Genetic Control Technologies for Invasive Pest Management: Postprint

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Abstract

"Biological invasion" refers to the phenomenon where an organism, following natural introduction or artificial introduction from other regions, becomes established in the wild and poses certain threats to local ecosystems. In the absence of natural enemies and other limiting factors, invasive species can rapidly reproduce and spread, inflicting serious losses on local ecological environments, biodiversity, and the economy. With the advancement of globalization, biological invasions have become increasingly prevalent and their impacts more severe, rendering the strengthening of invasive species management critically important. As traditional physical and chemical methods for invasive species control entail several intractable issues, the development and utilization of pollution-free and environmentally safe genetic regulation technologies have become particularly urgent for the integrated management of biological invasive species. This paper summarizes the current status of invasive pests in China, their primary invasion pathways, and existing control measures, while also elaborating on genetic regulation technologies and their applications in the integrated management of biological invasive species, with the aim of providing a reference for the scientific management of biological invasions in China.

Full Text

Genetic Regulation Techniques for Invasive Pest Management

Special Issue: Targeted Prevention and Control of Crop Pests and Diseases

Biological invasion refers to the phenomenon where a species, after being introduced naturally or artificially from another region, becomes established in

the wild and causes harm to local ecosystems. In the absence of natural enemies and other limiting factors, invasive species can reproduce and expand rapidly, inflicting severe damage on local ecological environments, biodiversity, and economies. As globalization accelerates, biological invasions have become more frequent and destructive, making effective management of invasive species critically important. Since traditional physical and chemical control methods present numerous challenges, developing and utilizing pollution-free, environmentally benign genetic regulation technologies has become an urgent priority for integrated management of invasive species. This article summarizes the current status of invasive pests in China, their primary invasion pathways, and existing control measures, while also elaborating on genetic regulation technologies and their applications in invasive species management, aiming to provide references for scientific governance of biological invasions in China.

Keywords: biological invasion, integrated management, genetic regulation
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Biological invasion severely threatens agriculture, forestry, ecosystems, and human health, representing the second leading cause of global biodiversity loss. With accelerating globalization, biological invasions have become increasingly serious. Among documented invasive species cases in China, 75% occur in economically developed coastal regions and border ports, causing substantial economic losses. Currently, China primarily relies on chemical and physical measures to manage invasive species, but these approaches bring severe environmental pollution and high control costs while suppressing invaders. Therefore, developing and utilizing new pollution-free, environmentally friendly control measures has become urgently needed for integrated management of invasive species. This paper summarizes the current status of biological invasion in China, major invasion pathways, and existing control measures, while also prospecting the use of genetic manipulation for invasive species regulation, hoping to provide references for prevention and scientific management of biological invasions in China.

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Pathways of Biological Invasion

Biological invasion pathways fall into two main categories: natural and human-mediated [2]. Natural pathways involve species dispersal through wind, water currents, or other animals, independent of human activity, making the spread area nearly uncontrollable and the resulting damage difficult to estimate. In China, *Eupatorium adenophorum* represents a classic case of natural invasion. This plant, native to Central America, was introduced to Myanmar and subsequently entered China along the China-Myanmar border, becoming a severely damaging invasive plant. Human-mediated pathways include both intentional introduction and accidental transport [3]. Intentional introductions often serve specific purposes. For example, during the 1960s-1980s, China introduced smooth cordgrass from the UK and US to protect tidal flats. Its strong stress tolerance and extremely rapid reproduction eventually caused severe consequences, including tidal flat ecological imbalance, destruction of nearshore habitats, and blocked waterways. With global development and increased international exchange, accidental transport through trade, tourism, and transportation has also increased invasion risks. In the 1970s, the fall webworm was accidentally introduced to China via aircraft and ships. As a polyphagous insect that can feed on nearly all green trees, it has caused enormous damage to Chinese agriculture and forestry.

Current Status of Biological Invasion in China

With its vast territory encompassing tropical monsoon, subtropical monsoon, and temperate monsoon climates, China is among the countries most severely affected by biological invasions. As early as 2003, surveys by the former State Environmental Protection Administration identified 283 invasive species, with numbers increasing annually at an alarming rate [4]. Statistics from November 2014 showed that China already hosted 515 invasive plant species. These invaders cause immeasurable economic losses annually. Table 1 lists direct economic losses and control costs associated with invasive species in China. For instance, just 11 major invasive diseases, pests, and weeds cause over 50 billion RMB in annual losses, with damages from other species being even more difficult to quantify [5].

Hazards of Biological Invasion

1.3.1 Causing Massive Economic Losses Biological invasion has become a global issue, drawing government attention worldwide due to its enormous economic impacts [6]. Invasive pests and diseases cause massive annual destruction to agricultural and forestry production. In China alone, several invasive pests —including the pine wood nematode, pine bast scale, pine needle scale, and fall webworm—cause millions of hectares of forest damage annually, while pests like the Colorado potato beetle and giant African snail affect millions of hectares of farmland. Additionally, annual control costs reach hundreds of millions. Conservative estimates indicate China spends billions of RMB annually just on water hyacinth removal. Similar severe damages occur in other countries; the United States suffers over $100 billion in annual losses from invasive species [7].

1.3.2 Destroying Biodiversity Without natural enemies and environmental constraints, invasive populations often grow exponentially. They occupy habitats of native species or prey upon them, causing native populations to decline and eventually disappear, thereby reducing local biodiversity. Further biodiversity loss can alter ecological structure and trigger ecological disasters [5]. For example, in the 1970s, the US introduced Asian carp to improve local ecosystems. Under conditions lacking natural enemies and with abundant food, this "guest from Asia" proliferated rapidly, occupying habitats and survival spaces of other fish, leading to sharp declines in native fish species and severely damaging local biodiversity. Another plant example confirms this pattern: *Caulerpa taxifolia*, native to Australia and introduced to the Mediterranean as an ornamental species, forms dense monocultures that prevent native seagrass establishment and outcompete nearly all other marine life, causing severe ecological damage. Cases of biodiversity loss due to biological invasion are countless, with new examples continuously emerging.

1.3.3 Threatening Human Health Invasive organisms also pose serious threats to human health [8]. Ragweed, native to North America, produces pollen containing water-soluble proteins that trigger allergic reactions upon contact, causing "hay fever" in autumn. The red imported fire ant, discovered in Taiwan in 2004, has since spread to Guangdong, Hong Kong, Macau, Hunan, Guangxi, and Hainan. Besides damaging crops, animals, and infrastructure, they directly harm human health, causing burning pain, suppurative swelling, and in rare cases, anaphylactic shock. Invasive species also indirectly harm humans by transmitting infectious diseases. Many diseases—including malaria, Japanese encephalitis, and dengue fever—are vectored by invasive organisms, posing serious threats. Dengue fever, transmitted by *Aedes* mosquitoes, infects over 50 million people across more than 100 countries annually, with numerous fatalities [8].

Current Major Control Methods for Invasive Species

Prevention is crucial for invasive species management. Various measures should be implemented to reduce harmful organism entry and minimize impacts on environment, economy, and human health. Common control methods include mechanical/physical, chemical, and biological approaches, often used in combination to restore and rebuild ecosystems.

Mechanical/Physical Control This approach uses specially designed equipment to directly remove invasive organisms based on their characteristics. These methods can quickly clear invaders within certain areas and are environmentally friendly [9]. In Shaanxi and Liaoning provinces, manual mechanical removal of larval webs and high pruning successfully controlled fall webworm outbreaks. However, equipment costs are high and these methods are labor-intensive. Additionally, improper disposal of plant residues after mechanical removal may create new propagation sources through asexual reproduction [9].

Chemical Control Chemical methods work quickly, are convenient, and can be applied over large areas. However, they are costly and often harm native species, ecosystems, and human health while eliminating invaders, and can induce resistance. Chemical pesticides are often impractical for large forest areas or low-value ecosystems, and restricted for use in lakes, rivers, and reservoirs. Herbicides are important chemical tools, classified as systemic or contact types. Systemic herbicides, generally used for perennial weeds, work slowly but provide lasting effects. Contact herbicides act quickly and suit annual weeds. Since many invasive plants are perennials, systemic herbicides are more effective. While chemical control has achieved some success, resulting environmental pollution and resistance issues present new challenges.

Biological Control For large, stable invasive populations, biological control is recommended—introducing natural enemies (insects and fungi) from the invader's native range. After safety, specificity, and ecological adaptability studies, these enemies are released to control the invasive species [10]. Biological control is environmentally safe, cost-effective, and provides lasting results. The world' s first successful case was introducing the vedalia beetle (*Rodolia cardinalis*) from Australia to control cottony cushion scale in California, saving the citrus industry. China has also achieved successes, such as using *Alternaria alternata* to control *Eupatorium adenophorum*, planting ryegrass (*Lolium perenne*) or pigeon pea (*Cajanus cajan*) and raising lac insects (*Laccifer lacca*) to prevent *Eupatorium* growth, and introducing water hyacinth weevils to manage water hyacinth [11].

However, biological control requires careful avoidance of the control agent itself becoming invasive. In 1935, Australia introduced cane toads (*Bufo marinus*) from Hawaii to control native cane beetles (*Dermolepida albohirtum*). The toads reproduced rapidly, competed with native wildlife, secreted lethal toxins, and preyed on various organisms—but not cane beetles when given choices [7], resulting in complete failure. Therefore, safety testing of natural enemies is essential before release [12].

Genetic Regulation: An Ideal Technology for Invasive Pest Management

Introduction to Genetic Regulation Technology While physical and chemical methods can suppress invasive species, their problems are evident. Long-term chemical pesticide use enhances pest resistance, causes environmental pollution, and leaves residues. Therefore, new pollution-free control measures represent the future trend. Genetic regulation technology offers multiple advantages: (1) It targets only the specific organism through intrinsic mechanisms, affecting no other species (especially crops). (2) It uses no chemical pesticides, avoiding environmental harm. (3) Once established in nature, the genetic capacity spreads within the target pest population for sustained effect without impacting other species. Thus, genetic regulation of pest populations represents an ideal future solution.

Although no successful cases of genetic regulation for invasive species exist yet, applications in pest populations provide valuable lessons.

Key Elements of Genetic Regulation Technology

3.2.1 Genetic Transformation and Genome Editing Technology Since the first successful transgenic fruit fly in 1982 [13], genetic transformation technology in insects has advanced steadily. Genetic transformation introduces target gene DNA sequences into early embryos via microinjection or other mediated techniques, integrating foreign DNA into the recipient genome through enzymatic action. Due to limitations from transposons (mobile genetic elements, also called jumping genes or transposable factors) and microinjection technology, successful transgenesis with stable inheritance remains limited to few species, mainly in Diptera and Lepidoptera.

Genome editing is a crucial recent technology. Despite its short application history, it greatly advances life science research and applications. From zinc finger nuclease (ZFN) technology around 2000 [14], to transcription activator-like effector nuclease (TALEN) technology [14,15], and the CRISPR/Cas9 system emerging in 2013, genome editing has become possible for many organisms including non-model species, with potential future applications in invasive species genetic regulation. These technologies have been successfully applied in insects. Wang et al. [16] injected Cas9 mRNA and in vitro-transcribed sgRNA to successfully knock out the *BmBLoS2* gene in silkworm, obtaining heritable translucent larval phenotypes. Compared to ZF or TALE array construction, sgRNA construction offers overwhelming advantages in difficulty, time, and cost, with clearer benefits for multi-site editing. Genome editing provides unlimited possibilities for gene function research and applications due to its potential for targeted knockout and insertion, and should have unlimited application potential in invasive species management. Combining genetic transformation with genome editing further advances these applications.

3.2.2 Discovery and Application of Key Target Genes and Elements Both transgenic and genome editing technologies for invasive species control require identifying specific regulatory elements and target genes. The following uses insects as examples.

3.2.2.1 Transposable Elements

Insect transgenesis primarily relies on transposons for foreign sequence delivery. The earliest used transposon was the P element in fruit flies. Subsequently, Minos [17], Hermes [18], and piggyBac [19] were widely applied. Among these, the piggyBac transposon from the lepidopteran *Trichoplusia ni* is most commonly used. It can carry relatively large foreign genes, functions across species and germ lines, and has successful applications from single-celled organisms to mammals, making it the most widely used transposon vector. To date, piggyBac has successfully transformed various insects across Diptera, Lepidoptera, Coleoptera, and Hymenoptera [20].

3.2.2.2 Sex-Specific Regulatory Elements

(1) Sex-Specific Promoters. Drawing from successful pest control cases, effective invasive species management requires obtaining spatiotemporal-specific promoters. These drive expression of lethal or toxin factors in specific sexes, tissues, or developmental stages. Commonly used insect-specific promoters include: Sex-specific promoters such as vitellogenin (*Yp* and *Vg*) promoters [21] and testis-specific 2-tubulin promoters [22]. Stage-specific promoters like *nullo* and *serendipity * expressed during the embryonic blastoderm stage in fruit flies [23,24]. Tissue- and environment-specific promoters such as fat body-specific* Yp3* [22], germ cell-specific *Vasa* [25], *Aedes* indirect flight muscle-specific *Act-4*, and temperature-regulated *Hsp70* [26]. These specifically expressed promoters effectively regulate sterile and lethal systems, important for sex separation and population regulation in transgenic colonies.

(2) Sex-Specific Splicing Systems. New-generation genetic regulation systems integrate sex-specific splicing elements from insect sex determination pathways. In 2007, Fu et al. [27] first applied sex splicing systems to the Mediterranean fruit fly (*Ceratitis capitata*). They integrated female-specific splicing signals (the female-specific intron fragment of the *tra* gene) with a single-component Tet-off system, causing female lethality while males survived. Ant et al. [28] achieved similar female-specific killing in olive fruit flies using Mediterranean fruit fly female-specific splicing signals (*Cctra*). Laboratory cage experiments showed that after 24 weeks of releasing female-specific lethal transgenic populations, wild population oviposition rates and female numbers nearly dropped to zero. These experiments demonstrate that effective use of sex-specific alternative splicing signals can achieve female-specific lethality for population density regulation.

3.2.2.3 Specific Lethal and Defective Genes

Identifying specific lethal or defective genes is crucial for constructing insect population genetic regulation systems. Currently used genes include apoptosis-related genes like *hid* (head involution defective) [29], *reaper* [30], and *grim* [31] from fruit flies; cytotoxic genes like *Ras64B*; and systems like Tet' s own tTA.

In 2011, Thailayil et al. [32] discovered that female malaria mosquitoes undergo behavioral changes after mating, including lifelong refractoriness to remating and blood meal-induced oviposition. Through RNAi of the *zpg* gene, they successfully generated spermless male malaria mosquitoes that severely impaired wild female reproduction when mated. This system provides an excellent example for achieving insect population genetic regulation through species-specific gene screening and RNAi.

Application of Genetic Regulation Technology

Genetic transformation research has succeeded in at least 20 insect species across four orders. Combining transgenic technology, UK-based Oxford Insect Technologies (Oxitec) developed RIDL (Release of Insects Carrying a Dominant Lethal)—a DNA recombination technology producing genetically modified insects. This technology introduces a controllable "dominant lethal" gene that kills insects unless suppressed, enabling artificial rearing. Building on this, Fu et al. [27] constructed female-specific lethal systems in 2007 by inserting the *transformer* (*tra*) gene exon into the transactivator controlled by the Tet-off system. Recent trials in the Cayman Islands and relatively isolated environments have achieved ideal results.

To reduce labor requirements, scientists are developing gene drive technology. Gene drive enables exogenous genomic fragments to spread rapidly through target pest populations via non-Mendelian inheritance, quickly reducing population density. Combining this with CRISPR/Cas9, researchers developed the Mutagenic Chain Reaction (MCR) system, obtaining two strains with rapid exogenous fragment amplification in *Anopheles stephensi* mosquitoes [33]. This system shows enormous potential for reducing mosquito populations carrying dengue and Zika viruses. Gene drive technology can rapidly introduce lethal or toxin factors into pest populations, offering great potential for invasive species management. Successful transgenic applications in some invasive species have laid the foundation for this approach.

In beneficial insect applications, the Zhejiang Academy of Agricultural Sciences used sex-linked balanced lethal strains imported from Russia to construct male-only silkworm strains (which provide over 15% benefit improvement over female strains), with some production-scale promotion. To better establish male-only silkworm systems, Tan Anjiang et al. collaborated with Oxitec to develop female-lethal silkworm strains (Figure 1 [FIGURE:1]) [34]. With continuous optimization of silkworm genetic transformation and genome editing platforms, new male silkworm strains are being continuously developed for industry.

Summary and Outlook

Facing increasingly severe biological invasion problems, we must not only continuously improve existing integrated management technologies but also strengthen new technology development. New technologies must meet multiple requirements: harmless to non-target organisms, non-polluting, and capable of long-term effectiveness. Genetic regulation technology can essentially meet these strict requirements. Invasive species are not native to begin with; removing these outsiders after settlement causes no special ecological harm to the invaded region. The theories and methods accumulated through genetic regulation of invasive species will undoubtedly serve population regulation of other invasive pests.

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Figures

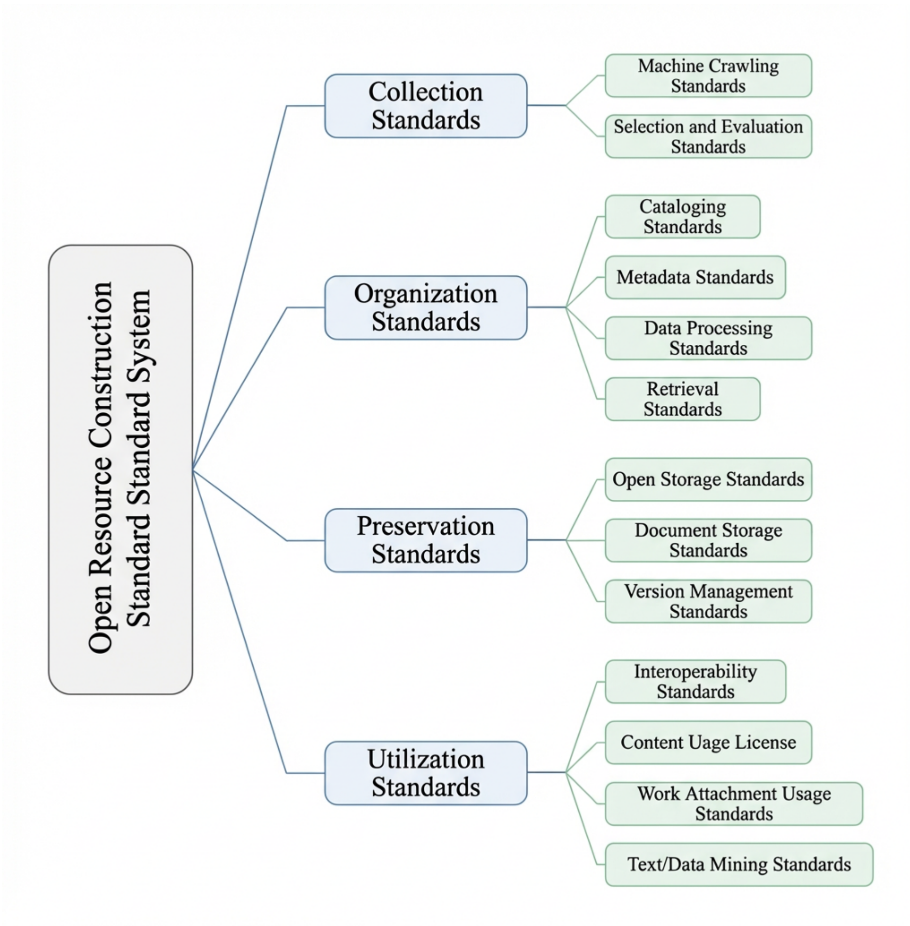

Figure 1: Figure 2

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