

Fungal bioinsecticide with a sting

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A fungus engineered to express a scorpion toxin may prove useful in combating insect pests.

Biological pesticides are often touted as being safer and more sustainable than their chemical counterparts. But they also tend to be less effective and more costly, limiting their widespread use¹. In this issue, Wang and St. Leger² report that engineering a fungal entomopathogen to express an insect-specific neurotoxin upon contact with insect hemolymph makes the fungus more deadly to specific insect pests. The boost in insecticidal activity could improve the cost effectiveness of biological control using fungi.

Fungal pathogens naturally attack many insect species, and in some respects they are well-suited to development as biopesticides. Fungi can be mass-produced *in vitro*, stored for long periods and their spores applied with conventional spray equipment¹. Unlike viruses and bacteria, which must be ingested to infect insects, they infect simply through external contact (Fig. 1a). And compared with most chemical insecticides, fungi are less toxic to mammals and have negligible environmental impacts^{3,4}.

One obstacle in exploiting fungi for insect control, however, is that they may kill their host too slowly. Even highly virulent isolates take 2–5 days to kill an insect, and infected hosts can survive much longer, depending on dose and environmental conditions. Wang and St. Leger have developed a method to accelerate the killing speed. *Metarhizium anisopliae* was modified to express an insect-specific neurotoxin (from the scorpion *Androctonus australis*) under the control of

a promoter that is active only in the presence of insect hemolymph. This restricts expression of the neurotoxin to the time after the fungus has penetrated the cuticle (Fig. 1a).

Wang and St. Leger tested the effects of the modified fungus on tobacco hornworm—an agricultural pest and a model for several other defoliating lepidopterans—and on the mosquito *Aedes aegypti*—the principal vector of several important tropical diseases, including yellow fever and dengue. The toxin

dramatically increased pathogenicity (the capacity to cause disease) and virulence (the capacity to kill). For example, compared with the wild-type fungus, the modified fungus achieved the same mortality rates in tobacco hornworm at 22-fold lower doses, and, at certain concentrations, reduced survival times of infected mosquitoes by >40%.

Increasing pathogenicity and virulence has implications for the cost effectiveness of a fungal biopesticide. More rapid killing of

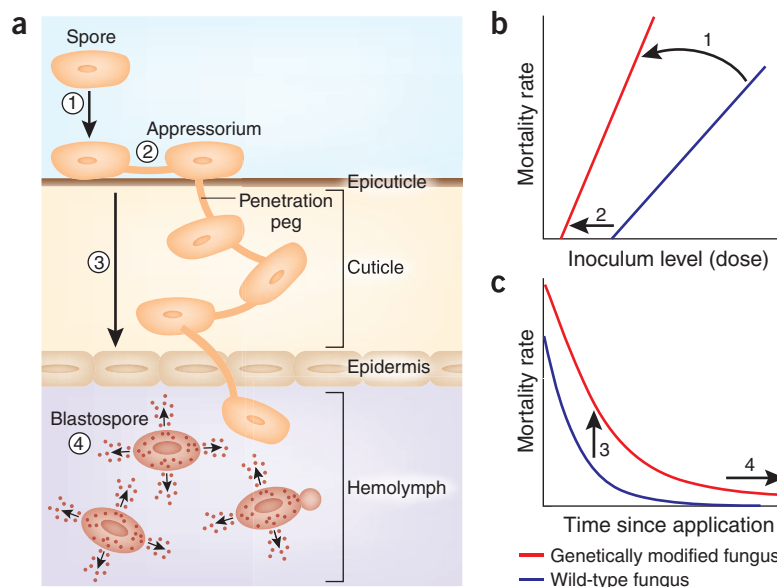


Figure 1 Mode of action of a genetically modified entomopathogenic fungus and the consequences for managing insect pests. (a) After physical contact between a fungal spore and the insect cuticle (1), recognition of the host by the fungus leads to spore germination and production of a penetration structure, the appressorium (2), which grows a penetration peg and a series of hyphal bodies to cross the cuticle and epidermis (3). Once inside the insect, the fungus produces blastospores that bud and spread through the hemolymph (4). Hemolymph-specific expression restricts release of an insect-specific scorpion toxin to the period after infection (from step 4 onwards). (b) Increasing pathogenicity and virulence of a pathogen should alter the dose-response relationship by increasing the killing rate for a given dose (altered slope indicated by arrow 1) and reducing the threshold level of pathogen required to cause lethal infection (shift along intercept of the dose axis indicated by arrow 2). (c) A steeper dose-response relationship should increase the relative pathogenicity of the modified fungus at any given time point (indicated by arrow 3) and so increase the duration of efficacy after biopesticide application (indicated by arrow 4).

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insects should provide better control, and equivalent control could also be achieved using less product (Fig. 1b). The effective persistence of the biopesticide should also increase, even if spores of the genetically modified and wild-type fungi decay at the same rate (Fig. 1c). Long-term efficacy is a key determinant of the frequency of retreatment needed to control vector-borne diseases such as malaria and dengue¹.

More generally, the concept of modifying fungi to express transgenes within the insect host hemolymph could pave the way for innovative control strategies in which pathogens are used to deliver molecules that combat insects, microbes or viruses. Such 'paratransgenic' approaches have yet to be fully explored, but some exciting possibilities include expression of insect-specific immune suppressants, of antimicrobials to suppress endosymbionts and of antiparasite molecules to block the agents of disease within the mosquito vector^{1,5}.

So what are the challenges? One uncertainty is whether increased virulence will enhance selection for resistance¹. Relatively little is known about heritable variation for fungal resistance in insects or about what trade-offs might limit the spread of resistance alleles. It also remains to be seen whether more virulent fungi could impose selection pressures approaching the strength of those imposed by chemical insecticides. Developing sustainable alternatives requires that the depressing fate of earlier generations of pesticides be avoided.

One of the greatest potential advantages of entomopathogenic fungi as biopesticides is their relative environmental safety. Although the overall host range of *M. anisopliae* is broad, individual strains can target particu-

lar hosts. Initial host infection is determined largely by the ecological and physiological processes outlined in the first three steps in Figure 1a. Moreover, because the species does not have a sexual cycle, transfer of an engineered transgene between strains is unlikely.

The authors elected to use a strain with a relatively broad host-range to test the potential of the approach for controlling two different pests. At the same time, they showed that the modified fungus had no effect on two 'nontarget' species. If an insect cannot be infected by the wild-type fungus, any modification of downstream processes should be irrelevant. Nonetheless, certain hosts may become infected by the wild-type fungi but mount an effective immune response, and even highly resistant hosts can succumb to infection given sufficient disease challenge⁶. As with the target species, post-invasion expression of toxin in these species would be expected to increase mortality (Fig. 1b).

That said, risk to a nontarget species derives not only from susceptibility to infection but also from likelihood of exposure. The authors note that this could be minimized by further modifying hypervirulent strains to limit their environmental persistence after their job is done (e.g., by preventing subsequent sporulation on cadavers). Fully quantifying risk to non-target species requires detailed ecological analysis^{7,8}. Of course, determining both the 'potential' and the 'realized' or 'ecological' host ranges of modified fungi will be essential before this approach could be implemented. However, in many contexts, risk-benefit analyses are seldom straightforward; for instance, DDT is still used for malaria control, despite its potential harm to non-target insect species and human health.

Whether transgenic fungal pathogens will become a practical method for insect control remains to be seen. Analogous research to increase the virulence of insect viruses, including genetic modification of baculovirus to express scorpion toxin⁹, has yet to be translated into practice, largely owing to commercial and political issues rather than to technical limitations. Social resistance to genetically modified fungi may prove a bigger hurdle than the emergence of biological resistance. But with increased calls for more sustainable production methods and concern that the effectiveness of current disease-control strategies are being undermined by resistance to synthetic insecticides¹⁰, the need for alternatives to conventional products is clear. Genetic modification to alter a range of traits affecting the cost-effectiveness of fungal insecticides may therefore prove valuable for future pest control.

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