

纳米技术在害虫绿色防控领域的应用与展望*

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摘 要 近些年, 纳米技术在农业领域发展迅猛, 推动了传统农业在交叉学科领域的不断发展和深化。目前已利用纳米载体实现了四个方面的功能与应用: 纳米载体携带外源 dsRNA 突破害虫体壁屏障, 调控害虫生长发育; 纳米载体携带专性病毒 DNA 毒杀非寄主害虫, 扩大病毒防治谱; 纳米载体携带 Bt 毒蛋白高效杀死非敏感害虫, 治理害虫抗药性; 纳米载体携带杀虫剂提升利用率、降低使用量、拓展防治谱。本文结合最新研究进展, 重点介绍了纳米技术在害虫绿色防控领域的研究进展和应用现状, 并对纳米技术在绿色防控领域的研究与应用作了展望。

关键词 纳米材料; RNA 干扰; Bt 毒蛋白; 核型多角体病毒; 纳米杀虫剂

Prospects for the application of nanotechnology in green pest control

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Abstract Agricultural nanotechnology has developed rapidly in recent years, promoting the gradual development of traditional agriculture as an interdisciplinary field. Nanocarriers have four functions and applications. The first is to deliver dsRNA to penetrate the body wall, thereby regulating the development and growth of pests. The second is to deliver viral DNA to kill non-host pests, thereby broadening the host-range of viruses. The third is to deliver Bt toxin to kill non-sensitive pests, which provides new possibilities for the management of pesticide resistance. The fourth is to deliver pesticides, which can increase the rate of pesticide use while at the same time, decreasing the amount of pesticides applied and broadening the range of pests under control. Based on recent researches, this paper reviews progress on the research and application of nanotechnology in green pest control, and discusses future prospects for research on, and utilization of, this technology.

Key words nanomaterial; RNA interference; Bt toxin; nuclear polyhedrosis virus; nano insecticide

近年来, 我国农作物病虫害总体处于频发、多发态势, 长期威胁着我国农业生产安全和国家粮食安全。据全国农业技术推广服务中心统计, 2006-2015 年, 我国农作物病虫害发生面积在 4.60 亿-5.08 亿 hm^2 次, 年均实际损失粮食 1 965.49 万吨, 年均挽回粮食损失 9 684.68 万吨(刘万才等, 2016)。作为重要的农业生产资料, 农药在病虫害防控工作中发挥着举足轻重的作用, 但其不科学的使用也给农业可持续发展、农

业现代化推进, 乃至人类健康带来了许多负面影响(王佳新等, 2017), 如农药残留与食品安全、生态环境污染、害虫抗药性、生物多样性下降等热点问题已经引起了社会的广泛关注和讨论(El-Shahawi *et al.*, 2010; Power, 2010; Gill *et al.*, 2012; Köhler and Triebkorn, 2013; 张帅等, 2016), 成为了制约我国农业绿色发展、实现乡村振兴的突出问题。因此, 研发高效、安全、环保的新型病虫害绿色防控技术已成为全球的重

*资助项目 Supported projects: 国家重点研发计划(2017YFD0201200); 北京市自然科学基金项目(6182020)

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收稿日期 Received: 2019-05-27; 接受日期 Accepted: 2019-06-04

大战略需求。

自纳米技术在微电子学、半导体工业、生物医药领域中成功应用之后,它在农业领域发展迅猛,为现代农业科学提供了新的科学方法论,推动传统农业在交叉学科领域不断发展。纳米粒子是指粒径在 1-100 nm 的超细颗粒,因其独特的三维尺度,具有小尺寸、比表面积大、可修饰性强、水溶液分散性好、粘附性强、光催化降解等特点(何碧程等,2013)。以纳米材料为载体高效携带外源杀虫因子,有望起到提升毒力、扩大杀虫谱、减少使用量、延长持效期、减少环境污染等作用。中国农业科学院崔海信研究团队系统开展了农业纳米材料关键制备技术及新型纳米农业投入品的研究,利用纳米材料的小尺寸效应、大比表面积与智能响应等特殊属性,构建具有靶向传输与智能释放功能的农药、兽药、疫苗和饲料等新产品,改善了药物生物活性与持效期,实现了传统农业投入品的提质增效与节能减排(Cui *et al.*, 2009, 2010, 2013; Wang *et al.*, 2016; Yu *et al.*, 2017)。近几年,中国农业大学沈杰团队研发合成了一系列核酸型、蛋白型、药剂型纳米载体,以纳米材料为载体的新型杀虫剂及 RNA 喷雾剂可以扩大杀虫谱、提升杀虫效率、降低农药使用量,实现了良好的害虫控制效果,纳米载体的应用提供了一种全新的病虫害绿色防控技术。

1 可喷洒型 RNA 农药的研发进展

随着昆虫基因组测序不断深入,大量与害虫生长发育、免疫、抗性、代谢、生殖、化学感应等生命活动相关的基因不断地被发现, RNA 干扰技术不仅被广泛地应用于昆虫基因的功能鉴定和解析,还为害虫的遗传学控制提供了可能(Wang *et al.*, 2011, 2014; Xue *et al.*, 2014; 张传溪, 2015)。深度解析调控昆虫生长发育、繁殖和行为等方面的关键基因,利用害虫特有的基因和特异性基因序列,是基于 RNAi 的害虫防治策略成功的关键。目前,实验室条件下,人们通过干扰害虫关键基因的表达,对鳞翅目、鞘翅目、直翅目、双翅目、半翅目、蜚蠊目、等翅目

等多种害虫的遗传学控制取得了一系列进展(Mutti *et al.*, 2006; Baum *et al.*, 2007; Mao *et al.*, 2007; Whyard *et al.*, 2009; Mao and Zeng, 2012; Christiaens and Smagghe, 2014; Tang *et al.*, 2016)。尽管大量的潜在 RNAi 靶基因不断被筛选,但 RNAi 在害虫防控领域的应用还处于起步阶段。害虫的免疫系统会阻止外源 dsRNA 进入自身细胞并将其降解,从一定程度上降低了基因的干扰效率(Terenius *et al.*, 2011; Allen and Walker, 2012; Christiaens *et al.*, 2014; Wynant *et al.*, 2014; Peng *et al.*, 2018), 现有的 dsRNA 递送系统均存在自身局限性,并不能满足田间病虫害防控的实际需求,如饲喂法中 dsRNA 分子穿透害虫肠道围食膜和肠细胞膜到达靶标细胞的效率低,导致干扰效率低、成本较高;浸泡法多用于昆虫细胞系的研究(March and Bentley, 2007; Sivakumar *et al.*, 2007; Mon *et al.*, 2012), 对于体壁特化变厚的活体昆虫干扰效果较差;利用转基因植物表达 dsRNA 是一个很好的递送方式,但目前来看效果并不稳定(Pitino *et al.*, 2011; Zha *et al.*, 2011; Thakur *et al.*, 2014), 考虑到转基因技术的潜在风险,在国内短期内推广转基因作物还存在很多困难(Yan *et al.*, 2015, 2018)。因此开发一种经济简便高效的 dsRNA 递送系统显得尤为重要。

Xu 等(2014)以苝酰亚胺(Perylene diimides, PDI)为荧光核合成了周围携带正电荷树枝状大分子星聚物,较窄的吸收峰位避开了生物体的自发荧光吸收区域,通过检测载体荧光强度可以在生物体内进行实时追踪。作为阳离子聚合物, PDI 荧光纳米载体可以通过静电作用结合负电性的核酸,形成稳定的复合体,携带并保护外源核酸,通过细胞的内吞作用跨越细胞膜进入细胞内,转运进入溶酶体,经过溶酶体逃逸至细胞质中。PDI 荧光纳米载体能够迅速进入离体培养的棉铃虫活体组织,显示出较强的组织吸收率,同时,利用 PDI 荧光纳米载体建立的高效递送系统可以打破昆虫体内的器官基底膜、细胞膜和肠道围食膜等屏障,通过饲喂的方式递送 dsRNA 进入玉米螟 *Ostrinia nubilalis* 活体,干扰几丁质酶基因

的正常表达,导致害虫发育迟缓、停止蜕皮甚至死亡(He *et al.*, 2013)。考虑到饲喂法的局限性,进一步研发了更加简便的体壁渗透法,纳米材料可以快速携带外源 dsRNA 穿透大豆蚜 *Aphis glycines* 体壁,进入活体细胞(<1 h),大幅提升 *hemocytin* 基因干扰效率(48 h 后基因干扰效率达 95.4%),起到了良好的种群控制效果(5 d 后种群抑制效果达 80.5%)(Zheng *et al.*, 2019)。通过进一步筛选大豆蚜高效致死基因,大豆蚜在背板点滴 3 d 后,致死率高达 81.67%;在喷洒 3 d 后,致死率也可以达到 78.50%,起到了良好的种群控制效果(未发表)。纳米载体介导的 RNAi 提供了一种全新的 dsRNA 递送平台,突破了体壁渗透的技术瓶颈,其操作简便,试虫机械损伤小,基因干扰效率高,不仅为昆虫基因功能解析提供了全新的方法,而且为研发 RNA 制剂、控制害虫发生危害提供了技术储备。

2 核酸型纳米载体提升昆虫病毒 DNA 对非寄主毒力

昆虫杆状病毒是实际生产中开发最早、应用最广的一类昆虫病毒,其重要的成员包括核型多角体病毒(NPV)。因为昆虫病毒制剂专性强、易降解、低残留、环境友好等优点,应用前景乐观(Bonning and Hammock, 1996; Moscardi, 1999; Herniou *et al.*, 2003; Glare *et al.*, 2012)。目前,NPV 等生物农药已成功应用于棉铃虫 *Helicoverpa armigera*、斜纹夜蛾 *Spodoptera litura*、大豆夜蛾 *Anticarsia gemmatilis*、舞毒蛾 *Lymantria monacha* 等害虫的实际防控中(Steinhaus and Thompson, 1949; Fuxa, 1987; Shapiro, 1992; Chen *et al.*, 2000; Sieqwart *et al.*, 2015)。自棉铃虫 NPV (HaNPV) 病毒制剂登记为微生物制剂以来,已经在棉花、大豆、玉米、马铃薯等作物上使用,每年喷洒面积达到 10 万 hm^2 (Zhang *et al.*, 1995; Sun and Peng, 2007; Ye *et al.*, 2014)。虽然 HaNPV 对棉铃虫的防控效果出色,但由于其成本高昂,杀虫谱窄,实际生产中主要还是使用化学农药,HaNPV 制剂只能作为害虫防控的第二选择,防治谱窄也成为了

制约其进一步推广应用的重要因素(Steinhaus and Thompson, 1949; Moscardi, 1999; Herniou *et al.*, 2003)。2018 年我国发现并分离了广谱甘蓝夜蛾核型多角体病毒中国株,该病毒可防治 32 种害虫,在一定程度上打破了 1 种病毒只能防治 1 种害虫的技术难题。NPV 与寄主细胞的识别主要取决于细胞膜上的受体,如果可以绕过膜上受体的作用,直接将病毒带入细胞,可能会进一步提高病毒致病力及扩大杀虫谱(Cheng and Lynn, 2009; Jakubowska *et al.*, 2010)。

利用核酸型纳米载体结合棉铃虫 NPV 病毒的 DNA (HaNPV-DNA),单独纳米载体粒径小于 10 nm,复合体粒径增加到 100 nm,二者主要通过氢键和范德华力作用结合,复合体的尺寸有利于活细胞的内吞和摄取。单独 HaNPV-DNA 并不能够穿透果蝇 S2 细胞膜屏障进入到非寄主细胞中,然而核酸型纳米载体能够成功携带 HaNPV-DNA 进入 S2 细胞,携带的 HaNPV-DNA 能够引起细胞凋亡,3 d 细胞致死率达到 70%以上,携带的 HaNPV-DNA 经过 3 d 潜伏期,完成大量复制和增值,引起进一步细胞凋亡。饲喂非靶标鳞翅目害虫小地老虎 *Agrotis ypsilon* 的实验表明:核酸型纳米载体携带 HaNPV-DNA 可以引起小地老虎严重的感病表型,幼虫行动迟缓、拒食,幼虫体壁变软,液化死亡,饲喂 10 d 后致死率达到 70%以上,存活的小地老虎体长缩短 40%以上;饲喂非靶标直翅目害虫飞蝗的实验结果类似,携带的 Ha-NPV-DNA 导致飞蝗生长受限、活动下降、拒食、体壁变软,死亡后伴随身体溃烂,与液化的死亡表型接近,饲喂 10 d 后致死率达到 75%以上(Liu *et al.*, 2016)。综上所述,核酸型纳米载体可以携带 HaNPV-DNA 绕过非寄主细胞的识别过程,HaNPV-DNA 在核酸型纳米载体的携带下可直接进入非寄主细胞并复制增值,导致细胞凋亡,诱发非寄主细胞及害虫感病死亡。这种纳米载体介导的递送方式使得专性寄生的昆虫病毒变为广谱性昆虫病毒,为扩大杀虫病毒的应用范围提供了一种全新途径,同时,该技术是利用病毒 DNA,而不是病毒活体,在一定程度上可以提高农药合成、运输、施用过

程中的稳定性, 保证病毒制剂防效。

3 蛋白型纳米载体提升 Bt 蛋白毒力、毒杀非敏感害虫

苏云金芽孢杆菌 (*Bacillus thuringiensis*) 可产生多种形态的伴胞晶体, 随着母细胞的裂解释放到环境中, 杀虫晶体蛋白主要分为 Cry 和 Cyt 两类, 其中 Cry 类毒蛋白应用最广, 对鳞翅目、鞘翅目、膜翅目和双翅目等多种害虫及螨类、线虫等有害生物具有特异性毒杀作用, 因此 Bt 杀虫蛋白作为微生物杀虫剂或构建转基因植物被广泛地应用于害虫生物防治 (Lu *et al.*, 2012; 彭琦等, 2015; 束长龙等, 2016)。Cry 类杀虫蛋白的作用机制较为明确, 主要分为两类: (1) “孔洞形成”模型: Cry 类毒蛋白插入细胞膜形成离子通道, 改变了中肠细胞的渗透压, 最终导致靶标害虫死亡 (Grochulski *et al.*, 1995; Bravo *et al.*, 2004); (2) “信号转导”模型: 该模型认为 Cry 类毒蛋白并不是形成孔洞的毒素, 而是它与特异性受体相互作用进而激活了一系列的信号转导途径 (Zhang *et al.*, 2006)。虽然两种模型有些不同, 但起始步骤相同, 即 Cry 类毒蛋白在被取食摄入后, 被靶标害虫中肠特异性蛋白酶剪切和活化, 形成的 Cry 类毒蛋白活化毒素进一步与害虫中肠细胞膜上受体结合并发挥作用。随着 Bt 微生物制剂和转基因作物的商业化推广, 鳞翅目、鞘翅目、双翅目等多种害虫已对 Bt 蛋白产生了较高水平的抗性, 通常认为抗性的产生是由于受体基因突变或表达量下调所引起的 (Liu *et al.*, 1999; Tabashnik *et al.*, 2008; Wei *et al.*, 2014; Tabashnik and Carrière, 2017)。因此, 延缓抗药性的产生、保护 Bt 蛋白这一优良的生防资源可能要在细胞膜上受体做文章。

中国农业大学沈杰团队研发合成了蛋白型纳米载体, 其是一类荧光星形聚合物, 稳定的结合可以保证蛋白的长距离运输, 在害虫体内复杂环境下, 避免极端 pH 的拆解、肠道蛋白酶作用以及溶酶体吞噬等不利因素的干扰, 保证活性蛋白高效到达靶标组织和细胞。选取 Cry1Ab 原毒

素为研究对象, 蛋白型纳米载体与 Cry1Ab 主要靠氢键和范德华力作用, 纳米载体可以携带 Cry1Ab 穿透果蝇中肠围食膜, 进入肠道细胞。较单独使用 Cry1Ab 饲喂小地老虎, 复合体对小地老虎毒性大幅提升, 幼虫生长出现严重缺陷并死亡。饲喂复合体 6 d 后, 0.1、1.0 和 10.0 μg 处理的小地老虎存活率分别下降至 66.7%、53.3%和 25.0%, 幼虫体长较野生型下降 53.4%、64.7%和 87.2%。同时, 蛋白型纳米载体携带 Cry1Ab 对果蝇 S2 细胞无毒安全, 因为被取食的 Cry1Ab 原毒素需要特异性蛋白酶的水解活化, 才能发挥毒素作用, 细胞系中并不存在特异性的蛋白酶, 由此证明对于缺乏特异性蛋白酶的非靶标生物来说, 复合体是安全的 (Zheng *et al.*, 2016)。纳米载体介导的蛋白递送系统提升了 Bt 蛋白对非敏感害虫的毒力, 推测蛋白型纳米载体可以携带毒蛋白绕过细胞膜受体, 细胞内可能存在新的 Bt 毒杀机制, 亦或是蛋白型纳米载体可以保护毒蛋白活化毒素进一步被中肠蛋白酶水解剪切。这项工作有利于治理和延缓害虫抗药性的产生, 为大量使用 Bt 农药和长期种植转基因 Bt 作物提供了抗性治理的新思路。

4 纳米农药的研究进展与推广应用

化学农药在病虫害防控、保障粮食生产安全等方面发挥着重要作用, 我国农药目前多以乳油、可湿性粉剂等剂型为主, 传统农药剂型存在大量使用有机溶剂、粉尘漂移、水分散性差、有效利用率低、生物活性不高、农药残留与环境污染严重等问题 (王安琪等, 2018)。因此, 发展高效、安全的绿色农药是保障国家粮食生产安全、食品安全、生态安全的重大战略需求, 对于缓解农药残留、环境污染、促进农药产业健康可持续发展具有积极意义。纳米粒子可以经过修饰作为一种药物载体, 快速包裹药物分子, 提高大颗粒、难溶农药分子的分散性和穿透力, 提升农药分子的附着力和利用率 (Ghormade *et al.*, 2011; 何碧程等, 2013; Peters *et al.*, 2016; Luksiene, 2017; 郑洋, 2018)。目前关于纳米

农药新剂型的研究主要集中在纳米微乳、纳米微囊、纳米混悬剂等领域。微乳液是由水、油、表面活性剂和助剂等自发形成的一种粒径为 10-100 nm、透明或半透明的热力学稳定的均相分散体系,在提高农药水溶性、分散性、生物利用率等方面优势明显 (Frederiksen *et al.*, 2003; Wang *et al.*, 2007; Ao *et al.*, 2013)。目前先正达公司已经上市了纳米杀菌剂微乳液新产品,但是农药微乳液制剂存在表面活性剂用量大、控释和靶向传输性能不突出等问题 (余曼丽, 2017; 杨东升等, 2018)。纳米微囊是将农药 (或其他药剂)包裹在天然或合成高分子聚合物膜内形成的农药纳米载体,可以抑制农药分解或流失,降低农药接触毒性等药害,延长农药持效期,从而做到农药减量控害 (Amory *et al.*, 2002; Teixeira *et al.*, 2005; 李敏等, 2011; 郑和堂和张越, 2011)。此外,也可以将难溶性有机农药加工成纳米颗粒粉剂,或分散于水中的纳米混悬剂,进而提高改善药物的水溶性和分散性,提高生物利用率 (余曼丽, 2017)。目前,全球包括欧洲、美国、加拿大、巴西、日本、印度在内的多个国家和地区先后开展了纳米农药的研发工作,取得了一系列研究进展,纳米农药具有广阔的市场前景。

Liu 等 (2015) 研发合成了药剂型纳米载体,可以稳定、高效携带化学农药分子进入昆虫细胞,从而大幅提升农药毒力,扩大防治谱。噻虫嗪是防治蚜虫、飞虱等半翅目害虫的一种疏水性化学农药,利用 3 种药剂型纳米载体分别与噻虫嗪混合孵育,在细胞和活体水平上均检测到农药毒力的提升。苦参碱是一种广谱性的生物制剂,药剂型纳米载体可以与苦参碱稳定结合,粒径在水溶液中从 800 nm 降低到 10 nm 左右,对刺吸式口器害虫桃蚜和咀嚼式口器害虫棉铃虫的毒力均有显著提升 (未发表)。因此,将药剂型纳米载体作为农药增效剂使用,有利于提升农药毒力,减少农药的使用量、扩大农药防治谱,符合国家“双减”战略,有广阔的应用价值和前景。

5 展望

随着纳米材料技术的不断完善和发展,不同

用途的纳米材料载体不断被设计合成,用于携带外源杀虫因子进入昆虫靶标组织和细胞,高效干扰害虫正常生长发育。基于纳米载体的昆虫瞬时转染技术,为农作物病虫害绿色防控提供了一种全新的思路,该技术的研究前景和应用价值十分广阔: (1) 纳米载体/杀虫因子复合体制剂毒力大幅提升,通过不断筛选优化纳米载体及杀虫因子,毒杀效果还将进一步提高,易于被农户接受; (2) 通过去掉荧光核以降低纳米材料成本,通过工程菌高效经济合成 dsRNA,纳米载体/dsRNA 制剂及其他纳米载体制剂生产成本低廉,预计批量生产后,成本接近植物源农药,符合田间生产实际需求; (3) 纳米载体/杀虫因子复合体制剂比其它生物制剂应用简便,受田间因素影响小,且效果更加稳定; (4) 纳米载体/杀虫因子复合体制剂比传统化学农药特异性强,与其它生物制剂相似,对非靶标安全; (5) 纳米载体/杀虫因子复合体制剂与传统化学农药使用流程相似,符合农户用药习惯。该技术的研发和应用将建立一种害虫绿色防控的新技术,有望促进病虫害绿色防控技术的实践和发展。

未来工作重点将放在以下三个方面: (1) 继续研发筛选高效、稳定、低成本、通用性的新型纳米材料,建立核酸型、蛋白型、药剂型纳米载体的田间应用技术平台; (2) 不断筛选高致死效果的 RNAi 靶标基因,通过优化助剂拓展 dsRNA 体壁渗透技术,利用纳米材料研发新型杀菌剂、杀卵剂; (3) 开展纳米载体介导的杀虫因子 (RNAi、毒蛋白、药剂等) 毒力提升的机理研究,进一步探讨提升害虫种群控制效果的可能。

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