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Transgenic Plum Tree Tribulations in Romania

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Scattered in an orchard in the Bistrita experimental research station of Transylvania, in the Carpathian Mountains, several dozen trees have their trunk marked with a 'T' in white paint. They are plum trees that have been genetically modified by the Institut National de Recherche Agronomique (Inra) laboratory of Bordeaux, France, so as to tolerate the plum pox virus. The first trees were tested there from 1996, with the support of the European Commission. Ten years later, after citizen pressure, there's talk of destroying some. This article looks back on an experiment far from citizens' eyes.

In spring 1996, a field trial of transgenic plum trees was established in the Bistrita region of Romania. This trial was carried out within the framework of a European research project. Yet, this trial does not resemble the majority of those carried out in Europe. Here, the country concerned is not a member of the European Union, and its legislation is not yet completely in accordance with European legislation. More officially, this trial has not been authorised according to the Romanian law on GMPs (genetically modified plants), and, above all, Romania is a genetic birthplace of plum tree varieties that international legislation must protect.

GMPs in Romania: an opaque and poorly controlled situation

In February 2006, the Romanian Ministry of Agriculture, Forests and Rural Development announced through a press release that the growing and production of genetically modified (GM) soy in Romania is to be banned from 1 January 2007, the date that the country joins the European Union. This decision was made by the Romanian government following a series of consultations with the European Commission and several discussions organised by the Ministry of Agriculture. During these discussions, various actors put forward their points of view. These included Inf'OMG, the Romanian citizen watchdog group on GMOs[1]; Greenpeace Romania; research institutes; consumer protection organisations; members of parliament; representatives of seed companies; companies that process GM soy seeds, etc...[2]

Until recently, Romania was completely on the sidelines of the GMO debates. At the Paris Saint-Denis European Social Forum in November 2003, Avram Fitiu, secretary-general of the Romanian National Federation of Ecological Agriculture (FNAE) gave an account of the spread of GM crops that revealed a serious and uncontrolled situation[3]. The prospect of contamination of the European Union via Romania was discussed. This contamination could represent a strategy to bypass European legislation, by making it face a fait accompli of contamination once the country will have joined. The difficulties faced by economic actors and social movements from Eastern Europe in opposing widespread pollution from non-authorised GMPs in the EU can be understood, given the recentness of the Brazil example. Brazil had resisted against its neighbour Argentina, but it was forced to legislate in favour of GMPs because of contaminations of its soy crops. Romania has a dual system of agriculture: 4 million peasant families whose 'identity has been erased for the last 50 years', according to Avram Fitiu, live alongside the former state farms of 2,000 to 15,000 ha that now operate as huge private enterprises on 2 million ha. The 4500 organic farmers, represented for about the last 10 years by the National Federation of Ecological Agriculture, cultivate 90,000 ha and feel directly threatened by the GMP contaminations.

In May 2003, an initial report[4] warned about an anarchical and worrying situation regarding transgenic crops in Romania. In November 2004, a mission led by the French organisation BEDE , at the request of FNAE, reported the following: *'Romania, third largest European soy producer, has been growing Roundup-resistant GM soy since 1999. Today, out of the 123,000 ha, between 75% and 100% (according to the source) of crops would be either GM or contaminated. In the national catalogue of varieties authorised for crops, 12 new GM varieties of potatoes, soy, corn, and beet could be noted in 2002. Today, no one is able to give a full and exact account of the crops and experiments, because the information is opaque. Among the research projects, two recent ones can be mentioned: in 2004, the Romanian biosafety commission gave a favourable decision concerning a type of genetically modified wheat; further, a big research project for transferring a Bt gene from the Monsanto Company into a local variety of potato is being carried out by the University of Timisoara, along with the support of the World Bank'*[5].

In 2005, a field study[6] pointed out that *'The public research stations are encountering considerable financial difficulties. That's why 80% of the surface area is devoted to seed production and products intended directly for sales for consumption, and only 20% devoted to research. These research centres are then made very weak and exposed to outside pressures that fund research programmes in the fields that interest them, such as GMOs. This is, for example, the case of the Bistrita research station, with its transgenic plum trees, or also that of the Lovrin research station, which devotes one fourth of its surface area (600 ha out of 2,400 ha) to the multiplication of transgenic soy seed for Monsanto. The university research situation is similar. Weakened by their limited capacity to invest in research, the universities sign contracts with private firms'.*

There is a ruling (214/2002) that adapts the Romanian legislation to the new European standards. The biosafety commission, under the supervision of the Ministry of Environment, is made up of 12 scientists, the great majority of whom are favourable to GMOs. It has only a consultative role. No growing conditions are specified for GMOs. Rules and possibilities for traceability and labelling are negligible and not provided for, nor are public participation or transparency in information about locations (despite repeated demand by the FNAE, in order to protect organic crops).

In this somewhat blurry context, there is a Romanian paradox, in which transgenic plum trees are present in a centre of biodiversity, even though transgenic soy has been banned. Furthermore, application of the decision to ban transgenic soy seems like a real challenge for the Romanian government. This situation brings together the elements of what appear to be a textbook study for biosafety in Europe.

Why transgenic plum trees?

The scientific research project devoted to the genetic modification of plum trees to make them acquire resistance to the plum pox virus brings together laboratories from different countries (France, Romania, Spain, the United States, etc.). This work has been financially supported by the European Union since 1996. The first European project[7], carried out between 1996 and 1999, was entitled 'Risk assessment with genetically engineered woody plants expressing virus coat protein gene' under the reference code BIO4960773 and was coordinated by Dr. Ravelonandro from the phytopathology section of the Inra research centre of Bordeaux. It was the occasion for the planting of many transgenic plum trees in Europe, in particular around 100 at the Romanian pomoculture research station of Bistrita, in Transylvania. Other field trials were also carried out in Skierniewice, Poland, and in Valencia, Spain. A second project - Project *'Transvir'* - then took over. This latter is a 3-year project that started in 2003 and that received a 1.47-million euro grant from the European Union. This programme, whose reference code is QLK 3-2002-02140[8], is being coordinated by the French Inra. It concerns the study of the impacts, at various levels, of the expression of these transgenic proteins on the viral populations present in the trial fields. This research programme obviously concerns the transgenic plum trees already in the fields, as well as transgenic grapevine.
The objective of the transgenesis made here is to make plum tree varieties resistant to the plum pox virus. This resistance is acquired through constant expression, inside the plant, of a viral protein whose gene is artificially introduced in order to inhibit the development of the pathogenic virus. Saturating the plant in RNA with the viral protein (RNA made by the transgene) would inhibit, by feedback, the RNA of the virus that would produce this protein and would thereby block the viral cycle.
This strategy was chosen by Inra's phytopathology researchers because, according to them, the other strategies that involve prevention of the disease or for perfection of resistant hybrid varieties are turning out to be ineffective.

After the phases confined to the laboratory, the transgenic plum trees were planted in the field in Romania without any formal authorisation. To date, no study of the impact on the environment with regards to spread of the transgene or of impact on health after consuming the trees' fruits has been conducted. Does this mean that there is no risk tied to this project and to growing the transgenic plum trees in fields?

What sort of disease is the plum pox virus?

The plum pox virus disease mainly targets stone fruit trees, such as plum trees, peach trees, or apricot trees. It originated in Bulgaria and quickly spread to orchards in neighbouring countries. Today, Central Europe and the Balkans are so-called *'endemic centres'*; i.e., the disease is always there residually. Along with large-scale international exchanges, the disease has spread to all industrial orchards. Today, Canada, Turkey, Egypt and Chile are affected[9]. In France, the plum pox virus is a real curse for single-variety industrial orchards. In January 2006, during the 60th conference of the Fédération Nationale des Producteurs de Fruits (FNPF), the French Minister of Agriculture and Fisheries, D. Bussereau declared, *'We are also continuing our struggle against plum pox virus, a significant pest in this region [Isère]. This approach comes fully within the framework of our search for health quality. [...] Funding has already been released: 600,000 euros for funding a research and experimentation programme for varieties resistant to the disease and 900,000 euros for moving tree nurseries outside of risk zones, in order to avoid planting trees struck with plum pox virus'.*

Romania is the world's third largest producer of plums. It lies at the centre of the area where the plum tree was originally domesticated, extending from Georgia to Hungary. As a melting pot of genetic exchanges between spontaneous populations of Prunus and of cultivated plum trees (*Prunus domestica*), it's a pool of genetic resources for the cultivated species. In Romania, plum trees have an important cultural role through the production of palinka, a plum eau de vie at the centre of social relations. Alongside several industrial plum tree orchards severely affected by the plum pox virus, Romania, and especially the Transylvania region, offers a diversity of peasant agrarian systems and of family gardens that include local varieties of plum trees stemming from stones that have a good level of tolerance to the disease. Furthermore, the related wild species that grow naturally around fields can also be healthy carriers.

Scientific basis for the modification of plum trees

Since the beginning of the project, several scientific articles on these experiments have been published[1]. The most revealing is probably the one published in the journal 'Virus Research' in 2000[2], which summarises the results obtained and the objects aimed for.
The programme of breeding transgenic plum trees first of all started through the isolation of a coding viral gene for the coating protein (CP) of the plum pox virus and its transgenic expression in a tobacco plant. Following this stage, a transgene expressing this CP was inserted in plum trees by means of infection by the soil bacterium *Agrobacterium tumefaciens.* This latter, responsible for tumours or galls, has the ability to transfer part of its genetic heritage to certain plants. Different levels of expression of this protein have been obtained. After three years of exposure in greenhouses, a clone from these transgenic plum trees named C-5 was isolated as being resistant to the virus, through extinction of the expression of the wild-type viral protein during the virus' replication cycle within the infected plant. Following these results, the research switched to the field (in 1996), in Poland, Romania, and Spain, where the virus spread. In the fields, these trees showed the same capacity for resistance to the virus. Even though virus replication occurred on some parts of the plant, the plants did not suffer significant development of the disease. This phenomenon was still unexplained at the time of writing this article, but the researchers suppose that the causes could probably be found in the environmental conditions that are different between greenhouse and field. It's thus important to note that the transgenic plants are not free from the virus, but are subject to weak replication of the latter. In its conclusion, this article announces that the researchers will work in the future on evaluating the toxicity and allergenicity of the transgenic fruits. Furthermore, transgenic trees will be developed for expression of the CP only in the vegetative parts of the plant not eaten by man.

1, europa.eu.int/comm/research/quality-of-life/gmo/01-plants/01-11-project.html
2, "The use of transgenic fruit trees as a resistance strategy for virus epidemics : the plums pox (Sharka) model", M. Ravelonandro et al., Virus Research 71 (2000), 63-69

The virus is propagated mainly by aphids that travel from tree to tree. However, there can be other forms of transmission of the virus, such as exchanges of contaminated plant material (rootstock), or the crossing of tree roots that then graft together, leading to transmission of the virus through this path. Finally, transmission through stones, even though possible, seems slight[10].

Ways to fight this propagation already exist. The first step considered is based on field surveys and the cutting down of infected trees and their replacement either by resistant varieties produced conventionally, or by crops that are not targets of the virus[11]. Spread of the virus can also be terminated by using virus-free multiplication material (grafts for example). This type of material can be provided from orchards certified as uncontaminated. These methods of control against the virus rely on a fundamental first stage: field observation. Concerning the use of insecticides against aphids, their effectiveness has been put into question due to the fact that they interfere with other pest-management programmes applied in the orchards, increasing resistance to insecticides and sources of secondary pests[12]. These programmes of pest management are especially developed in single-variety crop zones, which are more often subject to pest attacks.

What safety-first principle for risks that have already been identified?

In 1996, the experiment's establishment year, the Inra researchers had identified several limits to the production of transgenic varieties that are resistant to the virus. Hubert Laude, member of the French Biomolecular Engineering Commission (CGB) and research director at Inra, underlined several of the risks. These include: the irreversibility of breeding transgenic trees: *'a special aspect to take into account in the case of virus-resistant plants is that selection pressure, which determines biological advantage, cannot be suspended or modulated as is the case for herbicide, for example. Because of this, it's hard to imagine a return to the prior situation'*; ignorance: *'The question of the relative speed of the genetic drift of viral populations hosted by naturally resistant wild plants is unfortunately not documented'*; and heightened dangerousness for perennial plants: *'... certain specific situations of transgenic plants can take on a character of novelty that's important to pay attention to. With this regards [...] inducing an infectious state tolerated on a long-term basis on perennial species is an element that generates new interactions or that is likely to increase their frequency'*. The author recommended: *'carrying out specific evaluation of each host system/transgene/virus, given the diversity of epidemiological situations and of applicable strategies [and] and seeing to it that an epidemiology surveillance system can, when appropriate, be set up when a new virus-resistant variety is introduced"*[13].

In late 1999, the risks were identified by Mark Tepfer of Inra: *'The Inra teams from the phytopathology research stations of Avignon and Bordeaux were the first to show that heteroencapsidation (i.e., the forming of the capsid of the virus with proteins expressed by the plant) in transgenic plants does in fact produce a modification of the ability of aphids to transmit a related virus [...]. In transgenic plants, the viral sequences incorporated into the plant to give it a resistance can in turn be incorporated by recombination in the genetic material by an infective virus... The modifications due to the recombination are stable and can lead to the appearance and then the spread of new viruses with properties different from those of the parental viruses. The findings made by Inra (in cellular biology in Versailles and phytopathology in Avignon) have shown that recombinant viruses can produce aggravated viral infections"*[14].

Why then was the risk of experimentation taken? In Spain, a similar application was submitted in 1996 by the Valencia Institute of Agrarian Research (Instituto Valenciano de Investigaciones Agrarias), reference code B/ES/96/16. A second application is moreover on file in the same country, reference code B/ES/05/14, to request field trial authorisation for these plum trees from 1 January 2005 to 31 December 2010. In France, in 1999, Inra Bordeaux submitted an authorisation application to the CGB[15]. This request dealt with planting plum pox virus-resistant plum trees in a nursery. This application B/FR/99.02.10, involving a 4-year project, was postponed by the CGB, which required further information before making a decision. Those informations have never been provided nor was an application resubmitted to the CGB.

Scientific controversy over the appropriateness of the technical choice

There are already plum tree varieties resistant to plum pox virus that have been conventionally bred, such as the German variety *'Jojo'*. Furthermore, scientifically, the effectiveness and profitability of the transgenic technique on plum trees remains to be shown. Frédéric Laigret, director of the research unit for food species and grapevines (UREFV, a unit especially specialised in Prunus), of the genetics and plant improvement department of the Bordeaux-Aquitaine Inra centre, commented on this effectiveness in July 2005 during a visit to his laboratory by Romanian farmers: *'The domestic plum tree is genetically very complex. It's a hexaploid, and its genome is made up of three genomes of three different species. Knowing the abilities of viruses to bypass resistances, the introduction of a single transgene is probably not sufficient, and it would be better to accumulate resistances of different origins, even if partial. Furthermore, the material transformed by transgenesis is not adult material but juvenile material (hypocotyl - coming from the stone). Therefore, its genetic heritage is unknown and distinct from that of the original plant, as it has been obtained by pollination. Transgenesis of commercial varieties has not been mastered to date. It's thus necessary to recross the transgenic plants with these commercial varieties, and this several times, in order to obtain fruit varieties that show resistance to plum pox virus. There's therefore no significant time-saving compared to traditional genetic methods (aided by molecular markers), which consist in crossing individuals having natural resistances or tolerances with the varieties of interest. In my opinion, if there were a material for fruit trees for which transgenesis could provide advantages without risk, it would be rootstock, because it produces neither flowers nor leaves'*. And how will the tolerance evolve with time? How will be the quality of fruit produced by these initially obtained trees? The Romanian researchers of the project mentioned the poor quality of the fruits of Clone C5-10: *'The pulp can't be removed from the stone'*. That's why the researchers at the Bistrita research station immediately made crossings between the initial transgenic clone and local varieties.

The transgenic plum tree field trials in Bistrita are oriented towards effectiveness of the technique on the viral population rather then on the risks of contamination in the environment. But what are these risks ? The conditions of how they were cultivated within the framework of the trials proves that protection of the environment and of human health has been neglected.

A seemingly ineffective biosafty framework

On the legal level, the field trials of this project started in 1996, along with the planting of around 100 transgenic plum trees at the Bistrita research station, without authorisation. The Romanian legislation adopted in 2001 provides for authorisations to be issued by the Ministry of Agriculture and the Ministry of Environment after consultation with two biosafety commissions. When it was adopted, this law was declared retroactive: a request therefore should have been submitted, but this was not done.

Furthermore, the authorisation process theoretically meets European legislation, but its implementation is still partial. According to Professor Pamfil from the Cluj Biotechnology Laboratory, the Biosafety Commission, of which he is a member, never meets. Professor Badea, President of the Biosafety Commission of the Ministry of Agriculture writes the notifications by herself and passes them on as opinions to the entire Commission . Furthermore, it's usually required for the notifications and final decisions to be made public, which is not the case. Growing these transgenic trees in fields is therefore illegal today.

According to the project's scientific coordinator between 1996 and 1998, Maxim Laurel, around 100 trees stemming from the resistant clone were multiplied and planted on four plots of land at the Bistrita research station. Nevertheless, the cultivation itself shows security gaps, since no real buffer zone has been established. This lack of buffer zone leads to legitimate questioning about the risks of the transgene spreading, be it by pollen or by fruit stones that fall to the ground. Nevertheless, these questions do not seem to be part of this evaluation project. A theoretical buffer zone made up of apple trees must present a guarantee against spread by pollen; however, during a visit to the research station by Inf'OMG in November 2005, this zone was not observed. The only real differentiation between the transgenic and non-transgenic plum trees is a 'T' painted in white paint on the trunks of the plum trees (see photo). To better understand the fears generated by this lack of buffer zone, we must remember that scientific findings have shown that the pollen of some trees can travel up to 600 km (*cf. Inf'OGM issue 70*).

Finally, even though the researchers have never produced studies on the toxicity and allerginicity of the fruits, which had nevertheless been provided for in the beginning, Maxim Laurel has revealed that transgenic plums produced by these trees are sold at the local Bistrita market, mixed with other conventional plums. The sales of the plums produced helps to fund the research station; this is essential in the difficult economic context of Romanian public research.

Scientific responses at what price?

According to Marc Fuchs, coordinator of the *'Transvir'* project for Inra Colmar, 'Our preliminary results regarding the impact *of these transgenic plum trees on the viral populations are very promising. They show that the transgenic plum trees do not seem to modify the genetic diversity of the viral populations studied during the Transvir period, especially the emergence of recombinant viruses. [...] The authorisations for the setting up of field trials conducted within the framework of Transvir were issued independently of this project. These authorisations were moreover issued several years before Transvir started up. It's therefore logical not to find the environmental impact evaluations of transgenic grapevines and plum trees carried out within the framework of the process of issuing authorisations, in the Transvir specifications'*.

In the meantime, the *'promising'* results continue to contaminate the nearby crops freely. The researchers thus have a responsibility towards other economic actors with regards to damage that is not technically inevitable.

Further, with EU membership not far off, Romania is trying to reorient its agricultural policy towards organic agriculture. Its objective is 20% of the land in organic agriculture by 2013, which assumes no GM contamination. How can a project like that of the Bistrita plum trees be consistent with this policy? Furthermore, the situation of these plum trees is characterised by lack of formal authorisation; the conducting of a field trial in a country where the watch over GMPs is less strong than in France; lack of a buffer zone to protect the environment; sales of fruits that are unauthorised, unevaluated, and not scientifically studied; and, finally, the use of a technique that risks posing more problems than it seems to resolve. How can the European Union accept to fund this type of project without questioning the conditions under which it unfolds? Romania, like all countries that have signed the Biodiversity Convention, is obliged to protect its centres of biodiversity from any deterioration. How can the government take the risk of contaminating the centre of origin of the plum tree by conducting these trials, despite the irreversibility of contaminations that are so much feared? What is the current state of the spread of the transgene to nearby crops? How can Romanian researchers and farmers be prevented from crossing and multiplying the contaminated plum trees? All so many questions that will have trouble finding answers in the several months prior to EU membership. The research station is now considering the destruction of the source of contamination. This destruction will be carried out in two phases: an initial phase announced in an official declaration by environment wardens will take place in the spring, with the use of herbicides to get rid of some of the young plum trees. The second phase, which has been mentioned orally to D. Craioveanu of the FNAE by the scientific coordinator of the station Mr. Zagrai, will reportedly be held in June. The Bistrita Nasaud region where the experimental research station is located, was declared a GMP-free zone on 14 March 2006, heightening a Romanian paradox that has already been quite well affirmed.

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