氧虫啉; 取食量; 敏感性; 解毒酶; 蜜露

中图分类号: S482.3; TQ450.2

文献标志码: A

文章编号: 1008-7303(2018)04-0439-06

Sensitivity of *Sogatella furcifera* to cycloxyprid at different temperatures

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Abstract: In order to clarify the sensitivity of *Sogatella furcifera* to cycloxyprid at different temperatures, the toxicity of cycloxyprid to *S. furcifera* at different temperatures from 18 °C to 32 °C was determined by leaf dipping method. The effects of temperatures on the secretion of honeydew and detoxification enzymes (EST, P450 and GSTs) were also detected. The results showed that cycloxyprid was a positive temperature coefficient insecticide. The toxicity of cycloxyprid against *S. furcifera* increased with temperature. The toxicity of cycloxyprid against *S. furcifera* at 32 °C was 15.6 times as high as that at 18 °C. The feeding capacity and the amount of honeydew excretion increased with the increase of temperature. The activities of EST and P450 of *S. furcifera* also changed with temperature. With the elevation of temperature, the activity of EST increased first and then decreased. The P450 enzyme activity decreased at higher temperature. However, the activity of GSTs has no significant difference under different temperatures. The susceptibility variation of *S. furcifera* to cycloxyprid under different temperatures is closely related to its feeding capacity and detoxification activity (EST and

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樊钰虎 薛 霏

三、授权发明专利

证书号第 3243215 号



发 明 专 利 证 书

发 明 名 称：一种纳米二氧化硅接枝有机功能高分子有机磷杀虫剂缓释剂及其制备方法

发 明 人：何顺;李建洪;万虎;马洪菊;游红;张小磊;高云昊
沙黑拉什·拉马赞

专 利 号：ZL 2016 1 0191275.1

专利申请日：2016 年 03 月 30 日

专 利 权 人：华中农业大学

地 址：430070 湖北省武汉市洪山区狮子山街一号华中农业大学

授权公告日：2019 年 02 月 05 日

授权公告号：CN 105831113 B

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其他事项参见背面

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发明人：

何顺；李建洪；万虎；马洪菊；游红；张小磊；高云昊；沙黑拉什·拉马赞

证书号第 3245635 号



发 明 专 利 证 书

发 明 名 称：纳米二氧化硅接枝有机功能高分子大环内酯类杀虫剂缓释剂及其制备方法

发 明 人：何顺;李建洪;万虎;马洪菊;高云昊;沙黑拉什·拉马赞
张小磊

专 利 号：ZL 2016 1 0191303.5

专利申请日：2016 年 03 月 30 日

专 利 权 人：华中农业大学

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其他事项参见背面

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申请人：

华中农业大学

发明人：

何顺；李建洪；万虎；马洪菊；高云昊；沙黑拉什·拉马赞；张小磊

证书号第 3245636 号



发 明 专 利 证 书

发 明 名 称：一种纳米二氧化硅接枝有机功能高分子双酰胺类杀虫剂缓释剂及其制备方法

发 明 人：何顺;李建洪;万虎;游红;高云昊;张小磊;廖逊

专 利 号：ZL 2016 1 0192026.X

专利申请日：2016 年 03 月 30 日

专 利 权 人：华中农业大学

地 址：430070 湖北省武汉市洪山区狮子山街一号华中农业大学

授权公告日：2019 年 02 月 05 日

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其他事项参见背面

证书号第 3245636 号



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申请日时本专利记载的申请人、发明人信息如下：

申请人：

华中农业大学

发明人：

何顺；李建洪；万虎；游红；高云昊；张小磊；廖逊

证书号第3156456号



发明专利证书

发明名称：一种复配杀菌剂及制备方法

发明人：朱福兴；马洪菊；万虎；何顺；马伟华；杨中华

专利号：ZL 2016 1 0807377.7

专利申请日：2016年09月07日

专利权人：华中农业大学

地址：430070 湖北省武汉市洪山区狮子山街一号

授权公告日：2018年11月20日

授权公告号：CN 106472503 B

本发明经过本局依照中华人民共和国专利法进行审查，决定授予专利权，颁发本证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。

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专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



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四、科研项目

国家自然科学基金资助项目批准通知

何顺 先生/女士：

根据《国家自然科学基金条例》和专家评审意见，国家自然科学基金委员会（以下简称自然科学基金委）决定批准资助您的申请项目。项目批准号：31972302，项目名称：纳米载体高效递送dsRNA系统的构建及毒理学效应研究，直接费用：60.00万元，项目起止年月：2020年01月至2023年12月，有关项目的评审意见及修改意见附后。

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- 2、提交电子修改版计划书截止时间为**2019年9月18日16点**；
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附件：项目评审意见及修改意见表

国家自然科学基金委员会
2019年8月16日

关于国家自然科学基金资助项目批准及有关事项的通知

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31601654，项目名称：多功能氯虫苯甲酰胺纳米缓释剂的制备及生物活性研究，直接费用：20.00万元，项目起止年月：2017年01月至2019年12月，有关项目的评审意见及修改意见附后。

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附件：项目评审意见及修改意见

国家自然科学基金委员会
生命科学部
2016年8月17日

子课题编号： 2016YFD0200506-8

国家重点研发计划 子课题任务书

子课题名称：蔬菜害虫化学农药协同增效技术与产品研发

子课题承担单位（丙方）：华中农业大学

子课题负责人：何顺

所属课题：蔬菜化学农药协同增效技术及产品研发

课题主持单位（乙方）：中国农业科学院蔬菜花卉研究所

所属项目：化学农药协同增效关键技术及产品研发

项目牵头单位（甲方）中国农业科学院植物保护研究所

执行期限：2016年01至2020年12月

中国农业科学院植物保护研究所


2016年9月25日

七、合同签约方

甲方：中国农业科学院植物保护研究所（公章）

负责人（签章）：  2016年11月21日

首席专家：（签字）  2016年11月11日

财务部门（公章） 负责人（签章）：  2016年11月11日

联系人：张燕宇 联系电话：010-62815616, 18611346668

乙方：中国农业科学院蔬菜花卉研究所（公章）

负责人：（签章）  2016年11月11日

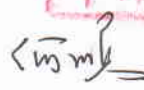
财务部门（公章） 负责人：（签章）  2016年11月11日

课题主持人：（签字）  2016年11月17日

丙方：

负责人：（签章）  2016年10月25日

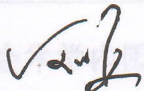
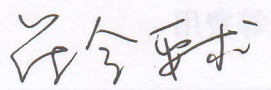
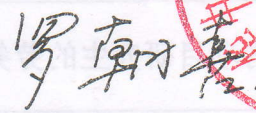



财务部门（公章） 负责人：（签章）  2016年10月25日

子课题负责人：（签字）  2016年10月25日

2019 年华中农业大学自主科技创新基金项目任务书

项目名称	层状双氢氧化物递送靶向二化螟 dsRNA 对宿主生长发育影响			负责人	何顺
项目类别	高水平科研论文培育专项			项目批准号	2662019PY052
考核期	2019 年 1 月-2021 年 12 月			资助金额 (万元)	24
学院	植科	学位	博士	职称	副教授

经费 预 算 表	科 目	金额 (万元)	计算依据与说明 (不能为空, 每栏限 100 字)
	1. 设备费	0.00	已有设备能够满足实验需求
	(1) 设备购置费	0.00	无
	(2) 设备试制费	0.00	无
	(3) 设备升级改造与租赁费	0.00	无
	2. 实验材料费	7.00	购买纳米材料合成和基因克隆等试剂和耗材
	(1) 原材料/试剂/药品购置费	7.00	购买纳米材料合成和基因克隆等试剂和耗材
	(2) 其他 (无特殊情况原则上不列支) **	0.00	无
	3. 测试化验加工费	2.50	纳米材料表征
	4. 差旅/会议/国际合作与交流费	4.50	5 人次参加国内学术会议
	5. 出版/文献/信息传播/知识产权事务费	1.00	论文发表及专利申请等费用
	6. 劳务费	8.00	支付参与项目研究生的劳务费
	7. 专家咨询费	1.00	项目专家咨询等费用
	8. 其他支出	0.00	无
	合 计	24.00	

<p>预期研究目标 (含具体科学问题、论文、专利、奖励、应用推广指标、争取国家级科研项目等)</p>	<p>(请对照申报要求中各类项目培育目标逐条列出。应具体、明确、详细可行、可考核。每栏限 200 字。)</p> <p>1: 拟解决的科学问题: LDHs-dsRNA 如何增强 RNA 干扰效率。</p> <p>2: 拟发表文章情况: 发表 Top 5% SCI 论文 1-2 篇。</p> <p>3: 拟获得专利、奖励、应用推广指标等: 无。</p> <p>4: 团队和人才目标: 培养硕士研究生 1-2 名。</p> <p>5: 争取国家级科研项目目标: 无。</p> <p>6: 其他: 无。</p>
<p>项目主持人承诺</p>	<p>我承诺:</p> <ol style="list-style-type: none"> 1. 严格按照《华中农业大学自主科技创新基金管理办法》及《任务书》中的研究内容和目标, 认真开展研究工作(科研原始记录将作为项目考核的重要内容, 请注意规范保存); 2. 若作为人才、团队、科技奖励培育类项目负责人, 将每年坚持申报对应的国家或省部级项目或奖励; 3. 按照经费预算及相关规定, 及时合理的使用经费, 当年拨付的经费在当年 12 月 20 日前执行完毕; 4. 严格按《中央高校基本科研业务费专项资金管理暂行办法》(财教[2016]277 号)文件规定的范围开支经费(开支范围主要包括: 材料费、测试化验加工费、设备费、差旅费、会议费、国际合作与交流费、出版/文献/信息传播/知识产权事务费、劳务费、专家咨询费等。经费不得用于: 购置单件≥40 万元的大型仪器设备, 开支有工资性收入的人员工资、奖金、津补贴和福利支出, 支付招待费、罚款、捐赠、赞助、投资等); 5. 在研究成果中标注“中央高校基本科研业务费专项资金资助项目(项目批准号: XXX)”或“Project XXX supported by the Fundamental Research Funds for the Central Universities”。 <p>项目主持人(签名):  2019 年 5 月 9 日</p>
<p>所在单位审核意见</p>	<p>请审核项目的研究任务和目标是否具体、明确、详细可行、可考核。</p> <p> 审核人(签名):  单位(公章):  2019 年 5 月 9 日</p>
<p>学校审核意见</p>	<p>审核人(签名):  单位(公章):  2019 年 5 月 10 日</p>

注: 请用 A4 纸打印, 盖章页请作为封底页打印。

项目编号: 2016ABA104

湖北省技术创新专项任务书 (重大项目)

项目名称 双酰胺类杀虫剂缓释剂的制备及生物活性研究

承担单位 华中农业大学

项目负责人 李建洪

起止年限 2016-07-01 至 2019-06-30

湖北省科学技术厅

四、承担单位、参加单位、主要参加人员及联系人

项目承担单位：华中农业大学									
项目参加单位：湖北省植物保护总站；									
项目负责人									
姓名	性别	出生年月	职务/职称	学历	为本项目工作时间(月/年)	在本项目中承担的主要工作	所在单位	办公电话	手机
李建洪	男	1964-02-15	教授	研究生	4	主持人	华中农业大学	027-87286968	13554628625
主要参加人员									
姓名	性别	出生年月	职务/职称	学历	为本项目工作时间(月/年)	在本项目中承担的主要工作	所在单位		
罗汉钢	男	1961-05-06	推广研究员	研究生	4	推广示范	湖北省植物保护总站		
何顺	男	1987-02-01	讲师	研究生	4	缓释剂的制备及表征	华中农业大学		
万虎	男	1984-10-29	讲师	研究生	4	生物活性测定	华中农业大学		
谢原利	男	1982-07-05	农艺师	研究生	4	推广示范	湖北省植物保护总站		
张小磊	男	1986-05-06	博士研究生	研究生	8	缓释剂的制备及表征	华中农业大学		
廖逊	男	1989-09-29	博士研究生	研究生	8	生物活性测定	华中农业大学		
毛凯凯	男	1989-08-31	博士研究生	研究生	8	生物活性测定	华中农业大学		

八、签订任务书各方意见

甲方（湖北省科学技术厅）：

主管处室负责人（签字）

项目管理责任人（签字）



乙方（项目承担单位）：

法定代表人（签字）

项目负责人（签字）



丙方（项目组织/推荐单位）：

法定代表人（签字）

项目管理责任人（签字）



国家重点研发计划 子课题任务书

子课题名称：农药对靶剂量传输调控指标与粮食等作物活性
关系

子课题编号：2017YFD020030803

子课题承担单位：华中农业大学

子课题负责人：杨中华

所属课题：农药对靶剂量传输调控指标与活性关系

课题编号：2017YFD0200308

课题承担单位：贵州大学

所属项目：化学农药对靶高效传递与沉积机制及调控

项目牵头承担单位：中国农业科学院植物保护研究所

执行年限：2017 年 7 月-2020 年 12 月

二〇一七年十月

五、参加单位及主要研究人员

子课题承担单位： 华中农业大学						
子课题负责人						
姓 名	性别	年龄	职务职称	业务专业	为本课题工作 时间（%）	所在单位
杨中华	男	32	副研究员	农药学	40	华中农业大学
主要研究人员						
何顺	男	30	讲师	农药学	40	华中农业大学
赵越	女	24	硕士生	农药学	80	华中农业大学
熊亚兵	男	24	硕士生	农药学	80	华中农业大学
张园	女	23	硕士生	农药学	80	华中农业大学
秦雪莹	女	22	硕士生	农药学	80	华中农业大学

六、子课题任务书签署

项目牵头承担单位：中国农业科学院植物保护研究所（公章）

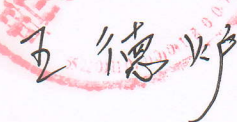
项目负责人（签字）：



2017 年 12 月 1 日

课题承担单位：贵州大学（公章）

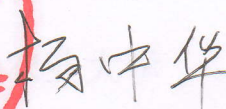
课题负责人（签字）：



2017 年 11 月 28 日

子课题承担单位：华中农业大学（公章）

子课题负责人（签字）：



2017 年 11 月 24 日



项目编号：2019020701011424
计划类别：应用基础前沿专项
管理处室：农村与社会发展处

武汉市科技计划项目任务书

项目承担单位：华中农业大学
项目名称：靶向蔬菜病虫害纳米载药体系的构建与应用
项目负责人：李建洪
联系电话：13554628625
项目编号：2019020701011424
下达文号：武科〔2019〕39号
起止年限：2019年7月至2021年12月

武汉市科学技术局
二〇一九年制

四、项目主要研究人员

项目负责人					
姓名	身份证号	所在单位	职务职称	现从事专业	手机
李建洪	430111196402150418	华中农业大学	教授	农药学	13554628625
项目组主要成员					
姓名	身份证号	所在单位	职务职称	现从事专业	联系方式
何顺	430681198702011773	华中农业大学	副教授	农药学	
万虎	360426198410294018	华中农业大学	副教授	农药学	
朱福兴	37292819700424361X	华中农业大学	副教授	农药学	
毛凯凯	429006198908310617	华中农业大学	博士研究生	农药学	
李钊	370181199003112453	华中农业大学	博士研究生	农药学	
李东林	413026199511012710	华中农业大学	硕士研究生	农药学	

五、教学奖励



荣誉证书

何顺老师:

荣获植物科技学院第十三届青年教师讲课竞赛

二等奖。

特发此证,以资鼓励。

华中农业大学植物科技学院

二〇一七年六月



六、其他材料

■大话农科

科技兴农 数据赋能

■本报记者 李晨

“种子是农业的芯片,农田则是农业的母版。”在中国科学院院士周成虎看来,中国农业的智慧大脑正在形成。在已可通过遥感和地面观测确认地球上有着 3.04 万亿棵树的今天,发展新技术,实现对每一块农田的精准测量和动态监测,构建地块级农田大数据,促进智慧农业科技创新发展,已经触手可及。

近日,在“2019 年智慧农业科技创新研讨会暨示范观摩会”上,与会专家实地观摩考察,并围绕“科技兴农、数据赋能”的主题深入探讨了我国智慧农业发展的现状和前景。

“天空地海网”动态立体时代

周成虎指出,当前,世界进入了智能化与绿色化、网络化、全球化相互交织的时期。把握时代的脉搏,抓住历史性机遇,发展现代农业信息事业,是智慧农业科技创新发展的重要内容,具有战略意义重大。

“农业信息的获取进入‘天空地海网’动态立体时代。”周成虎说,亿级互联的地面传感网,获取了海量的农情数据;高低轨、光学与微波组合的综合对地系统,组成全球覆盖的农情观测天网。在一切都可以数据化的世界,在一切数据都可以业务化的时代,农业信息化、农业大数据成为现代农业科技的核心组成部分。

农业遥感传统上服务于农作物生长状态监测、农作物种植面积监测与估算、农作物单产监测、灾害监测及损失评估、农作物产量估算、粮食供需平衡与安全预警等。随着物联网、云计算、大数据、深度学习等技术的不断进步,国际上已经开始将农业遥感技术应用于生态农业、订单农业、绿色农业的快速发展;我国也将农业遥感的服务范围扩展到承包地确权登记、耕地质量监测与保育,以及农业补贴支撑等方面。

“高空间分辨率影像为农田地块的精确测量提供了可能。”周成虎



史云供图

说,借助于现代航天航空技术,可以精确划定地块边界、精确监测播种面积,了解每一地块的内部细节。

这样的精准农业航空技术,被华南农业大学教授兰玉彬视作实现智慧农业的重要组成部分,是智慧农业的直接体现。

兰玉彬认为,“智慧农业”与现代生物技术、农艺技术等高新技术的融合,对我国赶超发达国家,建设世界水平农业具有重要意义。“生态无人农场”应运而生。

“生态无人农场”整合农艺和农机装备、绿色植保技术、无人机、人工智能、大数据、3S、物联网等技术;采用天空地一体化技术获取农情信息,实现农业信息的精准感知;使用地空一体化智能农机装备等协同作业,提高农业生产率;实现绿色生态农业生产的精准化种植、智能决策、可视化管理和智能化操控。

“生态无人农场”通过一系列技术实现循环农业模式,包括基于精准施药的农药减量技术、基于水肥一体化精准管理的减肥节水技术、生态沃土技术、生物防治技术、秸秆综合再利用技术、畜禽粪便有机化处理与施用技术、农场闭环优化管理技术。

“生态无人农场”融合生物防控、绿色植保、无人机、农业机器人、人工智能、物联网、大数据、云计算等众多

高新技术。“以后的农民不再是体力劳动者,而是新农民。一个人管理整个农场的目标将会成为现实。生态无人农场是农业产业变革的第一步。该领域的全球化竞争刚刚开始。”兰玉彬说。

助力农业“知天而作”

在成都市新都区泰兴镇四川省农业科学院新都现代农业科技创新示范园内,数百名专家聚在一块刚刚收割小麦后平整的土地上,目不转睛地看着屏幕上的现场直播。

农情无人机起飞,电脑屏幕上实时显示它的飞行轨迹。当无人机的飞行轨迹逐渐覆盖整个目标地块后,科研人员开始根据无人机传回的数据解析地块信息,了解果园面积、地形,果树数量、位置、树冠大小、长势,以及杂草分布等生产信息。

农情无人机起飞,电脑屏幕上实时显示它的飞行轨迹。当无人机的飞行轨迹逐渐覆盖整个目标地块后,科研人员开始根据无人机传回的数据解析地块信息,了解果园面积、地形,果树数量、位置、树冠大小、长势,以及杂草分布等生产信息。

从天上看到的信息还不够精确,每棵果树有多少果实,果实成熟度、大小、有没有病虫害等更加精细的信息该如何获取呢?这时候,“果园侦察兵”智能巡田机器人出动了,它能代替人类走进果园感受作物的细微变化。

通过智能设备收集的数据很快传递到“智慧农业大脑”——农业大数据挖掘与服务平台中。这个“大脑”包括天空地一体化农情信息处理一体机、

智慧农业大数据挖掘与可视化系统、云边端一体化田间服务一体机,具有数据管理与可视化云边端协同计算的深度人工智能算力。它经过智能分析判断,向果园智能作业装备发出正确的操作指令。

例如,水肥一体化灌溉系统接收到处方图,对水肥精准控制,按需智能化灌溉,省钱省时省力。

一台红色的无人喷药机器人根据处方图走起来了,在有病虫害发生的地方,它停下喷药,既可以提升农药利用效率,又能避免作业人员农药中毒情况发生。

无人除草机器人马力强大,就算是一棵小灌木挡在它面前,它也能毫不犹豫地碾压过去,迅速将其粉碎。在它身后,什么杂草也没留下。

中国农业科学院农业资源与农业区划研究所研究员史云告诉《中国科学报》,中国农科院智慧农业创新团队的这套农业云操作系统,包括天空地农情信息时空数据库系统、物联网观测系统、农业大数据多维可视化系统、农情智能诊断与监测系统、智能装备对接与管理系统、智慧农业云平台等,能助力农业生产“知天而作”。

智慧农业需要内外兼修

农业生产劳动力成本占 70%,而目前农村劳动力平均年龄 55 岁,农村老龄化持续加大。“20 年后谁来种地、种菜、养猪、养鱼?”中国农业大学信息与电气工程学院教授李道亮如是说。

面对资源节约、产出高效、环境友好、产品安全的现代农业需求,我国农业生产面临转型升级。李道亮认为,未来方向是生产装备化、装备数字化、监管网络化、管理智能化和作业无人化。

智慧农业的出现,就是要“让人类能以更加精细和动态的方式管理农业生产和生活,提升人对农业物理世界实时控制和精确管理能力,从而实现农业的资源优化配置和科

■环球农业

获奖的葡萄酒有点甜

获奖的葡萄酒往往成分更复杂,最好的葡萄酒则含有高浓度的乙醇和糖。这是美国华盛顿州立大学与葡萄牙里斯本大学科学家的最新发现。该研究论文近日发表在《葡萄酒杂志》上。

一款葡萄酒获得大奖会对其市场营销产生巨大影响,因此著名的国际葡萄酒赛事竞争都是比较激烈的。无论是科学家还是生产商,他们都想知道在国际葡萄酒比赛中获得最高奖项的葡萄酒普遍存在的特征。

为了找出答案,研究人员分析了在德国举办的 MUNDUS VINI 世界葡萄酒大赛的几年数据,该赛事每年举办两次。如 2018 年 MUNDUS VINI 春季品酒会共有来自全世界 150 多个葡萄酒产区的 6770 款葡萄酒参赛。

研究人员采用聚类分析和 Logistic 回归分析,对葡萄酒的化学和感官属性加以细化,以确定大金奖和金奖的青睐对象。分析表明,大型葡萄酒挑战赛倾向于选择乙醇和糖含量高的葡萄酒。

论文作者、华盛顿州立大学食品科学学院教授 Carolyn Ross 表示,这项研究

帮助科学家找到了获奖葡萄酒与评委重视程度最相关的感官特征,与所谓的国际商业葡萄酒风格大体一致。

通常,与甜味相关的口感,包括白葡萄酒中的奇异水果、红葡萄酒中的干果和辣味,增加了葡萄酒的获奖机会。相反,带有酸度和涩味的白葡萄酒、植物性或红色浆果的红葡萄酒往往得不到最高奖项。

但是,仅仅使葡萄酒更甜或者更少植物性,可能也不会获奖。

此前已有科学家研究了诸如获奖葡萄酒的 pH 水平或酸度等因素,但是葡萄酒特征的复杂性使得结果很难简单量化。新的数据分类帮助科学家和生产商确定了更具体的特征。

“定义葡萄酒的复杂性和和谐感不是一件容易的事,仅有数据是不够的。”Ross 说,“根据数据,你觉得添加更多的



图片来源: Ralf Ziegler, AdLumina/ MUNDUS VINI 大赛官网

果味或辛辣,可能就会获奖。但这可能会对葡萄酒更广泛的属性产生影响,反而酿造不出一款在大赛中令人印象深刻的葡萄酒。”

“获奖的重要性对葡萄酒来说不言而喻。有些人在两款不同的葡萄酒中选择,仅仅因为其中一款葡萄酒瓶上有奖品标签,就将其收入囊中。因此,这项研究对酿酒厂有着重大的积极影响。”Ross 补充说。(王方编译)

相关论文信息: <https://doi.org/10.1080/09571264.2019.1652154>

科学家首次建议科学布局 Bt 作物跨境防控迁飞害虫

本报讯 近日,中国农业科学院植物保护研究所转基因作物安全评价与管理创新团队系统分析了我国转基因抗虫作物的研发现状、应用经验,探讨了发展前景、面临的挑战及应对策略。该研究成果在线发表于《昆虫学年评》。

该项成果系统阐述了我国转基因抗虫作物的开发和应用现状,分析了 Bt

棉花商业化种植带来的生态、经济和社会效益,重点探讨了我国在 Bt 棉花靶标害虫抗性治理及非靶标害虫综合防控方面积累的重要经验。在此基础上,系统分析了转基因抗虫玉米、水稻等重大作物在我国商业化应用的前景、经济社会和生态效益及面临的挑战。

研究团队在国际上首次探讨了通过转基因抗虫作物的科学合理布局,对

重大迁飞性靶标害虫的区域性治理和跨境防控的重要意义。

论文还探讨了进一步提升我国生物技术和应用的策略,相关建议不但对我国农业生物技术的发展具有重要指导意义,对其他发展中国家也具有重要的参考价值。(刘明娜)

相关论文信息: <https://doi.org/10.1146/annurev-ento-011019-025039>

华中农大研究团队研制成功温度响应性纳米载药体系

本报讯 近日,《化学工程杂志》在线发表华中农业大学植物科学技术学院教授李建洪领衔的农药毒理学与有害生物抗药性研究团队的最新研究成果。

该研究团队在前期研究中发现,新烟碱类杀虫剂对褐飞虱的毒力表现为显著的正温度效应。基于该特性,研究人员利用具有正温度效应的杀虫剂噻虫嗪作为模式农药,以温度响应性聚合

物修饰的中空介孔二氧化硅纳米复合物为载体材料,巧妙设计制备了温度响应性纳米载药体系。

该体系表现出显著的温度控制释放性能和优异的光稳定性。研究表明,该体系对褐飞虱的杀虫活性与温度呈正相关,且较噻虫嗪传统剂型具有更长的持效期,可显著提高杀虫剂利用率。同时,纳米载体材料具有良好的生物相容性,对水稻十分安全。

以上研究结果可为全球变暖背景下农药的科学使用提供数据支撑,为智能型农药制剂的研发及农药高效利用提供重要参考。

(高云昊 毛凯凯)

相关论文信息: <https://doi.org/10.1016/j.pestbp.2019.02.009>

<https://doi.org/10.1016/j.cej.2019.123169>

抗病育种新途径 为手中“饭碗”保驾护航

■本报记者 黄辛

第 39 个世界粮食日前夕,上海交通大学发布的最新研究成果为解决水稻抗病性丧失问题、保障粮食安全与食品安全提供了新的思路。

该校教授陈功友领衔的植物与病原菌分子互作研究团队通过多年研究,揭示了病原菌效应蛋白这个“间谍”与植物感病基因“接头人”之间的协同进化关系,并提出利用基因编辑技术阻断两者之间的协同进化进程,从而使植物因丧失效应蛋白诱导的感病性(ETS)而获得广谱抗病(RLS)的新途径。该研究成果近日在线发表于《分子植物》。

大量筛选:发现病原菌新“间谍”

由模式生物稻黄单胞菌引起的水稻白叶枯病是水稻三大病害之一,是危害水稻生产的头号细菌“杀手”。该病在亚洲、拉丁美洲和西非数十个国家的水稻种植区广泛发生。国内目前除了新疆和东北北部地区以外,其他省市的水稻种植区均有分布。

水稻白叶枯病通常导致水稻减产 10%-20%,发生严重的地区可达 50%以上,甚至是绝收。同时,水稻白叶枯病也是植物病理学和植物免疫学领域最重要的模式系统之一,在科学上具有重要研究价值。

“植物病害发生的过程,就是病原菌与植物双方投入大量‘兵力’进行的一场没有硝烟的战争。”论文通讯作者陈功友说,为了抵御病原菌的入侵,植物通过角质、蜡质等多种生化物质构筑起一系列坚固的物理和化学“堡垒”。

而病原菌一方面通过合成胞外降解酶、毒素等物质“明修栈道”,从外部一层层瓦解这些“堡垒”;另一方面还通过组装多种被称为分泌系统的装置“暗度陈仓”,将大量效应蛋白“间谍”直接分泌到植物体“堡垒”内部。这些“间谍”在植物体内部大肆破坏,最终使“堡垒”从内部被“攻破”,植物发生病害。

近 20 年来,陈功友团队从全国各水稻产区的发病田块收集分离到数百个白叶枯病原菌株,在不同的水稻品种

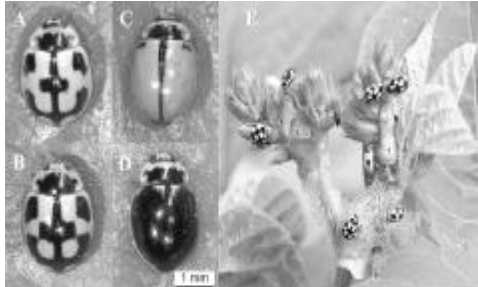
上测定了这些菌株的毒性,并进行分子标记分析。他们发现,这些中国菌株具有丰富的遗传多样性和毒性差异。研究人员通过基因组学和分子生物学工具,从其中几个强毒性菌株中分离到数十个 TALE 蛋白编码基因并分别测定毒性,最终“识破”了一类被称为 PthXo2-like 的新型主效 TALE 蛋白“间谍”,并且在不同的菌株中发现了这类蛋白的五种类型。

反复验证:确定水稻“间谍接头人”

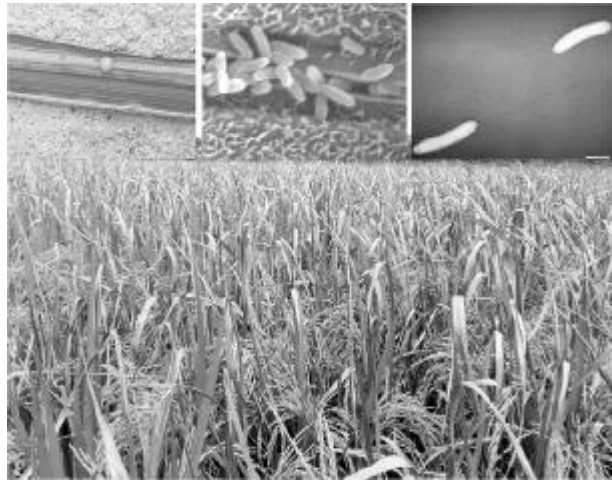
论文第一作者徐正银告诉《中国科学报》,水稻白叶枯病菌分泌的 TALE 蛋白进入植物细胞后,能够躲过植物免疫系统的侦察,伪装成水稻自身的转录激活因子,与“潜伏”在水稻细胞内的一类感病基因接头,“暗号”就是双方特定氨基酸序列与 DNA 序列之间的一套密码。一旦对上了暗号,TALE 蛋白就能够识别并结合在感病基因启动子的相应区域,激活感病基因的表达,从内部“攻破”植物的防线。

研究人员通过对新发现的“间谍”蛋白序列进行比较分析,初步确定水稻感病基因 OsSWEET13 具有“接头人”嫌疑。接下来经过不同分子生物学实验的反复验证,最终确定该基因即为该类“间谍”的靶标基因“接头人”。

同时,对 3000 份水稻种质资源基因组进行的分析发现,该基因在启动子识别区域也存在 10 种不同的类型。徐正银介绍说,这次发现的不同 PthXo2-like 类 TALE 蛋白,作用于感病基因 OsSWEET13 的不同单倍型,将病原菌与植物的“军备竞赛”认识上升到了一个新的层次。



棉花所供图



水稻白叶枯病的症状及其病原菌 上海交大供图

善用利器:建立抗病育种新途径

据该论文共同通讯作者、副教授邹丽芳介绍,通过增强水稻自身防御体系,培育广谱持久抗病水稻品种,是一种既经济又绿色的病害防控措施。

该团队研究人员根据水稻感病基因“接头人”OsSWEET 与病原细菌 TALE 蛋白“间谍”之间的关系,采用新兴的第三代基因编辑技术(CRISPR/Cas9),对水稻品种 Kitaake 的 3 个感病基因的效应蛋白识别位点进行编辑,干扰“间谍”与“接头人”相互识别。

由此获得的水稻新品系广谱抗白叶枯病,最终开辟了阻断植物感病性而获得广谱抗病性的育种新途径。

南京农业大学教授窦道龙对此给予高度评价:“该研究在理论上阐明了不通过利用抗病基因而通过编辑多个感病基因从而实现作物的广谱抗病性,为水稻白叶枯病的防治提供了新的材料和方案,为作物抗病育种和抗性丧失治理提供了成功案例,具有重要理论和实践意义。”

陈功友表示,团队目前正在将该技术应用于优良杂交水稻亲本的改良,预计 1-2 年内可育成稳定遗传的品种。这将大大缩短杂交水稻抗病育种的年限。

相关论文信息: <https://doi.org/10.1016/j.molp.2019.08.006>