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Vector Control: *Wolbachia* Expands Its Protective Reach from Humans to Plants

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RNA viral titers are often suppressed in insects co-infected with the bacterial endosymbiont *Wolbachia*. This property has been used to suppress transmission of the ragged rice stunt virus from its insect host, the brown planthopper, to the rice plant.

On March 17th, 1954, a World War II training plane with a cargo of thousands of sterilized screwworm pupae lifted off from an airstrip on the island of Curacao. This was the first strike in an extraordinary US Department of Agriculture (USDA) operation to eliminate the screwworm, a flesh-eating parasite targeting cattle and other livestock [1]. A member of the blowfly family, it seeks open wounds in which to lay its eggs. The resulting larva gorge on the livestock flesh, expanding the area for new rounds of infection [2]. Rather than using insecticides, USDA scientists took advantage of H.J. Muller's Nobel-prize worthy discovery that X-irradiation causes mutations and sterility [3]. Insect eggs fertilized by extensively X-irradiated males are inviable as a result of the induction of dominant lethal mutations in the sperm DNA. The USDA's plan was both ingenious and simple: eradicate screwworm infestations through massive releases of sterilized male screwworm pupae [4]. The success of this strategy is evidenced by the fact that the screwworm has been eradicated from North and Central America. Currently, the northern range of the screwworm population is kept at bay through continuous releases of pupae across the Isthmus of Panama.

A new study by Gong, Li *et al.* [5] in this issue of *Current Biology* puts an innovative twist on this biologically based strategy of suppressing insect populations. Rather than population suppression, the strategy is to replace existing insect populations with a beneficial variant. Gong, Li and colleagues introduced a specific strain of *Wolbachia*, a bacterial endosymbiont of insects, into the brown planthopper, an insect vector of a devastating virus of rice plants. They demonstrate that this strain of *Wolbachia* rapidly spreads through brown planthopper populations, dramatically reducing planthopper RRSV viral loads and viral transmission to the rice plants.

The strategy used to eradicate the screwworm is known as the 'sterile insect technique' (SIT) and has been successfully used to suppress numerous pest-insect populations [6]. A key advantage of this strategy over chemical approaches is that it targets a single species, unlike the latter, which results in collateral destruction of beneficial insects. In addition, as populations are suppressed, the chemical approach becomes less effective, whereas the SIT becomes more effective. Until recently, the public and many scientists were largely unaware of the virtues and successes of SIT-based vector control strategies, which were simply an ordinary part of doing business. However, for reasons described below, vector control and SIT-based technologies are now enjoying considerable attention in the scientific and lay press. Students are seeking graduate programs in this field and venture capitalists, ever hopeful for the next unicorn company, are funding vector-control-based start-ups.

A number of factors are responsible for this quiet renaissance in combatting insect-borne diseases. Campaigns led by the Gates foundation and the World Health Organization have greatly increased awareness of the global health impact of insect-borne diseases [7]. According to the World Health Organization, half of the world's population is at risk for a vector-borne disease. One sixth of all infectious diseases are the direct result of transmission by an insect vector, resulting in over 700,000 deaths annually. In addition, pest insects are directly responsible for 20 to 40% of annual crop loss globally. With global warming expanding the northern range of many species, developed nations now must combat a broader range of insect pests and disease vectors [8]. For example, Chagas disease is now common in the southern United States [9]. Concomitant with this increased concern and awareness of vector-borne diseases has been the development of CRISPR-based technologies facilitating genetic modification of pest and disease-bearing insect species. Consequently, there is a growing arsenal of innovative, genetically modified insects, many of which have shown promise in suppressing insect populations [10].

The benefit of insect-vector control through genetically engineered insects is countered by real and perceived perils of these technologies [11]. As a result, development of alternative vector-control strategies that do not rely on genetic modification are also being pursued. Foremost among these is harnessing *Wolbachia*, a naturally occurring bacterial endosymbiont of insects. *Wolbachia* has proven an excellent vector-control agent because it induces a conditional sterility in infected insects. Known as 'cytoplasmic incompatibility', matings between *Wolbachia*-infected males and uninfected females are sterile because the resulting eggs fail to hatch [12] (Figure 1A). Unlike X-irradiation SIT, *Wolbachia* infection does not weaken the

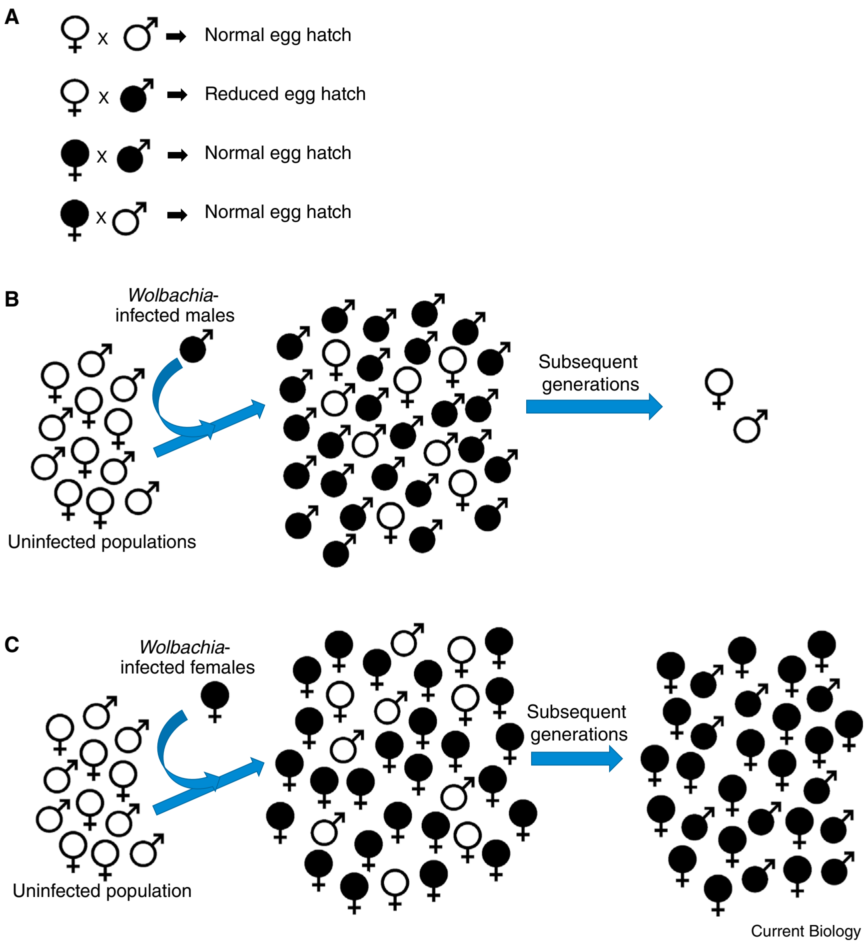

Figure 1. *Wolbachia*-induced sterility and population control strategies.
(A) *Wolbachia*-induced male sterility. Cytoplasmic incompatibility refers to the reduced egg-hatch rates resulting from matings between *Wolbachia*-infected (filled symbols) males and uninfected (open symbols) females. However, matings between infected females and either infected or uninfected males produce normal hatch rates. This conditional male sterility endows infected females with a selective advantage over uninfected females, driving the maternally transmitted *Wolbachia* through the population. (B) *Wolbachia*-mediated population suppression. The male sterility associated with *Wolbachia*-mediated cytoplasmic incompatibility provides an efficient method of suppressing insect populations through releases of large numbers of infected males (filled circles) into an uninfected population (open circles). Whereas X-irradiation induced sterility compromises male vitality and competitiveness, *Wolbachia*-infected males are robust. (C) *Wolbachia*-mediated population replacement. Cytoplasmic incompatibility endows infected females with a selective advantage over uninfected females. Consequently, release of large numbers of infected females (filled circles) into uninfected populations (open circles) results in maternally transmitted *Wolbachia* rapidly spreading through the entire population. The *Wolbachia*-infected population suppresses replication and transmission of pests and disease-causing RNA viruses.

males, thus making it an extremely efficient technique. Recent field trials in Guangzhou, China employing a potent combination of *Wolbachia* and X-irradiation-based SIT, resulted in the near elimination of resident mosquito populations [13] (Figure 1B).

The work presented by Gong, Li *et al.* [5] in this issue takes advantage of a second property of *Wolbachia*—specifically—its effects on viral replication. The strategy originated from pioneering studies in *Drosophila*, screening for host factors that inhibited viral replication. Using *Drosophila*, Teixeira and colleagues discovered that the presence of *Wolbachia* suppressed single-stranded RNA viral titers in its insect host [14]. That is, *Wolbachia* is in an evolutionary battle with very different foes: insects and viruses.

Although much remains to be learned concerning the mechanisms of *Wolbachia*-mediated viral suppression [15], this finding led to the development of an alternative vector-control strategy. Rather than releasing large numbers of *Wolbachia*-infected males to suppress insect populations, *Wolbachia*-infected females would be released in order to transform an insect population from uninfected to infected (Figure 1C). The infected females would suppress replication and transmission of disease viruses present in the insect population. The success of this strategy relies on the fact that, like mitochondria, *Wolbachia* is maternally transmitted and *Wolbachia*-induced cytoplasmic incompatibility provides a selective advantage to infected over uninfected females, promoting the rapid spread of *Wolbachia* through the population. Funded by the Bill & Melinda Gates Foundation, the ‘Eliminate Dengue Project’ has successfully applied the *Wolbachia* replacement strategy in a number of cities throughout the world to reduce the spread of Dengue, a mosquito-transmitted RNA virus [16].

To date, the *Wolbachia* success stories have been limited to mosquitos. Because mosquitoes are vectors for the most prevalent and deadliest of insect-borne diseases, they have been intensively investigated for over a century and much is known about mosquito biology, ecology and husbandry. Fortuitously, the disease-carrying mosquito species also manifest the robust cytoplasmic incompatibility required for the success of both *Wolbachia* suppression and replacement strategies. Thus, it was an open question of whether this approach could be applied more broadly to other disease and pest insects.

The work presented in this issue of *Current Biology* presents a compelling case for the broad applicability of the *Wolbachia*-based replacement strategy and also provides a road map for its successful implementation. The authors focus their studies on the brown planthopper (*Nilaparvata lugens*), a hemipteran insect that carries and transmits the double-stranded RNA ragged rice stunt virus (RRSV) into the rice-plant phloem, resulting in the annual destruction of 25 to 50 million acres of this essential crop [17]. *N. lugens* is naturally infected with a *Wolbachia* strain that does not cause cytoplasmic incompatibility, making it unsuitable for population replacement and suppression strategies. However, the authors took advantage of the fact that a closely related species *Laodelphax striatellus* (small brown planthopper) is infected with a strain (sStri *Wolbachia*) that causes strong cytoplasmic incompatibility, resulting in extremely high *Wolbachia* infection rates. Using fine needles and steady hands, sStri Wolbachia was extracted from *L. striatellus* embryos and transinfected into *N. lugens* embryos. Remarkably, sStri *Wolbachia* quickly established itself in this foreign species, exhibiting perfect maternal transmission and near 100% cytoplasmic incompatibility. Thus, the sStri strain appeared to be well suited for rapid invasion and spread through *N. lugens* populations; indeed, the authors demonstrate as much in laboratory populations.

The next issue was whether infection with the sStri *Wolbachia* strain suppressed RRSV viral titer and viral transmission to rice plants. Although *Wolbachia*-mediated suppression of single-stranded RNA viruses had been well documented, it was unclear whether it would have a similar suppressive effect on dsRNA viral titers. By inoculating *Wolbachia*-infected and uninfected *N. lugens* with equivalent amounts of RRSV, the authors demonstrate a 75% decrease in viral load in *Wolbachia*-infected insects. In addition to the obvious applied implications of these studies, this is the first demonstration that *Wolbachia* suppresses double- and as well single-stranded RNA viruses. This result may shed light on the vexing issue of the mechanism of *Wolbachia*-based viral suppression and suggests that *Wolbachia* likely targets host components or processes required for both single and double-stranded RNA viral replication.

The authors found that the *Wolbachia*-mediated reduction in viral titer is also associated with a dramatic reduction in viral transmission to the rice plants. Rice seedlings attacked by sStri *Wolbachia*-infected *N. lugens* resulted in a dramatic 82% lower incidence of viral infection relative to seedlings attacked by native *N. lugens*. Consistent with these results, 87% and 27% of rice plants attacked by sStri *Wolbachia*-infected and native *N. lugens*, respectively, were asymptomatic and healthy. These experiments were performed in a lab setting and it still must be determined whether similar results will be obtained in the field. However, previous studies in mosquitos on the spread of *Wolbachia* infection and viral suppression indicate that results in the lab are borne out in field studies [18].

The studies by Gong, Li *et al.* [5] are certain to have a tremendous impact in the field of pest management. With increasing awareness of the environmental harm of pesticides and an ever-increasing number of pesticide-resistant insect populations, there is a great need for alternative approaches to controlling insect disease and pest vectors. Although CRISPR technologies hold much promise, there is still unease concerning the release of highly mobile, genetically modified insects into nature. *Wolbachia*-based strategies offer an environmentally friendly, safe alternative. As with all vector-control strategies, the potential and probability of insects and their viruses developing a resistance to the effects of *Wolbachia* remain unknown. However, given the success of the airplane releases of screwworms over ranchland, I imagine a day when satellite-controlled drones routinely release *Wolbachia*-infected pest insects over farmlands.

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