Biological Confinement of GEOs: Opportunities for Reducing Environmental Risks?

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With the introductions of genetically engineered organisms (GEOs) into the environment over the past 20 years, there has been a growing number of questions and concerns about consequences for natural and managed ecosystems. An emerging body of research evidence shows that GEOs are viable and capable of reproduction with wild relatives in natural ecosystems. There is also evidence that transgenes can move from one domesticated variety to another. Also, given the fact that future GEOs may feature traits that have the potential to significantly modify the ecological niche that these organisms occupy, there has been interest in developing methods and approaches to confine certain GEOs and their transgenes to specifically designated areas. Of the various methods available to confine GEOs, those that are biological in nature are of particular interest. These include induced sterility and related methods—approaches that, in some cases, have been applied to non-engineered organisms such as shellfish and crop plants.

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In 2000, the federal government completed an interagency review of its regulatory oversight of biotechnology products. This review revealed that ensuring confinement could become a regulatory requirement for approval of some GEOs. In 2001, the US Department of Agriculture requested the National Academies to review and evaluate biological methods of confinement for GEOs and report on their application in confining transgenic crop plants, shellfish, trees, grasses, fish, microbes, insects and other organisms. This paper summarizes that report (*Biological Confinement of Genetically Engineered Organisms*) with the hope that biological confinement methods for current and future GEOs will be given adequate consideration as mechanisms to reduce environmental risk.

The expert committee convened by the National Academies was asked to address a set of six questions:

- What is the status of scientific understanding about various biological confinement methods for genetically engineered organisms?
- What methods are available, and how feasible, effective, and costly are they?
- What do we know about when and why methods fail, and what can be done to mitigate such failures?
- When these methods are used in large-scale applications, what detection and culling procedures can be used if the biological confinement methods have failed? What is the cost-effectiveness of these procedures?
- What are the probable ecological consequences of large-scale use of biological confinement methods on wild populations, biological communities and landscapes?
- What new data and knowledge are required for addressing any of these important questions?

The report is organized into six chapters. The introductory chapter provides definitions and historical context for GEO confinement. The second chapter addresses the questions of when and why biological confinement ("bioconfinement") should be considered. Chapters 3, 4 and 5 are the heart of the report: bioconfinement methods for plants, animals, and microbes are analyzed and reviewed. The final chapter explores biological and operational opportunities and constraints for bioconfinement, examines the potential for confinement failure and mitigation, and looks to the future in terms of unanswered research questions and needs that should be addressed in order for bioconfinement methods for GEOs to be successful.

Definition of Bioconfinement

There is a fundamental assumption in the report that the movement of GEOs or their genetic material might need to be restricted to designated areas, which in turn creates the need for confinement. Three types of confinement are defined—physical, physicochemical and biological—each involving barriers that prevent the survival, spread, or reproduction of the organism in the natural environment. Bioconfinement refers to the methods that utilize biological mechanisms to achieve confinement.

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When and Why to Consider Bioconfinement?

The report repeatedly acknowledges that many, if not most, GEOs will not require bioconfinement. Each should be determined on a case-by-case basis that takes into consideration the risk associated with the escape of the GEO or its transgenes. The most commonly known environmental risk is that of GE crop alleles being sexually transferred to wild relatives and conferring new traits that result in increased weediness. Other risks include effects of a GEO on non-target species (including humans). These range from potential food-safety concerns associated with crop plants engineered with genes expressing novel proteins not intended for the food chain to GE animals out-competing or driving related and locally rare taxa into extinction.

The report notes that researchers and GEO developers need to consider the role of preventative action; in other words, are there options that might eliminate or mitigate the need for bioconfinement or that may also prevent failure of a given confinement effort? Such an assessment often presents a greater set of options in contrast to the often higher expense of remedial action. The question of "How much bioconfinement is enough?" must also be asked; an appropriate risk assessment, exploring the need for stringency and redundancy, will be influenced by the risks posed by the organism, its biology, the transgenes and other factors.

The consequences of failure of bioconfinement are varied and are difficult to determine in advance. Potential consequences include negative ecological impacts at local to regional scales, and political impacts in instances where bioconfinement failure results in significant impacts for human or environmental health, followed by public outcry or concerns that the failure was due to negligence or inadequate regulatory oversight.

Plants

Many approaches are possible for the bioconfinement of plants, due in large part to the diversity of reproductive strategies they employ. Of the methods discussed in the report, a few are based on existing agronomic and horticultural practices. Other methods are newly developed and untested or are merely working hypotheses. An evaluation of the strengths and weaknesses is included in the discussion of each method. As a whole, the methods typically target sexual and/or vegetative reproduction.

The report also evaluates these methods for their effectiveness at different special and temporal scales, given that they are the equivalent to natural mechanisms of reproductive isolation that act to maintain species barriers. In nearly every instance, bioconfinement of reduced numbers of GE plants planted in smaller regions for shorter periods of time is likely to be more successful than efforts with larger numbers planted over larger areas and when confinement needs to be maintained for longer periods of time.

These issues become more important as genetic engineering is applied to new types of plants, including long-lived species such as trees. Furthermore, new types of GE traits are being developed including traits that produce medicinal and chemical precursors for such products as vaccines and natural rubber. Our ability to combine multiple GE traits that make plants hardier and more prolific also presents potential environmental risks that might warrant bioconfinement.

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Overall, the outlook for confining plants is positive. When recommended methods work as planned, there is no reason to expect environmental problems. However, the report acknowledges that nearly all of the methods have limitations, mostly in the areas of availability and reliability. This is particularly true for long-lived, clonal plants. Another concern that might impact the use of bioconfinement involves the level of public acceptance of sterile seeds in staple food crops. This issue was raised some years ago with the public perception of "terminator" technology that a company considered using to protect their technology and investment.

Animals

For the discussion of bioconfinement of animals, the report limited its focus to insects and aquatic species, in large part because these two categories are active areas in current GE research and development efforts. Consequently, they are likely to be among the early GE products considered for commercialization. Finally, the potential for negative environmental effects from confinement failure is much higher relative to terrestrial livestock species. Of the methods evaluated, the best understood systems are those applied to fish and shellfish species, due to the success of aquacultural programs employed around the world. These methods focus on disrupting sexual reproduction by a trio of approaches:

- sterilization through induction of triploidy;
- combination of triploidy with monosex lines; and
- interspecific hybrids alone or with triploidy.

In most instances, successful bioconfinement relies on the ecological characteristics of the GEO and the production site to reduce escape.

The best developed methods for animals involve the induction of sterility. However, no method is 100% effective, and the success of any method relies heavily on effective screening for failures prior to the release of the GE animals. Some early data involving GE salmon reveal that a high level of screening for triploidy appeared to be cost-effective. As for transgenic methods of confinement, the report acknowledges that they are at very early stages of research, so much needs to be understood before such approaches become commercially viable. One lesson from current aquaculture is that it is very difficult to monitor for failures after commercial release. Overall, animals pose some unique challenges for confinement, but given the level of experience gained from aquaculture, our understanding of bioconfinement methods may be more advanced for GE animals than for the other taxa discussed in the report.

Considerable caution is warranted since relatively little is known of the ecology and evolution of GE microbes.

Microorganisms

The use of GE microbes offers significant potential benefits, given that viruses, bacteria, and fungi are pathogens of insects and of a variety of other pests. Microbes are also capable of degrading certain pollutants. With genetic engineering, this capability could be extended to a broad list of toxins and pollutants in the environment. However, considerable caution is warranted since relatively little is known of the ecology and evolution of GE microbes. Furthermore, since reproduction is asexual in bacteria and often clonal in viruses, the methods developed for inducing sterility in plant and animals are not appropriate for many microbial species.

There are two major categories of bioconfinement methods. One focuses on "fitness reduction" for microbes. With "phenotypic handicapping," the energy costs of expressing the GE trait causes a loss in competitive ability relative to naturally occurring microbes in the environment. The success of this confinement effort depends on the persistence of the GE-microbe population under such a handicap. However, a limitation with this method is the fact that microbes rapidly reproduce and can mutate, so subsequent generations may be more adapted to the environmental conditions and may be capable of coexistence.

The second category, "suicide" genes, is oriented to confining the GE microbes in the wild. The mechanism involves the GEO carrying a suicide gene that is repressed while the microbe is "working"—for example metabolizing a pollutant in a lake—and is activated when the microbe is no longer metabolizing the chemical in question, resulting in programmed death.

One reality regarding GE microbes must be acknowleged. It is virtually impossible at this time to completely eliminate specific genotypes (GE genotypes for this discussion) in natural populations of microbes. This needs to be considered when deciding whether a GE microbe should be released into the environment.

Biological and Operational Considerations

The bioconfinement methods characterized for plants, animals, and microbes share three features:

- all methods have strengths and weaknesses;
- all vary in efficacy depending on circumstances; and
- no method will achieve 100% confinement.

As noted earlier, in many cases GEOs will not require bioconfinement. For all three taxa the efficacy of bioconfinement will depend on the organism, the environment, and the temporal and spatial scales over which the organism is introduced.

Report Recommendations

Given these shared features of bioconfinement methods, the report provides a number of recommendations (in italics):

- *Evaluation of the need for bioconfinement should be considered for each GEO separately.*

The report emphasizes making biosafety a primary goal from the start of developing any new GEO. This will be an efficient and effective way to prevent safety failures.

- *The need for bioconfinement should be evaluated in the early stages of development of a GEO or its products.*

Because methods can fail, and because it is unlikely that 100% confinement will be achieved by a single method, it is thought that redundancy in confinement methodology will decrease the probability of failing to attain the desired result. It is also understood that the spatial or temporal scale of a GEO field release can influence the potential for confinement failure. The appropriate confinement option will depend on scale. Therefore,

- *Bioconfinement techniques should be assessed with reference to the temporal and spatial scales of field release.*

The question of "How much bioconfinement is enough?" is challenging to answer with most GEOs, but with a systematic risk assessment and management approach,

- *An adequate level of bioconfinement should be defined early in the development of a GEO, after considering worst-case scenarios and the probability of their occurrence.*

Following the decision to develop a new GEO, after a risk assessment, and the research to validate the assessment,

- *An integrated confinement system (ICS) approach should be used in deployment of the GEO.*

The ICS includes a number of features familiar to those who use best-management practices in the workplace. The recommended ICS approach includes

- Commitment to confinement by senior decision-makers
- Establishment of a written plan for redundant confinement measures to be implemented, including documentation, monitoring, and remediation
- Training of employees
- Dedication of permanent staff to maintain continuity
- Use of good management practices for applying confinement measures
- Periodic audits by an independent entity to ensure that all elements are in place and working well
- Periodic internal review and adjustment to permit adaptive management of the system in light of lessons learned
- Reporting to an appropriate regulatory body

Looking Ahead

Much of the report focuses on the front end of the process where determining whether, what kind and how much bioconfinement is needed. There is also the need to follow up once a bioconfinement strategy has been deployed with a GEO. Given the relative inexperience we have with GEOs and the deployment of confinement methods in the environment, the efficacy of the confinement system must be monitored. However, this is where our current knowledge is lacking and where our needs are perhaps greatest.

- *Easily identifiable markers, sampling strategies, and other methods should be developed to facilitate environmental monitoring of GEOs.*

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The fact is, the current lack of quality data and science is the single most significant factor limiting our ability to assess effective bioconfinement methods. Methods need to be tested in a variety of appropriate environments and in representative genotypes of the GEO under consideration. In order to implement effective bioconfinement of GEOs, the report recommends support for additional scientific research that

- *Characterizes the potential ecological risks and consequences of a failure of bioconfinement*
- *Develops reliable, safe, and environmentally sound bioconfinement methods, especially for GEOs used in pharmaceutical production*
- *Designs methods for accurate assessment of the efficacy of bioconfinement*
- *Integrates the economic, legal, ethical, and social factors that might influence the application and regulation of specific methods*
- *Models the dispersal biology of organisms targeted for genetic engineering and release, where sufficient information does not exist.*

The objectives for this and any other research on bioconfinement are to minimize the risk or damage to human and environmental health. The success of these efforts will do much to bolster public confidence in the continued growth, development, and opportunities presented by biotechnology.

Further Reading

NRC (2004) Biological Confinement of Genetically Engineered Organisms. Washington, DC: National Academy of Sciences. http://www.nap.edu/catalog/10880.html.

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An ecologist by training, he completed his undergraduate degree in agroecology at the University of California at Santa Cruz in 1990 and his PhD in biological sciences at the University of South Carolina in 1996. He then did postdoctoral research in entomology and taught at the University of Maryland, College Park.

Prior to these academic pursuits, Waddell spent 15 years as a waiter, wine steward, and wine buyer for a number of restaurants throughout the Caribbean and in the San Francisco bay area. He still enjoys good food and wine and is excited to be living on a small farm in the Napa wine country.