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Analysis of the Impacts of Public Agricultural Research

Pre-breeding lines for the creation of potato varieties resistant to late blight and cyst nematodes

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Within the framework of the French Ecophyto Plan, the development of potato varieties resistant to biotic stresses is now an absolute need in order to reduce the use of pesticides. Indeed, potato crops require a considerable use of pesticides (mean Treatment Frequency Index = 16.6), because of their susceptibility to numerous pathogens, including *Phytophthora infestans* (late blight agent) and cyst nematodes. This document highlights the work that has been achieved by INRA since the 1980s in order to find and use appropriate resistance genes present in different wild accessions of *Solanum* tuberous species. During the past 20 years, this research activity has generated 729 innovative pre-breeding lines, 80% of which carry new resistance genes to various pathogens. Since 1995, these lines have been released to French potato breeders, who can now develop new resistant varieties using this innovative germplasm. The potential environmental impact of such varieties is considerable, because they may enable reductions in very heavy chemical applications to soils contaminated by nematodes, speed the recovery of fields placed under quarantine control and assist in the control of potato late blight using only half the amount of pesticides compared to susceptible genotypes.

Context

Potato is the 4th staple food crop in the world behind wheat, rice and maize (FAO-Stat, 2013). There has been a sharp increase in both total world production and global market demand, mainly from developing countries such as India, Bangladesh, Pakistan, China and Brazil. In Europe (the second largest producer in the world behind China), production remains quite stable and and 30% of it is located in a geographical triangle running from London to Paris and to Berlin. France ranks tenth for potato production in the world (7 million tonnes produced on about 170,000 ha by 10,000 growers) mainly in the northern part of the country (Nord Pas de Calais, Picardie, Champagne Ardennes), in Brittany, and in the central region of the country (Centre). About 340 different varieties now cultivated in France by various production sectors: seed potatoes for national potato production or export, potatoes for fresh consumption, or processing potatoes (French fries, crisps, potato flakes and starch). France is the leading country in the world for fresh tuber exports and ranks second for seed tuber exports, behind The Netherlands. French seed potato quality is widely acknowledged and demand from importing countries is much greater than supply, which offers good development prospects if the current acreage (about 18,000 ha) could be increased. However, this would be reliant on different factors:

- The ability of the French seed producer organisation (FN3PT) to maintain a high health status for exported seed tubers and ensure the successful establishment of new potato seed growers;
- The registration and development of new and innovative varieties requiring fewer pesticide applications, meeting the needs of different market sectors and enabling the successful development of French seed potato merchants.

In terms of potato consumption, current French production is sufficient to cover national fresh market demand and exports, but there is a marked disequilibrium between imports and exports with respect to processed potato products, where France is dependent on other European countries.

Potatoes are vegetatively multiplied and are affected by a large number of pests and diseases that may affect yield and/or tuber quality. In intensive production areas (oceanic islands, coastal zones), local sanitary problems are increasing due to shorter rotations. Thus, cyst nematodes (*Globodera sp.*) are an increasing problem in these regions, mainly for seed growers. Indeed, these pathogens are quarantine pests, which results in a complete ban on seed production for many years in fields where these nematodes have been detected. To maintain ware tuber production in highly infested areas, nematicides are used. The three compounds still permitted in France are not very specific and are highly toxic to humans, animals, and also to the environment (Table 1). The development of resistant varieties would reduce the need for these expensive applications (about €3000 per ha in France, including environmental taxes of about €1000 per ha).

However, the most damaging disease for potato production remains late blight. Indeed, the late blight pathogen *Phytophthora infestans* (Pi), which causes foliage and tuber necrosis, is able to destroy an entire susceptible crop in less than one week if climatic conditions are suitable for rapid epidemic development. Pi is present in all potato growing regions. For conventional and organic potato growers, this disease still constitutes a major cropping risk. The crop is therefore heavily sprayed, with a mean Treatment Frequency Index (TFI) of more than 12 treatments that specifically target Pi. This very high TFI was highlighted in the Ecophyto Plan, where potato crops appeared to be one of the arable crops receiving the largest quantities of pesticides. Not only from an environmental point of view, but also in terms of human health, it is now clear that the use of pesticides on potatoes must be reduced. Developing new Pi resistant varieties is therefore a major research objective in most of producing countries.

Table 1 : Risks associated with use of the three compounds permitted to control potato cyst nematodes.

Name of active compound	Associated risk	DL 50 or CL 50 if available
Oxamyl	Harmful in contact with the skin (R21) Highly toxic by inhalation (R26) Highly toxic if swallowed (R28) Toxic to aquatic animals (R51) May cause long-term adverse effects in the aquatic environment (R53)	 170 mg/m^3/h (rat)
Ethoprophos	Toxic if swallowed (R25) Highly toxic by inhalation (R26) Highly toxic in contact with the skin (R27) May cause sensitization by skin contact (R43) Toxic to aquatic organisms (R50) May cause long-term adverse effects in the aquatic environment (R53)	55 mg/kg (rabbit) 2.4 mg/kg (rabbit)
Fosthiazate	Harmful in contact with the skin (R21) Toxic by inhalation (R23) Toxic if swallowed (R25) Danger of very serious irreversible effects (R39) Risk of serious damage to eyes (R41) May cause sensitization by skin contact (R43) Toxic to aquatic organisms (R50) May cause long-term adverse effects in the aquatic environment (R53)	>2000 mg/kg (rat) 230-440 mg/kg (rat)

Demand concerning potato varieties has become increasingly complex because of the constantly growing segmentation of the potato market (fresh market, processing, export). There are now strong political and societal concerns that favour production strategies which limit the use of chemicals, rendering these demands even more challenging. For both the fresh and processing markets, demand is mainly for quite early maturing varieties (80-120 days), with tasty tubers displaying good technological properties and resistance to biotic and abiotic stresses, ensuring a stable and attractive economic return. Overall, more than 70 traits are important and taken into account by breeders when selecting a new potato cultivar. For industrial purposes (starch production), late maturing varieties (120 days or less) are also required because of their high dry matter content. Some Pi resistant varieties have already been released; they are either late maturing types (Hinga) or varieties with specific monogenic resistance genes which are known to be often rapidly overcome by the pathogen. Considering nematode resistance, a major R gene (H1) has been widely used for over 40 years in breeding crosses against *G. rostochiensis,* with very few failures being observed. At the beginning of our work, no varieties were registered with resistance to *G. pallida*.

Inputs and productive configuration

Research in the BAP Division aims to develop new resistant potato germplasm and innovative agricultural practices in order to reduce chemical inputs and achieve sustainable production. Wild species of *Solanum* were introduced at INRA Ploudaniel from different genetic resource centres during the early and late 1980s. This material has been characterised for its resistance to both Pi and nematodes (mainly *G. pallida* and *Meloidogyne sp*.). Amongst the accessions that were introduced, we mainly focused on wild potato species from South America, but some hybrids were also obtained from different research centres and used in the research programmes. Table 2 gives an overview of the different projects during which this research took place and was financially supported. Institutional partners and the close collaboration of plant geneticists, pathologists and nematologists (IGEPP-UMR, RGCO-UE), molecular biologists from INRA in Avignon (GAFL-UR), and collaboration with French private breeding companies, were crucial to developing the necessary knowledge on resistance genes. Indeed, these projects gathered on the same subject people having different abilities and skills which enabled real breakthroughs and stimulated innovation.

The four private potato breeders in France: Germicopa, Bretagne Plants, Comité Nord and Grocep, are all members of a not-for-profit association named ACVNPT (*Association des Créateurs de Variétés Nouvelles de Pomme de Terre*, or Association for Breeders of New Potato Varieties), which was set up in 1966 and aims to promote useful genetic resources for the breeding of new, innovative and resistant cultivars. Since 1995, INRA has stopped its potato breeding activities and new relationships have been established between INRA and the ACVNPT, whereby INRA mainly carries out basic research and pre-breeding, while the private companies breed the new cultivars. An agreement was signed between INRA and ACVNPT in 1995 where the role of each partner was clearly described. Since then, the research carried out by INRA has received significant financial support from the ACVNPT, and in return, ACVNPT members have free access to the pre-breeding material generated by INRA during its research activities with a 5-year exclusivity. A new version of this agreement was discussed and signed in 2008, but the principles remained the same.

The material used in the INRA research programme is quite diversified. For nematodes, a collection of more than 32 *Solanum* species was introduced by INRA from the US potato gene bank at Sturgeon Bay in 1987, and was screened for nematode resistance by the nematologists at INRA Le Rheu under controlled conditions (greenhouse and laboratory accredited to work on quarantine pests). Resistance to Pi was found in a potato population (called A population) assembled at the International Potato Center (CIP) in Peru. This population was developed from a number of wild and cultivated tuber-bearing species of *Solanum*, including *S. andigena*, *S. phureja*, and *S. bulbocastanum*. Different specific clones from this population were introduced into Ploudaniel in 1982 and were screened during field trials for their adaptation to European growing conditions and late blight resistance. Other accessions (mainly old European cultivars partially resistant to Pi) held by the BryACySol Biological Resource Centre (BRC) at INRA in Ploudaniel, were also used during this research programme in order to decipher the genes involved in partial quantitative resistance. More recently, a set of 30 wild tuberous species was screened for Pi resistance in order to broaden genetic diversity and the sources of resistance.

Part of this material was also occasionally screened for resistance to other pathogens: *Fusarium*, PVY and PLRV viruses, *Streptomyces, Pectobacterium* and *Dickeya*, but this work is not the central focus of the present case study.

In breeding schemes, molecular markers are helpful to follow useful alleles. A collaboration between INRA and the FN3PT (*Fédération Nationale des Producteurs de Plants de Pomme de Terre*, or National Federation of Seed Potato Growers) was established 10 years ago to develop such markers for different traits: resistance to Pi, nematodes, bacteria and viruses. This collaboration was reinforced in 2012 with the opening of the INNOPLANT Joint Technology Unit (Unité Mixte Technologique (UMT) INNOPLANT).

Table 2: Some of the research projects important to the development of new resistant pre-breeding lines and knowledge on genetic resistance to pathogens in potato.

Period	Funding sources and contract types	Aim	Principal result(s)	Main partners concerning potato pre-breeding
1989-1993	MAAF - Contrat de Branche	Genetic study of a diploid potato population	Identification of nematode resistance sources	Pathologists and nematologists INRA Le Rheu
1995-2015	ACVNPT - private	Genetic resources preservation and characterisation. Pre-breeding	Creation of 729 pre-breeding lines	ACVNPT
1995-1999	Europe - ResGen	Genetic resources characterisation	Description of varieties for organic and conventional production - European database	JHI Scotland
1997-2000	MAAF - Contrat de Branche	Establishement of correlation between pathogen resistance and suitability for food	No relation between resistance levels and glycoalcaloid contents	Arvalis, Pathologists and nematologists INRA Le Rheu
1999-2003	Europe - EcoPapa	Joint program between Latin America and Europe for breeding for resistance to late blight	Exchange of material and test for P. infestans resistance in various countries and pedo-climatic conditions	WUR, INTA Argentina, Pathologists INRA Le Rheu
2003-2006	Europe - Eucablight	Establishement of a late blight (LB) network in Europe	Establishment of common protocols for material evaluation - Creation of Euroblight network	JHI Scotland
2003-2006	MAAF - Contrat de branche	Preservation of environment by developping LB resistant cultivars	Re evaluation of different varieties for LB resistance	Arvalis, ACVNPT, Pathologists INRA Le Rheu
2003-2006	Europe - Dream	Durable resistance management of the soil-born quarantine pathogen Meloidoyne chitwoodii et M. fallax	Introduction and screening of new species for nematode resistance	WUR, nematologists INRA Le Rheu
2005-2010	Europe - FP6 - BioExploit	Development of LB resistant potato cultivars	14 sources of resistance to Pi identified, one locus identified that explains 20% of Pi resistance on stem	WUR, UGAFL INRA Avigon
2006-2008	ANR - ADD - CEDRE	Durable resistance management in plants	Damage function approach for potato late blight according to genotype resistance level	UMR AGIR INRA Toulouse, ACVNPT
2009-2012	ANR - ARCHIDEMIO	Plant architecture and resistance to aerial pathogens	Study of correlation between potato resistance to Pi and plant development and architecture	Pathologists INRA Le Rheu, BIA INRA Toulouse, ACVNPT
2006-2009	MAAF - Contrat de branche	Interest and limit of MAS for potato breeding	Molecular markers developed for resistance to nematods, late blight and viruses	UGAFL INRA Avignon, FN3PT

Research outputs

The principal results of INRA research were published and contributed to improving knowledge of potato/pathogen interactions. Indeed, we are now able to better understand not only how resistance works but also how it should be used in order to delay or reduce the risks of being overcome.

Three different sources of resistance to *G. pallida* were identified: *Solanum vernei, S. sparsipilum and S. spegazzinii*. The first interspecific crosses were performed in order to introduce the necessary resistance genes into the cultivated potato genome. The pseudo F1 mapping populations (resistant x susceptible) were used to map the loci involved in the expression of resistance (Caromel et al., 2003, 2005). A major Quantitative Trait Locus (QTL) was identified on chromosome V of the three different species and is markedly responsible for the resistance response. Fine mapping of the so-called *GpaV* region in *S. sparsipilum* led to the identification of two R-genes from the NBS-LRR family that are supposed to be involved in recognition of the nematode by resistant plants. Small QTLs also proved to be present in susceptible or resistant genomes and are responsible for a small proportion of the resistance observed. Epistatic interactions were detected between major and minor QTLs and the durability of resistance was shown to be increased when both QTLs (mainly *GpaV* and one minor QTL from *S. sparsipilum* located on chromosome XI, called *GpaXI*) were present in the potato genome. Indeed, when only the major QTL *GpaV,* is present in the material, virulent nematode strains appeared after eight multiplication cycles of the nematodes on resistant plants (under laboratory conditions). By contrast, it has not been possible so far to break the resistance procured by the combination of *GpaV* and *GpaXI* (Fournet et al., 2013). The efficiency of this resistance was tested in a naturally infested field and, whatever the resistance origin, the nematode population decreased after planting a resistant genotype (Mugniery et al., 2007).

Molecular markers were developed to follow the different alleles in progenies. So far, flanking markers have been developed for both the *GpaV* and *GpaXI* loci (Kerlan et al., 2012).

Several backcrosses were needed to remove undesirable wild traits from the first interspecific hybrids between cultivated species and the three resistant sources, while selecting at each step for their resistance to the nematode.

Concerning late blight, our research mainly focused on quantitative, polygenic resistance whose durability has been reported as being greater than the monogenic resistance governed by R-genes which are rapidly overcome by Pi (Pilet et al., 2005; Forbes et al., 2005). Indeed, Pi is an oomycete with strong evolutionary potential and an explosive demography, which gives it an opportunity to overcome the major resistance genes quite easily and rapidly. This work, which is still under way, has enabled the identification of several QTLs which can explain less than 20% of the resistance observed (Marhadour et al., 2014), possibly correlated with architectural traits (Esnault et al., 2014). Molecular markers have been developed to follow some of the alleles of interest. An analysis of the components of resistance (spore germination inhibition, reduction of pathogen growth on stem or leaf, a reduction in sporulation ability) was undertaken to obtain a more precise overview of the function and location of the resistance allele (Danan et al., 2009) on the chromosomes, and to cumulate alleles targeting different Pi traits. This work is still in progress, but improved pre-breeding lines have been released to the breeders throughout the research process. The first registered resistant varieties have all arisen from the A population obtained from CIP in Peru. In this material, Pi resistance is governed by a mixture of R-genes and QTLs.

During or after the research work, crosses and backcrosses enable the creation of pre-breeding lines which are evaluated during field trials conducted at INRA in Ploudaniel. Each year, an average of 30 additional improved lines are described phenotypically. This description concerns:

- The degree/efficacy of resistance;Flowering ability and the possibility to use the clone as a male or female in crossing experiments;
- The degree of ploidy;Agronomic characteristics (earliness, yield, number and size of tubers produced , dry matter content);
- A description of the tuber (skin and flesh colour, tuber shape);
- Tuber quality (frying ability, blackening after cooking, shock resistance).

This work receives specific support from the ACVNPT, and over 20 years this private-public collaborative system has enabled the creation of a collection of more than 729 pre-breeding lines, 80% of which carry new resistance genes (to late blight, nematodes, *Pectobacterium*, *Fusarium*, or viruses). Table 3 gives an overview of all desirable traits that have been released since 1995 by INRA, and the number of pre-breeding lines delivered. This valuable biobank is conserved in the BrACySol BRC at INRA in Ploudaniel, and French breeders can obtain access to the material whenever they express their interest in it. The fact that resistance to late blight, nematodes and bacterial soft rot in these accessions is largely unrelated to anti-nutritional compounds (glycoalcaloids; Andrivon et al., 2003) reinforces their value for breeding purposes.

Different techniques are used to maintain this innovative material at INRA in Ploudaniel. Pre-breeding lines may be field-cultivated through seed tubers. This enables the production and distribution of certified seed tubers that can be useful to obtain flowering plants for crosses in greenhouse conditions. The material is also kept as *in vitro* plantlets (at least 3 plantlets per clone), and a new technology was recently developed in our laboratory for the long term storage of meristems in liquid nitrogen.

Table 3: Number of pre-breeding lines offered by INRA to ACVNPT members between 1995 and 2015. New and innovative traits are carried by these lines.

	Resistant pre-breeding clones							**Pre-breeding clones carrying at least two resistances**			**Pre-breeding clones for good tuber quality**	**Total /Year**
	Late blight resistance		Resistance to nematodes			Resistance to *Pectobacterium*	Resistance to *Fusarium*	Resistance to late blight and *G. pallida*	Resistance to PVY and *G. pallida*	Resistance to *G. pallida* and *G. rostochiensis*		
	from crosses at the tetraploid level	from doubled diploid hybrids	Resistance to *Globodera pallida*	Resistance to *Meloidogyne sp.*	Resistance to viruses PLRV and PVY							
Year 1995	**48**		**30**									**78**
Year 1996												**0**
Year 1997	**22**		**12**									**34**
Year 1998	**12**		**10**			**11**	**12**					**45**
Year 1999	**12**		**8**			**6**	**7**					**33**
Year 2000	**11**		**8**		**4**	**6**						**29**
Year 2001	**20**		**10**			**10**						**40**
Year 2002	**18**		**10**			**8**						**36**
Year 2003	**13**		**10**			**3**	**5**			**6**		**37**
Year 2004	**12**		**8**					**6**		**7**		**33**
Year 2005	**17**		**15**				**4**					**36**
Year 2006	**8**		**7**			**8**		**8**				**31**
Year 2007	**14**					**18**					**14**	**46**
Year 2008	**20**										**7**	**27**
Year 2009	**16**		**11**	**7**								**34**
Year 2010	**18**										**14**	**32**
Year 2011	**19**	**4**			**7**							**30**
Year 2012	**16**	**5**	**6**								**8**	**35**
Year 2013	**10**	**3**		**14**				**1**				**28**
Year 2014	**13**		**11**								**6**	**30**
Year 2015	**12**							**12**	**11**			**35**
Total per type	**331**	**12**	**156**	**21**	**11**	**70**	**28**	**27**	**11**	**13**	**49**	**729**
	629							**51**			**49**	

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Knowledge flow and intermediaries

The pre-breeding lines generated by INRA during its research activities, together with related phenotypic characterization of the material, are delivered to French private breeders under an exclusivity agreement (5 years) regarding their introduction in breeding programmes. The lines are presented to ACVNPT members during an annual meeting that is organised at INRA in Ploudaniel every February. This distribution is granted subject to the payment by ACVNPT members to INRA of an annual participation in research costs and genetic resource preservation. Pre-breeding lines are chosen by the breeders for use in their breeding programmes as a function of their breeding objectives and target markets (fresh, ware, export, fries, crisps, starch) and the description of the material offered by INRA (source of resistance, genealogy, morphological description).

During these annual meetings, a maximum of information is also given to breeders concerning the source of resistance: the number of genes involved, the nature of the resistance, and how it should be used. Whenever possible, molecular markers associated with resistance alleles are also supplied with the pre-breeding lines in order to offer breeders an opportunity to follow the alleles of interest in their seed progenies. This evolution is more recent, and currently registered varieties were not obtained using these molecular markers.

Thanks to their close involvement in the exploitation of INRA pre-breeding lines, the French breeders are able to generate large numbers of seed progenies, among which selection for resistance and good agronomic characteristics can occur. During the step by step annual selection process, the number of observed clones is gradually decreasing (elimination of inappropriate material) while the number of plants per clone is increasing (more repetitions and observations on the most promising clones). This selection procedure is time-consuming, and a minimum of 11 years is needed to select a new cultivar from the initial pre-breeding line. During the last years of selection, the breeders use their own experimental fields to validate the usefulness of future cultivars across a range of pedo-climatic environments.

As indicated in the chronology, the distribution to ACVNPT members of pre-breeding lines carrying nematode or late-blight resistance genes started in 1995. This improved material has been progressively introduced into the crossing schemes of breeding companies, and has been at the origin of new resistant varieties registered since 2008. Six such new varieties were registered and released by the breeding companies between 2008 and 2014 (Table 4). Three of them are resistant to late blight, with a registration score of 8 on a scale from 1 (susceptible) to 9 (fully resistant). These cultivars are Coquine, Cephora and Passion. The other three varieties considered in the present study are resistant to the cyst nematode *G. pallida*: Iledher, Malou and Stronga. Stronga is the first variety to have been registered in France with resistance to both *G. rostochiensis* and *G. pallida*. At least one backcross (for Coquine, Cephora, Iledher, Malou, Stronga) was needed in the breeding schemes to get rid of unfavourable characteristics still present in the pre-breeding lines and obtain material with improved agronomic and technological characteristics. In one case (Passion), two backcrosses and nearly 20 years of selection work were required.

During the cultivar registration process, INRA is again involved in the evaluation of material because Ploudaniel is one of the field experimentation sites used by the GEVES testing network.

Potato scientists from IGEPP-UMR are also involved in the registration process in their capacity as CTPS experts, and they can argue regarding the usefulness of the materials in light of the resistance status of the cultivar and the origin of the resistance genes. They can also comment on how the material should be used and deployed in order to preserve the efficacy of resistance for as long as possible. CTPS registration is a fully formalised, multi-criteria evaluation process during which variety traits are tested and scored. One third of the score concerns yield and productivity, one third concerns plant resistance to biotic stresses and the last third deals with tuber quality. The first resistant varieties registered still had some depreciative traits from the agronomic and/or technological points of view (mainly lower yields and/or tuber blackening), but nevertheless succeeded in obtaining registration thanks to their resistance qualities. One cultivar (Iledher) was registered based on the value of its resistance to *G. pallida* (an entirely new trait among cultivated potato germplasm) despite yield and quality issues. More recently, INRA scientists present on the committees contributed to an adjustment of the CTPS registration rules by testing the effect of awarding bonus/malus points for resistance to enable an increased focus on resistance. This revised scoring system will be used for the first time for the 2015 registrations (see political impacts below).

Arvalis has tested five of these six newly registered potato varieties and the trials confirmed the considerable interest of their deployment in France: a sharp reduction in nematode populations in infested soils when resistant cultivars are used (similar to those obtained with nematicides) was observed, while Pi resistant cultivars enabled a longer delay before the first fungicide spray against late blight and modulation of the frequency of subsequent sprays in almost all production areas. Research programmes and models were developed in order to evaluate the best strategies for the use and deployment of resistance in terms of durability (ADD-CEDRE project, PhD project by Toky Rakotonindraina, financially supported by the ACVNPT and supervised by INRA and Arvalis). However, work is still needed to refine the Decision Support Software (DSS) Mileos in order to take better account of the high level of resistance present in these newly released varieties.

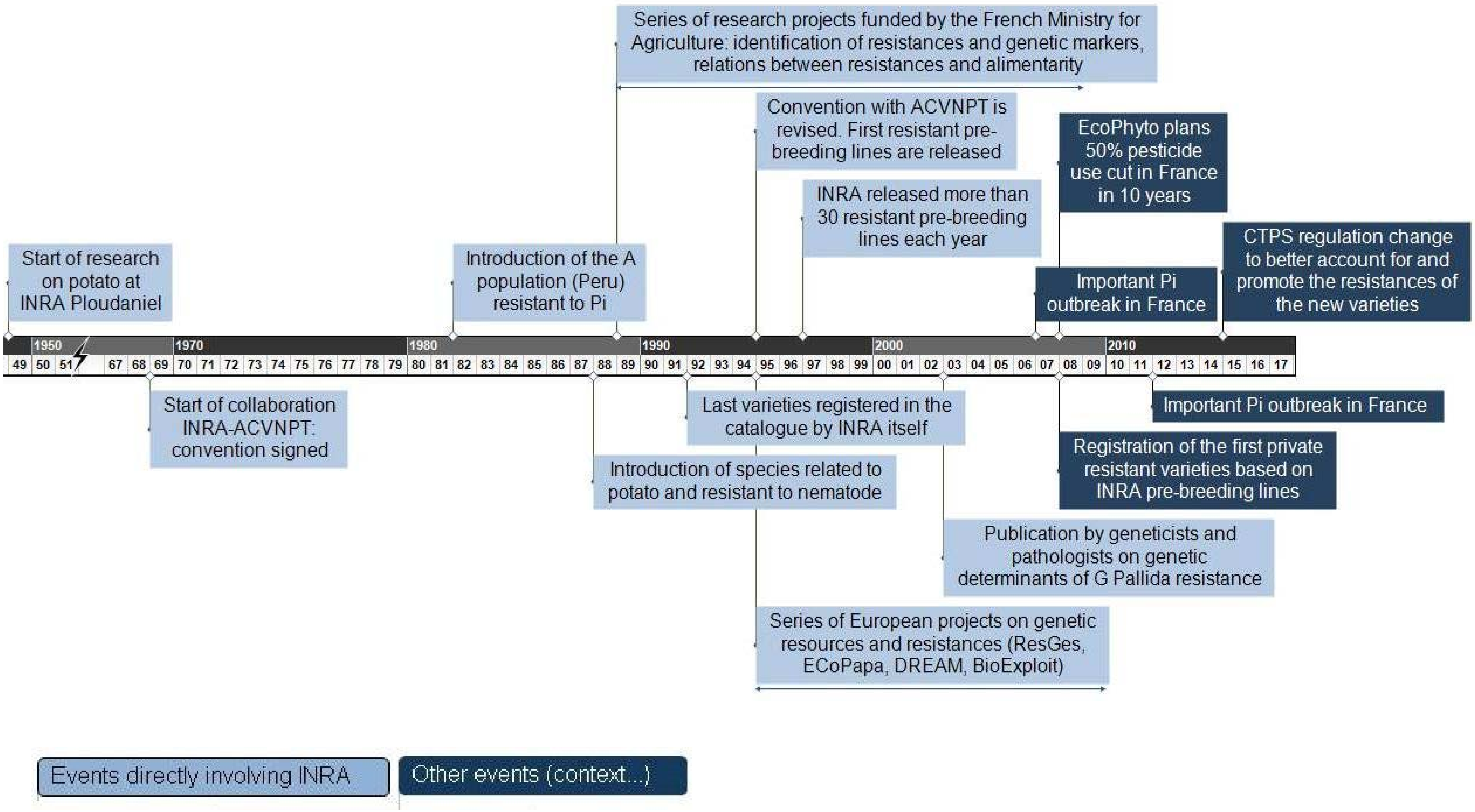
INRA, ACVNPT and Arvalis also deploy an active communication policy (papers, oral communications, technical assistance for producers) in order to promote good agricultural practices (rotations, fewer chemical treatments with the use of DSS, volunteers and the elimination of waste piles) aimed at preserving the efficacy of resistance for as long as possible.

For each new variety, breeders can choose an assignee (producer, producer group or seed merchant) who is responsible for promoting and developing the variety from a commercial point of view. This is not the case for Germicopa, which always promotes and sells its own material. Table 4 gives an overview of the potato breeders and their assignees for the six resistant varieties. The assignee has a major role in cultivar development and communication on how it should be used.

Table 4: List of the six newly registered resistant varieties, the breeders, assignees and principal markets targeted.

Variety name	Breeding company	Assignee	Resistance	Source of resistance	Targetemarket
Coquine	Grocep	Sementis	Late blight	A population	Fresh market, ware, organic production
Cephora	Grocep	Sementis	Late blight	A population	Fresh market, ware, organic production
Passion	Bretagne Plants	Douar Den	Late blight	A population	Organic production
		Van Rijn France			Fresh market, export
Iledher	Grocep	Coopérative de Noirmoutier	*G. pallida*	*S. vernei*	Early potato in nematode infested areas (Noirmoutier, Ile de Ré)
Malou	Germicopa	None	*G. pallida*	*S. vernei*	Mediterranean in nematode infested areas
Stronga	Comité Nord	Desmazières	*G. pallida* & *G. rostochiensis*	*S. vernei*	French fries in nematode infested areas

Chronology


Series of research projects funded by the French Ministry for Agriculture: identification of resistances and genetic markers, relations between resistances and alimentarity
Convention with ACVNPT is revised. First resistant pre-breeding lines are released
EcoPhyto plans 50% pesticide use cut in France in 10 years
INRA released more than 30 resistant pre-breeding lines each year
CTPS regulation change to better account for and promote the resistances of the new varieties
Start of research on potato at INRA Ploudaniel
Introduction of the A population (Peru) resistant to Pi
Important Pi outbreak in France
1950
1970
1980
1990
2000
2010
49 50 51 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 00 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17
Start of collaboration INRA-ACVNPT: convention signed
Last varieties registered in the catalogue by INRA itself
Important Pi outbreak in France
Introduction of species related to potato and resistant to nematode
Registration of the first private resistant varieties based on INRA pre-breeding lines
Publication by geneticists and pathologists on genetic determinants of G Pallida resistance
Series of European projects on genetic resources and resistances (ResGes, ECoPapa, DREAM, BioExploit)
Events directly involving INRA
Other events (context...)

Impacts 1

Economic

The economic impact of the six resistant varieties has been relatively limited for breeders and their assignees up to now, because their registration is quite recent. It should however increase in future years, given their innovation potential and according to the marketing efforts being made for their deployment. Moreover, these resistant varieties are only the first ones, and presage a more marked shift towards the registration of resistant varieties in France in the near future. Two more resistant cultivars, Tentation and Maiwen, were registered in 2015, both resistant to late blight. Other candidate varieties are currently undergoing their 2-year evaluation process in the GEVES experimental network before registration. Table 5 gives an overview of the agricultural areas dedicated to seed tuber production for the ten top French varieties and the new resistant varieties.

The royalties received by breeders concerning these varieties represent a maximum of 7% of the total royalty income of each company, and together amount to less than €50,000 for the 35 ha of seed tubers produced. The turnover achieved by assignees on these 35 ha of resistant varieties (consisting of royalties paid by multipliers) is of approximately the same value. Overall, an economic surplus of €100,000 was generated in 2014 alone in the seed production sector thanks to the resistant varieties.

For growers, the gain is (or could be) greater. Indeed, the actual seed production of 7.7 ha of varieties resistant to *G. pallida* in 2014 (Iledher, Malou, Stronga) will enable the planting of about 150 ha of potato in infested soils without any treatment. This represents a saving in treatment costs of about €460,000 for potato growers.

For late blight, this type of calculation is also possible. If we consider that the number of treatments needed to control late blight in resistant cultivars in association with the DSS ileos can be halved, then it is currently possible to save about €170,000 with 28 ha of seed of the resistant potato varieties available.

Table 5: Agricultural areas dedicated to potato seed production for the top ten potato varieties, and the newly registered resistant varieties. The total acreage for tuber seed production in France is more than 18,000 ha.

Variety name	Registered	Breeding company owning the Proprietary Variety Protection, if not yet in the public domain (<30 years)	Area of potato seed in 2014 in France	Percentage of the total	Trend
Top ten					
Spunta	1967	Public	1 568 ha	8.4%	Increasing
Bintje	1935	Public	934 ha	5%	Decreasing
Agata	1990	Geerstsema Zaden Pays-Bas	706 ha	3.8%	Stable
Charlotte	1981	Public	537 ha	2.9%	Stable
Monalisa	1982	Public	511 ha	2.7%	Increasing
Markies	1998	Agrico Pays-Bas	507 ha	2.7%	Increasing
Kaptah Vandel	1965	Public	493 ha	2.6%	Decreasing
Lady Claire	1997	Meijer BV Pays-Bas	485 ha	2.6%	Increasing
Amyla	1999	Germicopa	437 ha	2.3%	Stable
Amandine	1994	Germicopa	399 ha	2.1%	Increasing
Total			6 577 ha	35%	
Resistant varieties					
Coquine	2008	Grocep	20 ha	0.1%	Increasing
Cephora	2013	Grocep	2.6 ha	< 0.1%	Increasing
Passion	2014	Bretagne Plants	5 ha	< 0.1%	Increasing
Iledher	2009	Grocep	1.7 ha	< 0.1%	Decreasing
Malou	2011	Germicopa	5 ha	< 0.1%	Increasing
Stronga	2014	Comité Nord	1 ha	< 0.1%	Increasing
Total			35 ha	0.2%	
Other late blight resistant varieties					
Tentation	2015	Grocep			
Maiwen	2015	Bretagne Plants			

In general, breeders are cautious when considering the possibility to exploit and develop the new resistant cultivars in the near future. Some of these cultivars still retain certain undesirable traits inherited from the wild resistant parent (lower yields, tuber blackening). Moreover, and until now, the market did not consider disease resistance traits as being essential but, at most, as an additional and optional bonus which is frequently not even used as a commercial argument. The development of resistant varieties is still following the same route as other cultivars. However, the situation is evolving in this respect, mainly because of changes to the production rules that can accelerate the use of resistant varieties. For instance, since 2013, Dutch growers have been obliged to cultivate only resistant varieties in nematode infested soils, which will probably speed up the release and use of *G. pallida* and *G. rostochiensis* resistant cultivars in this country.

All six resistant varieties are now in a build-up phase. After some initial difficulties because the first assignee went bankrupt, 'Coquine' is now being cultivated on 20 ha for seed tubers, and developed by Sementis. It is being grown in France but has also produced good results in Switzerland. It is of interest to organic producers and the fresh market. 'Cephora' is more recent but it is also being tested in other countries (Belgium, Poland) where has produced some attractive results. It is tasty, with good tuber presentation. 'Passion' has some particularly attractive features. It is not only a late blight resistant cultivar but it is also highly productive (132% of the control). In France, Passion was first developed by Douar Den as an organic cultivar. However, it is now also being multiplied by Van Rijn France for conventional cropping and authorizations for its cultivation are currently being requested in Algeria, Morocco and Brazil. In this last country, a partner assignee has been found to develop the variety in South America. However, the resistance of this variety to late blight needs to be verified in this region because Pi isolates may differ in Europe and South America. Considering the *G. pallida* resistant varieties, 'Iledher' has experienced some problems in finding its market. On the French west coast it is not considered to be very attractive because it differs too much from the tubers that are usually grown and produced in these regions (Bonnotte, Charlotte, Sirtema, Lady Christl). After this failure, a new market is now being explored in the Netherlands where nematodes have been detected in fields, but Grocep is also looking for a new assignee. 'Malou' was registered in Portugal in 2011. However it is also grown in France, Switzerland, Italy and the United States of America. In this last country, it is appreciated for its good presentation and taste of its tubers (chalky). 'Malou' has a strong potential for development in Italy where it is well suited to the growing conditions, but development is also foreseen in Greece and Albania. 'Stronga' was registered very recently but has been tested by McCain for several years. It is a variety designed for industry (French fries) with resistance to both *G. pallida* and *G. rostochiensis,* and it is also resistant to PVY and common scab. Its target market is northern countries of Europe (United Kingdom, Netherlands).

One important point raised by breeders is that the resistant varieties they have obtained, even if they are not well developed for the moment, constitute a very efficient bargaining tool to obtain access to other variabilities present in the genepool of foreign breeding companies. This offers an "exchange currency" to obtain access to other resistance sources held by competitors. Moreover, the registration of a resistant variety in the EU offers an opportunity to broaden the range of trading partners for French breeding companies and obtain recognition at the international level that French companies are capable of doing a good job in terms of breeding.

For now, the new resistant varieties have not yet affected the organisation of breeding companies or their assignees. However, one person has been employed by Grocep to manage the resistant material. At Germicopa, the number of resistance tests for nematodes has increased from a dozen to several hundred each year during the past decade. The proportion of breeding activity based on INRA material ranges from 15% to 80% depending on the company, with an average of 44%. Without the input of INRA pre-breeding activities, the situation of the French companies would be quite different at present. For example, Grocep is very closely involved in exploiting INRA material, which accounts for 80% of its breeding activity and employs six people for this purpose. This percentage falls to 50% for Bretagne Plants, 30% for Germicopa and 15% for Comité Nord. This figure is rising in this last company, but has remained stable in the three others. The development of resistant varieties is too recent to have had a clear impact on the organisation of assignees or with respect to the recruitment of commercial staff.

Environmental

The pre-breeding lines produced by INRA represent a considerable broadening of the genetic diversity available for breeding purposes. The work achieved has not only permitted the exploitation of existing diversity; it has also created new biodiversity by associating alleles from interspecific crosses. Varieties resistant to cyst nematodes have a major advantage in terms of environmental value. Indeed, the field trials carried out by INRA, the Coopérative of Noirmoutier, the Chamber of Agriculture in the Nord-Pas de Calais region and Arvalis have clearly shown that resistant varieties can markedly reduce cyst nematode population levels. When planted in an infested soil, resistant tubers activate hatching of the cysts present in the soil due to secretion of active compounds by the roots of the plants. The juvenile nematodes that are released evolve into males or into very few females which are rapidly blocked in their development, due to the plant recognition and defence reaction. This leads to a tremendous reduction in the nematode population, comparable to that observed with pesticides. In the Netherlands, similar observations were made using the *G. pallida* resistant cultivar Innovator. In this country, the use of resistant cultivars is now mandatory when the soil has been proven to be infested by cyst nematodes. In view of these results, the use of resistant cultivars could entirely circumvent the need to use nematicides. However, in experimental conditions on cv Iledher (resistance from *S. vernei*), virulent nematodes were selected by INRA after eight consecutive cycles of multiplication of the nematode on the resistant cultivar (under artificial conditions in the laboratory). Therefore, good agricultural practices such as rotations lasting more than five years and gene stacking are needed in order to prevent the appearance of virulent populations. However, resistant material can be used in nematode infested areas, either to be able the maintenance of tuber production or as trapping plants to reduce the size of pest populations before potato plantation.

In the case of late blight resistance, quantitative resistance was favoured in our research strategy. In this situation, Pi is thus able to develop on resistant plants but much more slowly than on susceptible varieties. During this co-evolution, however, increasingly aggressive strains can appear over time (Andrivon et al., 2007). For this reason, chemical treatments are still highly recommended in the event of strong primary inoculum occurrences, to enable the combined management of host resistance and pesticide resistance. Table 6 (containing data from CTPS experts) provides an overview of the gains that can be achieved using resistant varieties, as a function of their initial resistance level.

Table 6: Number of chemical treatments required according to Pi resistance levels, as applied by CTPS experts during their work on the new rules on VATE.

Resistance score (1 susceptible – 9 resistant)	**Number of chemical treatments needed (estimates)**		
	Year highly favourable to an epidemic (e.g. 2007-2012)	Normal year	Year unfavourable to an epidemic (e.g. 2003-2005-2009)
7-8-9	**16**	**9**	**3**
6	**17**	**10**	**6**
5	**18**	**12**	**7**
4-3	**18**	**13**	**10**
2-1	**20**	**14**	**10**

Field experiments have proved that these estimations are not sufficiently optimistic. In practice, and under normal contamination rates, the number of chemical treatments can be decreased to 7 or 8, even 6 or 5; i.e. a saving of 2 to 7 treatments each year. In some situations (Centre), Coquine is produced on more than a hundred hectares without any applications of pesticide against Pi. However, this should not be recommended because i) this may favour the appearance of aggressive and virulent strains if Pi inoculum is present, ii) this also favours the appearance of other fungi and diseases such as early blight, and iii) there is no correlation between leaf and tuber resistance to late blight, so that a mild infestation on the leaves might result in tuber rotting at harvest or in storage. Varieties carrying different resistance genes are also required so as to be able to alternate the use of different, favourable alleles.

Political

From a political point of view, this new resistant material (pre-breeding lines) released by INRA offers a timely opportunity to encourage growers and the entire potato chain to reduce pesticide use within the framework of the National Ecophyto Plan. If the number of resistant varieties continues to increase during the coming years (and this should be the case), then it will be possible and easier to regulate this process.

INRA experts in the CTPS potato section made a major contribution to the formulation of new rules, which included the awarding of a bonus/malus for resistance characteristics. These new rules will be applied by the end of 2015 for potato variety registration. INRA has largely supported this implementation by releasing resistant pre-breeding lines during the past 20 years, thus facilitating the compliance of breeders with the new CTPS regulations.

Sanitary

From a sanitary point of view, varieties resistant to cyst nematodes are less dangerous because seed tubers are not supposed to carry the pests. Moreover, in a naturally infested field, the use of nematode resistant varieties enables a reduction in the size of the nematode population. However,, there is no proof that Pi resistant cultivars are not deleterious in terms of disseminating Pi, mainly because there is a lack of correlation between foliage resistance and tuber resistance. This requires further investigation during future research projects.

Social, territorial

On the islands off the French west coast (Ré, Noirmoutier), where agriculture is highly dependent on potato crops, nematicides are still allowed by special dispensation. However, they are highly toxic to humans and the environment, and they should be banned in the near future. The registration of new resistant cultivars will then be the only way to sustain potato production in these areas.

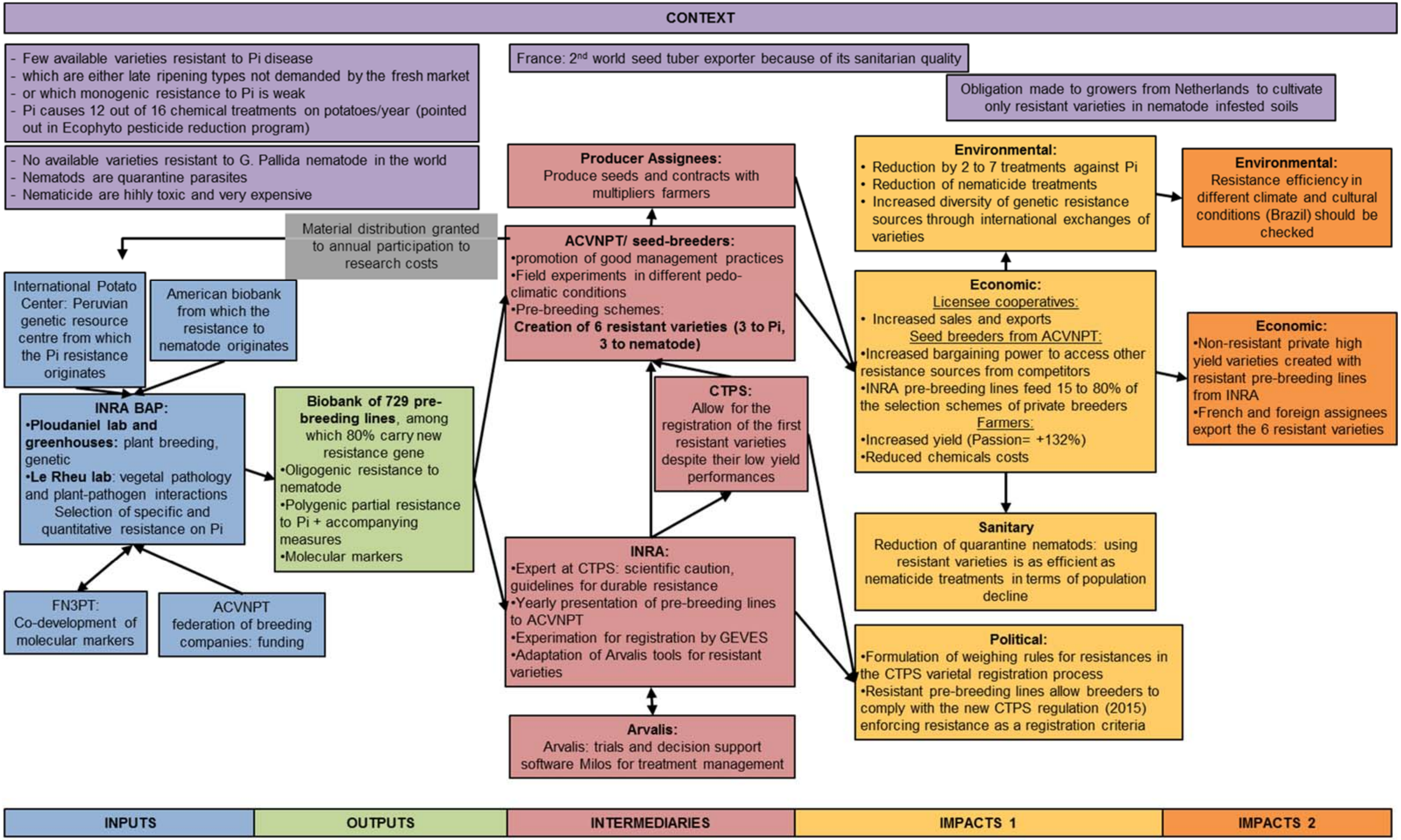
Impacts 2

The distribution of pre-breeding lines to French breeders has not only enabled the creation and registration of new resistant cultivars to Pi and cyst nematodes. It has also led to a broadening of the genetic variability available and the registration of new innovative cultivars that can be resistant to other pathogens. Indeed, three breeders each registered two new varieties, some of which have a potentially encouraging international career ahead of them. Current seed multiplication acreages are given in Table 7 for these new varieties. To develop 'Florice' (export type) for instance, a specific company was set up: Maritime Plants. For this cultivar, there is considerable potential for tuber exports to Turkey (1500 tonnes) and Algeria. 'Touareg' is considered to be better than its direct competitor cv Spunta (the leading variety for tuber export in France). 'Gwenne' is a very tasty cultivar that is resistant to *Fusarium* (this resistance is also arising from the INRA progenitor), and which is easy to use and stable, a high yielding in all European countries, from Sicily to the Netherlands, as well as in the United Kingdom and Japan. 'Captain' has been registered in Portugal and is well suited to southern European countries (Portugal, Italy and Greece). However it is also demanded by bakers in the UK. 'Gazelle' is registered in the Netherlands and also has a market in North Africa countries. 'Orlane' was more recently registered in the Netherlands and displays limited resistance to late blight.

Table 7: Agricultural areas dedicated to potato seed production for the varieties obtained from INRA genitors but with no (or limited) resistance, or resistance to *Fusarium* (cv. Gwenne)

Varieties' name	Registration date	Breeding company that owns the Proprietary Variety Protection	Area of potato seed in 2014	Percentage of total	Tendancy
Florice	2001	Comité Nord	77 ha	0.4%	Increasing
Touareg	2009	Comité Nord	34 ha	0.2%	Increasing
Gazelle	2013	Grocep	12 ha	< 0.1%	Increasing
Orlane	2014	Grocep			Increasing
Gwenne	2010	Germicopa	30 ha	0.2%	Increasing
Captain	2015	Germicopa	1.5 ha		Increasing
Total			154.5 ha	0.8%	

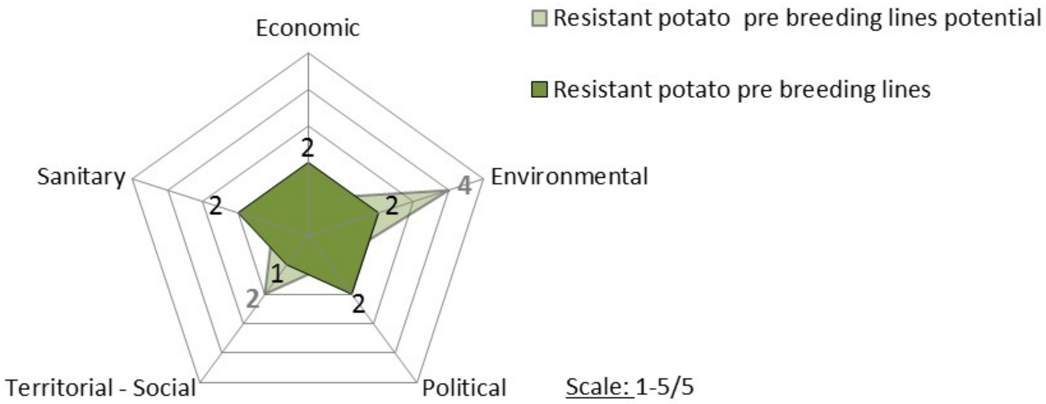
Impact pathway



INRA SCIENCE & IMPACT

Impact vector

Impact dimension	Importance (/5)	
Economic	2	• Between 2008 and 2014, six new varieties were registered in EU, all resistant to late blight or cyst nematode *G. pallida* and based on pre-breeding lines released by INRA between 1995 and 2002. They already accounted for 35 ha of seed production acreage in France in 2014 and are also being tested or cultivated in more than a dozen countries. Some of these resistant varieties can achieve high yield gains (e.g.: Passion, 132% of the control). • Around €100,000 of turnover were generated by breeders and assignees in 2014 with these six resistant varieties alone, • Potential savings worth €630,000 in terms of pesticide treatments against cyst nematode and Pi were achieved by potato producers in 2014 alone,Resistant varieties act as a powerful bargaining tool for exchanges between international breeders, • The percentage of breeding activity based on INRA material ranges from 15% to 80% among the four French breeders. Six jobs and turnover worth €300,000 for these SME
Environmental	2 Potentially 4	• Recommendations that can avoid 2 to 7 treatments against Pi when growing resistant varieties under normal contamination rates. • Nematicide applications may not be necessary at all, although resistance appears after eight cycles of nematode multiplication the resistant cultivars. Recommendations include appropriate rotations and the using of resistant varieties as trapping plants to reduce the parasite population. • The varietal creations based on resistant pre-breeding lines that have been built using two original sources from Peruvian and American databases have contributed to increasing domestic biodiversity. Moreover, the resistance to Pi and cyst nematodes originates from a mixture of R- genes and QTLs, thus increasing its durability. International varietal exchanges further increase the diversity of genetic resistance sources.
Political	2 / 5	• Contribution to formulation of the new CTPS regulation awarding a bonus to resistant varieties • Facilitation of breeder compliance with this regulation through the release of
Sanitary	2 / 5	Varieties resistant to nematodes can enable a reduction in the pest population similar to that achieved with pesticide treatments.
Territorial-social	Potentially 2	When synthetic nematicides are banned, resistant varieties will be the only way to maintain potato production on the islands off the French west coast.



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List of interviews performed:

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- Jean-Marc Abiven, Bretagne Plants
- Dominique Fagot, Comité Nord
- Frédérique Aurousseau, Comité Nord
- Jean-Denis Moal, Comité Nord
- Gisèle Lairy, Germicopa
- Hervé Dubreuil, Grocep
- Dominique Ruer, Coopérative de Noirmoutier