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THE IMPACT OF THE TRANSGENIC REVOLUTION ON AQUACULTURE AND BIODIVERSITY. A REVIEW

El Impacto de la Revolución Transgénica sobre la Acuacultura y la Biodiversidad. Una Revisión

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ABSTRACT

We discuss the aims and techniques of transgenics and examine some consequences of incorporating genetic modified organisms (GMs) into the aquaculture industry, as well as introducing them into native habitats. The detrimental effects of introducing transgenic organisms into native habitats include the extinction of indigenous species, the dispersal of transgenes to non-engineered organisms, and in some areas a negative impact on biodiversity. In view of possible adverse effects of genetically modified finfish, shellfish and crustaceans. Programs of monitoring that must carried out before the release of GMs into aquaculture installations, are strongly recommended. Transgenic research with altered temperatures or salinity tolerance should be avoided. Such GMs could enter and persist in communities that are not adapted to their presence. Furthermore it is clearly unwise to cultivate transgenic organims in environments where populations of the same or closely related species live.

Key words: Transgenics, transgens, environment, exotics, biodiversity.

RESUMEN

Se discuten las técnicas y alcances de los trangénicos. Se examinan los pro y contras de la incorporación de organismos genéticamente modificados (GMs) en la industria de la acuacultura y las consecuencias de la introducción de éstos en hábitats nativos que incluye la extinción de especies autóctonas, dispersión de transgenes a organismos normales y, en algunos casos, el impacto negativo en la biodiversidad. En vista de posibles efectos adversos de peces, moluscos y crustáceos geneticamente modificados, se recomienda ejecutar programas de monitoreo, antes de la liberación de GMs. La investigación con tolerancias a cambios de temperaturas o salinidades, debe evitarse. Estos organismos pueden entrar y persistir en comunidades que no están adaptadas a su presencia. Además se debe evitar cultivar organismos transgénicos en ambientes donde vive la misma o especies cercanamente relacionadas.

Palabras clave: Transgénicos, transgenes, ambiente, exóticos, biodiversidad.

INTRODUCTION

Aquaculture and the anticipated export of commodities such as shrimp and salmon have created high expectations with regard to reducing poverty, providing low-cost protein, increasing jobs, and strengthening foreign currencies

However, the fruits of aquaculture have not always been positive. In certain areas, poverty and nutritional deficiencies worsened and traditional patterns of family behavior were disrupted. Moreover, in some parts of South America, shrimp culture led to the destruction of extensive wetlands, in particular mangrove swamps [45]. Such impacts on the environment occurred even though some governments, being aware of the biological and economic importance of these mangrove

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swamps, encouraged the use of lands that have a traditional agricultural profile.

Even the conversion of traditional farmlands into aquacultural facilities is not free of environmental effects. Conversion of farmland often negatively impacts local populations: unemployment increases, the personnel employed in aquaculture is highly specialized and immigrate to the area, and peasants who take jobs as caretakers or cleaners, are poorly paid [40].

The current transgenic revolution—in which genetically modified organisms (GMs) are incorporated into populations of cultured organisms—has created new concerns that need to be dealt with. Technically, GMs, also called genetic engineered organisms or transgenics, are organisms whose genetic construction has been altered by the insertion of small segments of DNA from a different strain of the same or another species (or in some cases, DNA from a different genus, Family, Order, Phylum, or Kingdom).

On the other hand, there are indications that aquaculture is a possible solution, but also a contributing factor, to the collapse of fisheries stock worldwide. For some aquaculture species, as carps and mollusks, which are herbivorous or filter feeders, the net contribution to global fish supplies is great; but for some other species, as salmon and shrimps, potential damage to the ocean and coastal resources through habitat destruction, waste disposal, exotic species, pathogen invasions, and large fish meal and fish oil requirements may further deplete world fisheries stocks [32].

Currently, the US Food and Drug Administration (FDA) is evaluating an application from A/F Protein for a genetically modified salmon that will grow faster and consume less food than its wild relatives. If approved will be the first transgenic fish to reach the marketplace [35]. Also, Cooper and Enright [4], have obtained a patent to produce transgenic catfish and carp that contains a silk moth gene that produces cecropin-B, a lectin molecule which can function as a built-in fungicide and bactericide.

Although genetic engineering can clearly benefit the aquacultural industry, its development and application are tied to the needs of aquaculture industries in the First World. On the other hand, the benefits of genetic engineering to people in Third World countries are unclear for several reasons. Development of trangenic organisms is a high technological, costly enterprise that requires an intensive, controlled industry if adequate return on investment is to be realized. An adequate return on investment may be possible where aquaculture is practiced intensively, e.g., salmonid and prawn culture, but investments are much less unlikely to be viable in developing countries where aquaculture tends to be extensive.

The arguments used to support the Transgenic Revolution recall those that were used to justify the Green Revolution: it will not be possible to feed the growing human populations, expected to reach 8.3 billion by the year 2025, unless agriculture and aquaculture (in a lower scale) productivity rises through the cultivation of genetically modified plants and animals [41].

Although it is true that advances in transgenic organisms have taken place in aquaculture, they have been fewer and less promising than those in agriculture. Among the most successful of the many attempts to enhance fish growth is the work of [9], who report dramatic growth improvement in coho salmon (*Oncorhynchus kisutch*). They used an all salmonid construction, consisting of a sockeye salmon (*O. nerka*) metallothionein promotor spliced to a genomic sockeye salmon type 1 GH gene [10]. They obtained on average transgenic individuals eleven times heavier than the non-transgenic controls. Furthermore, most of the transgenics developed silver body color precociously. Gene transfer has also been accomplished in the channel catfish *Ictalurus punctatus*: transgenic fish containing salmonid growth hormone genes grew 20 to 40% faster than controls [48]. However, this accelerated growth in GH-channel catfish requires supplemental rations, and under a natural feeding regimen, no significant differences were observed between transgenic and control animals [14].

Growth hormone transgenic Atlantic salmon (*Salmo salar*) were produced using a gene construct comprised of an antifreeze protein gene promoter from ocean pout (*Macrozoarces americanus*), and the growth hormone gene from chinook salmon (*Oncorhynchus tshawytscha*). They were reared under temperature and photoperiod regimes which optimize growth, but which inhibit normal smolt development and post-smolt performance of non-transgenic salmon [44]. Fletcher et al [18] inserted an antifreeze protein (AFP) gene from the winter flounder (*Pseudopleuronectes americanus*) into Atlantic salmon (*Salmo salar*) embryos. This transgene integrated in 7% of the flounders. However, levels of AFP in the transgenic flounder salmon and in the F_1 progeny were about 100 fold lower than in the winter flounder. Thus, in order to produce salmon with sufficiently low temperature tolerance for them to be cultivated in cages located in cold areas, a different gene construct with a stronger promoter is required [46]. Significant efforts are underway to increase cold tolerance in other commercially important species so they may be cultivated in cold Atlantic waters [46]. Additionally, there are attempts to produce important pharmaceutical proteins in fish for subsequent purification, *i.e.*, the use of transgenic fish as bioreactors as well as to improve resistance to disease in cultured fish that are stocked in high concentrations [24]. Disease resistance is an important trait that hopefully will be improved through transgenesis, and several laboratories are working on this topic [46].

Accordingly, we would emphasize that in this article we are attempting to describe a balanced and realistic approach to the introduction of GMs in aquaculture—not merely to point out the negatives aspects. To assume the worst without scientific evaluation is as misguided as to assume that the creation and utilization of transgenic organisms will solve the world's nutritional problems.

A BRIEF REVIEW OF TRANSGENICS

Statements that genetic engineering can alter a trait by precisely identifying an individual gene that governs a desired trait, extracting it, copying it, and inserting the copy into another organism (and its offspring) are highly misleading. Such statements imply that genes are not influenced by the environment, by their position in the chromosome, or by other genes. Moreover, it is very important to appreciate that a gene may affect more than one phenotypic character (the so-called pleiotropic effects) and that the result could be positive or negative. For instance, some of the largest GH- transgenic coho salmon displayed a phenotypic syndrome consisting of head, fin, jaw, and opercular abnormalities arising from what superficially appeared to be excess cartilage and bone growth [11]. Effects can be sufficiently severe to impair feeding and respiration, and to cause reduced growth and poor viability [8]. Dunham and Devlin [14] mention another example of pleiotropic effects: GH-transgenic common carp, when compared to controls, develop larger heads as well as deeper and wider bodies.

Genetic engineering is hardly a precise operation, since the insertion of foreign genes into a host cell genome is a random process. One cannot designate the chromosome into which a transgene will be integrated, nor how many copies will be produced. Furthermore, changes in the chromosomal localization of a transgene can inhibit or modulate its expression (*i.e.*, cause a position effect) and, following random integration, transgenic expression may vary according to the chromosome integration site [26]. In addition, position effects are also thought to be one of the factors responsible for the lack of correlation observed between transgene copy number and the level of expression obtained [24]. The logical conclusion is that heredity does not reside solely in the constancy of the DNA in the genome, but in the complex network of intercommunications extending from the ecological environment to the genes. In this complex network, the expression of each gene depends on that of every other gene. That is why an organism tends to change in non-linear, unpredictable ways, even when a single gene is introduced. Unfortunately, current practices of gene biotechnology and biosafety risk assessment are too frequently influenced by the invalid reductionist paradigm in which genes are seen as stable units, separable from each other and from the environment [22].

We believe that the assumption on the FAO/WHO [16] report that genetic engineering does not differ from conventional selective breeding, is erroneous. As [21] indicates, genetic engineering enables exotic genes from viruses, bacteria, and other species to be introduced into GMs. Consider, for example, the introduction of a silk moth gene in catfish and carp [35]. Although using conventional selection Perez and Alfonsi [38] obtained positive results in growth of scallops, they were not as spectacular as those obtained in some experiments with transgenesis [9], however the improved lines obtained by conventional selection are far more stable than those obtained by transgenesis [22].

Moreover, when assessing transgenic organisms (during risk assessment), it is often assumed that transgenes behave in the same way as resident (or endogenous) genes, and that they follow the same principles that were developed from classic genetics. This is not always true. For example, the insertion of a rainbow trout GH- transgene for growth enhancement may alter survival in the common carp. However, the number of F_2 progeny inheriting this transgene is much less than expected. Differential mortality or loss of the transgene during meiosis probably best explain this effect. From fingerling size onwards, survival of the remaining transgenic individuals was higher than that of controls when subjected to a series of stressors and pathogens such as low oxygen, anchor worms, and dropsy [3].

Because risk assessment is based to a large extent on these premises, it is important to point out that transgenes do often display unusually high levels of expression and structural instability. In many cases, loss of transgene expression does not correlate with loss of the transgene but with transgene inactivation, mainly by methylation, a mechanism that probably evolved to neutralize invading DNA. Hence, in some circumstances the organism treats inserted DNA in a manner similar to the way it responds to invading pathogenic nucleic acids [5, 6]. It should be emphasized that is not easy to transfer genes between species because of endogenous cellular mechanisms that excise or inactivate foreign genes. These mechanisms are also responsible for the instability of transferred genes in transgenic organisms. However, vectors can be so well constructed that the cellular mechanisms of the recipient are no longer efficient; thus, the species' ability to resist invasion by some exotic genes is further undermined [22].

TRANSGENICS, NATURAL ENVIRONMENTS AND BIODIVERSITY

There is considerable concern about the unintentional (mainly from aquaculture installations) release of transgenic organisms into the wild and their possible undesirable ecological impacts, including a reduction in biodiversity. Transgenic lines of many kinds of terrestrial livestock, such as feedlot cattle, pose very few ecological risks when introduced into the wild because numerous generations of selection for domestic animals have substantially lowered their fitness in natural environments. However, it is important to point out that fish from artificially selected strains, in most cases, have not been domesticated enough to cripple their fitness in the wild.

Fifteen years ago, the first transgenic fish was produced [52]. Since then, transgenic fish research has grown vigorously. However, the effects of introduced transgenic fish, shellfish and crustaceans on natural habitats are essentially unknown. Whether or not transgenic fish will have a significant impact on the environment is debatable and difficult to predict, although we will try to get inside this problem. The mixing of transgenic fish with the wild-type population may have important implications on the survival of the native species through competition and the conservation of the natural genetic diversity of populations. As Pullin [42] states, the main problem for decision-makers and scientists is that the long-term effects on aquatic biodiversity of the escape of exotics and GMs from aquaculture cannot be predicted with certainty. On the other hand, there are sources from which we can obtain both direct and indirect knowledge that can be useful to deducing the effects of GMs on aquatic habitats. These sources include a) transgenic plants used in agriculture, b) aquatic (non-transgenic) exotics, and c) direct observations in transgenic fish.

Transgenic plants used in agriculture

The Green Revolution caused a reduction in biodiversity in agricultural regions when mixed crops and crop rotation were replaced with monocultures. It is possible that the adverse effects of the Transgenic Revolution on biodiversity will intensify because transgenic monocultures came from a very narrow genetic base. In any case, a GM may be expected to affect biodiversity if it has good abilities to self-propagate, and has locally occurring wild relatives; this will depend on the part of the world that is being seeded. Transgenic potatoes will have low risk of propagating in Europe, but the risk is high in South America, mainly in Peru, where many related species live. The equivalent in fish could be salmonids in South America and North America. Therefore, to cultivate transgenic organisms that easily interbreed with relatives is clearly irresponsible. In Maine (USA), home of a very important salmon aquaculture industry, there is a battle against declining wild stocks of the Atlantic salmon thanks in part to cross-breeding with escaped farm-raised fish of the same species. The farm-raised fish have trouble finding their way back to local streams to spawn and are diluting the wild stock [35].

Concern related to the potential risks associated with genetically engineered crops focuses on the possibility of pollen-mediated escape of engineered genes into populations of the crop's wild relatives. However, is the movement of pollen identical in normal and transgenic plants?. Hokanson *et al.* [23] address this question with regard to melon plants (*Cucumis melo*), where pollen dispersal of the native gene and transgenes are identical. Furthermore, field tests show a high frequency and wide range of gene flow between GMs and normal plants [7, 12, 30, 42, 47, 49, 50, 51]. Although in terrestrial habitats pollen has a greater potential to disperse than sperm, in aquatic habitats, sperm readily disperse in organisms with external fertilization, thereby providing an opportunity for transgenes to pass into natural populations.

The ecological impact of accidentally dispersed transgenes into the environment may be subtle, but it could be important. For example, although plants transformed with genetic material from the bacterium *Bacillus thuringiensis* (Bt) are generally though to have negligible impact on non-target organisms, *Bt* corn plants might represent a risk, because corn pollen is dispersed by the wind, deposited on other plants, and can be ingested by non-target organisms that consume these plants. Losey et al. [28] found that larvae of the monarch butterfly, *Danaus plexippus*, reared on milkweed leaves dusted with pollen from Bt corn, ate less, grew more slowly, and suffered higher mortalities than larvae reared on leaves dusted with unstransformed corn pollen or on leaves without pollen. Clearly, horizontal gene transfer—the transfer of genes by infection between species that do not interbreed—recognises no species barriers, and is inherent to many current transgenic technologies. This is why, to a large extent, transgenic organisms are different (and dangerous) from those obtained by conventional breeding methods [22].

Even though horizontal gene transfers have occurred in our evolutionary past, they were relatively rare events among multicellular plants and animals. Yet we may expect horizontal gene transfer to increase because the vectors constructed for genetic engineering are chimeras of many different vectors designed to transgress species integrity and species barriers; they are therefore capable of infecting many species. In the process, these vectors will recombine with a wide range of natural pathogens. That they have been "crippled" should not lull us into a false sense of security, because it is well-known that they can be helped by other viruses and mobile genetic elements to jump in and out of genomes. Otherwise, it would have been impossible to construct any transgenic organisms at all [22].

The use of genetically engineered crop plants has caused concerns about the transfer of their engineered DNA to indigenous microbes in soil. Evidence of such transfer has been detected in more than 40 species of soil bacteria [27, 33, 34].

Also, it has been demonstrated, in current transgenic experiments in plants, that introducing a single exotic gene into an organism can potentially effect an irreversible impact on the environment. For example, the release of transgenic plants with the *Bt* insecticide led to a rapid evolution of *Bt* resistance among major insect pests [20].

Aquatic (non-transgenic) exotics

We may reasonably expect that the introduction of transgenic organisms into natural environments will have similar (or even worse) consequences than non-transgenic exotics..

We can make no meaningful distinction, in ecological terms, between the release into the environment of a transgenic organism and the release of an exotic organism; hence, the well-known negative environmental effects of releasing exotic plants and animals do not augue well for the future releases of genetically manipulated organisms [37]. Actually, the implications may be of greater significance in the case of transgenic fishes, since most fish, not being domesticated like livestock, survive well in nature and have a high reproductive potential.

At this point we may ask if transgenic organisms would be "good invaders" in natural environments, and, if so, in what ways? Efforts to identify the characteristics of good invaders, such as fast growth rate, broad dispersal abilities, and so on, have been made, but such generalizations are often of little value because they do not take into account the particular community that is invaded. The success of introducing transgenic fish into a natural environment will depend on how transgenes influence specific traits relevant to fitness, as well as if the species has locally occurring wild relatives in the area.

In aquaculture, transgenic organisms are likely to escape from confinement into natural systems. Here they could survive, reproduce, and disperse to other systems, impacting conspecifics and the aquatic community at large.

One method suggested to avoid this situation is to sterilize transgenic fish by ploidy manipulation. However even then, there could be consequences on native fish. The triploid males could develop gonads and produce aneuploid gametes that fertilize eggs, thereby producing abnormal embryos that reduce the number of viable young produced by the population. This sequence of events could lead to a population crash. Yet even in cases where introduced transgenic fish do not reproduce, they could impact the ecology of native populations by competing for resources; and transgenic individuals that bear growth hormone genes could be especially formidable competitors. It follows that in an energy-limited community, the number of native spawners could diminish and the fitness of the population reduced. On the other hand, the sterilization technique is not 100% reliable.

As Perez and Rylander [39] stated, there exists potential for adverse genetic changes in fish populations following introgression, and as a result, potential for negative effects on community structure and species richness. Gene pools are altered by introduction of closely related species when reproductive isolating mechanisms are permeated. Most dangerous for endemic species is the breakdown of the postzygotic barrier. In this case, progeny show reduced viability or sterility. The resulting waste of gametes could be critical and could lead to extinction in the case of massive introductions or in cases where the introduced species becomes well established.

A fertile hybrid may backcross with one or both parent species in the natural environment, thereby genetically contaminating the parent species. Generations of such hybridization and backcrossing erode the genetic constitution of a rare species [39]. Moreover, the release of viable hybrids into the environment could be detrimental to species richness because the hybrids could introduce genetic material into the parental population. This could alter the genetic constitution of the population (especially with transgenes) and destroy the reservoir of necessary genetic variation for resistance of environmental changes.

Direct observations in transgenic fish

Transgenic fish, if mixed with wild types, will lower the variation of natural populations because transgenic lines, being cultured, will probably have been subjected to at least three generations of breeding. Production of aquaculture lines from a small number of transgenic founders would increase the inbreeding and genetic drift effects among non-transgenic cultured stocks. fishes. The escape of transgenic fish from culture facilities into populations of conspecifics could impact wild stocks by producing a diversity of transgenic phenotypes with varying adaptive values among their offspring.

Fortunately, studies indicate that in natural environments some transgenic fishes have a lower fitness than normal fishes. Growth hormone increases metabolic demand, which in turn elevates rate of feeding, thereby increasing an animal's propensity to risk exposure to predation during feeding. This risk does not exist in aquaculture conditions, but could determine a decrease in fitness in the wild. For example, transgenic channel catfish containing salmonid GH genes grow 33% faster than normal channel catfish in aquacultural conditions with supplemental feeding; however, relative to transgenic individuals, non-transgenic catfish fry and fingerlings are more likely to avoid predation by large-mouth bass *Micropterus salmoides* and green sunfish *Lepomis cyanellus* [14]. In the Atlantic salmon, Abrahams and Suitterlin [1] observed that GH-trangenic salmon increase the level of risk these fishes are willing to incur while foraging in the presence of a predator, therefore, increasing the risk of proliferating in natural environments. Furthermore the swimming ability of some GH-transgenic salmon is reduced compared with non-transgenic salmon, thereby making the transgenic salmon more vulnerable to predation [17].

Expression of an introduced growth hormone gene-giving rise to large size could favorably affect mating success for transgenic males. Rapid growth of escaped transgenics could disrupt established populations of similar or unrelated species through competition or even direct predation pressure [29]. Muir and Howard [31] found that Japanese medaka fish (*Oryzias latipes*) that were genetically modified to produce more growth hormone, also matured faster and carried more eggs than the non-GH-relatives. Male GH-transgenic fish, due to their larger size, attracted four times as many mates as smaller rivals. However, only two thirds of transgenics fish survived to reproductive age. Therefore the transgene simultaneously increased transgenic male mating success and lowers their viability. The authors, using a computer model, calculated that 60 transgenic individuals could lead to the extinction of a population of 60,000 fish in 40 generations. The transgene was appropriately called the "trojan gene."

Alternatively, should precocious maturation of growth hormone-bearing transgenic fishes prove common, it seems likely that many escapees from culture situations might reproduce as smaller, precocious males in natural systems.

CONCLUSIONS

Great uncertainty exists as to whether or not a gene-altered organism may adapt to conditions outside the laboratory; in particular whether it may be quickly eliminated, may be cultivated safely, or may encounter no natural controls to restrict proliferation. A problem created by a GM may be impossible to "clean up" in the same way as a problem created by a toxic agent, since, unlike toxic agents, GMs may reproduce and disseminate themselves throughout the environment. In view of possible adverse effects, the incorporation of risk assessment into public policies on genetically modified finfish and shellfish and crustaceans is strongly recommended. At the beginning of the 1980's, when the first GMs were developed, small-scale biosecurity experiments were implemented. However, closed environment experiments differ substantially from large-scale liberation. A test that is "safe" in for a particular environment with a certain climate may not be under other circumstances. On the other hand, the long-term monitoring the release of GMs can only record harm that has occurred, therefore, whereas such monitoring is not useful to prevent undesirable effects, it could be useful to improve risk assessment procedures. Therefore, we recommend programs of monitoring that are carried out before the release of GMs, as pointed out by Pasher and Gollman [36]. These procedures should have flexible designs, which allow their adaptation to developments that were unforeseen at their outset, and they should be conducted by organizations independent of those involved in the commercialization of transgenics fishes.

Our lack of knowledge regarding the reproduction-mediated impacts of transgenic fish is particularly distressing considering the potential importance of such impacts on native stocks. Because it is difficult to predict the spawning behavior of transgenic fish among native fish and the fitness of offspring so produced, measurements of reproductive success in well-isolated experiment systems is needed [22].

The American Fisheries Society recommended that given the current situation of uncertainty, intentional stocking of transgenic fishes in natural waters should be opposed and their use in production scale aquaculture should be restricted [25]. Ten years later we believe this statement is still valid.

Transgenic research with altered temperature or salinity tolerance should be avoided, such as the introduction of the antifreeze protein genes of the winter flounder into the Atlantic salmon [18], or the production of tilapia that are more tolerant to cold environments. Such transgenic fish could enter and persist in communities that are not adapted to their presence. It is clearly unwise to cultivate transgenic organisms in environments where populations of the same or closely related species live.

With regard to the perception of GMs as a health threat, there is a growing concern about the safety of consuming transgenic plants, as well as transgenic fish, especially salmon genetically manipulated to increase growth [2]. In aquaculture, as explained above, the use of viral DNA sequences in construction fusion genes will allow more efficiency in integration. However, its use is not recommendable in fish for human consumption owing to the lack of knowledge on the possible side effects of using viral sequences in genetically engineering food sources [46].

However to conclude that because consuming transgenic tilapia has no effects on human health, indicate no environmental implications for the introductions of this kind of transgenic fish [19] is clearly an exaggeration. The environmental impact of aquatic GMs depends on several factors, including the number of animals involved their geographic and phylogenetic proximity, and their fitness relative to their wild consespecifics.

Finally, we wish to point out that although some people, including scientists such as Dunham [13], have suggested that more efficient food production through the use of transgenic organisms may bring the price of food staples within the reach of more of the world's poor, we believe that the effects will be the opposite. We should keep in mind that GMs will be expensive (Interestingly, the high cost of GMs could be beneficial to the environment as it reduces slightly the risk of inadvertent escapes, [29].

The Transgenic Revolution in agriculture "is being driven by industrial corporations and as an editorial in *Nature* [15] observed: it is difficult to see how the interest of poor farmers will necessarily be protected during this transformation, particularly as most of the research and development currently under way is aimed at intensively commodity farming, primarily in the industrialized world".

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