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INRA and Apple Disease Research in the Loire Valley Region of France

The Loire Valley Region

France's Loire Valley region frequently evokes images of châteaux, those grand homes and historic structures encompassing immense medieval fortress-like castles and extravagant renaissance mansions alike. A visitor from abroad can't help but be impressed with each Loire Valley château: from the imposing castle of Angers, housing its immense medieval tapestry of the apocalypse; the elegant feminine mystique of château de Chenonceaux, built over a river; the sheer enormity of Chambord with its Leonardo da Vinci double-helix staircase; Chinon, site of the historic meeting between Joan-of-Arc and Charles VII; Sleeping Beauty's fairy tale castle, Ussé; to the geometric or playful gardens found at Villandry or Chaumont sur Loire, and many, many more (Fig. 1). *Mon dieu, qu'ils sont magnifiques!*

Although the Loire Valley is famous for its picturesque and historical châteaux, it is also a region rich in horticulture, often called the "*Jardin de la France*" (Garden

of France). The Loire, France's longest river, flows roughly east to west through central France past cities such as Roanne, Orleans, Blois, Tours, Angers, and Nantes before discharging into the Atlantic Ocean. *Voilà une très belle région!* The region is well known for its grapes and wine, pears, apples, strawberries, asparagus, lettuce, artichokes, and field and greenhouse nursery crops and ornamentals. Although apples are also grown elsewhere in France, such as in the Bordeaux region, the Rhone Valley, and Provence, the Loire region is the top producer; France produces about 25% of the apples in the European Union (3) and is a net exporter of apples.

During the late 1990s, the American authors (J. R. Hartman and S. Thomson), while on sabbatical leaves, worked in the laboratories of the French authors (L. Parisi and J.-P. Paulin) on apple diseases. In this article, we report on the French apple industry and describe the national institute for agricultural research and its role in understanding apple diseases. We review results of work on apple scab and fire blight in France and provide impressions from the point of view of American plant pathologists working in France.

Loire Valley Apple Production

Imitated worldwide, the densely planted, high hedgerow apple growing method, sometimes known as the "French axe" or *axe central* is used throughout the Loire Valley. This system, in which apple trees are planted little more than a meter apart and supported by a two- or three-wire trellis, induces precocious fruiting and increased yields per hectare. These intensively managed orchards are usually drip-

irrigated and often covered with extensive nets to protect the apple crop from hail (Fig. 2A to C). Golden Delicious and its variants are most commonly grown, but there are many other cultivars grown as well. Table 1 lists some of the more common apples grown in the Loire region (1).

Apples are sold in the supermarkets and in outdoor markets held regularly in every city and town. The tradition of outdoor markets (Fig. 2D), where one can buy breads, cheeses, spices, fresh seafood, and meats as well as fruits and vegetables, dates from medieval or earlier times. French apple growers are attempting to educate their customers on the use of different apple cultivars. A label on a bag of apples often contains useful consumer information (Table 2). *Bon appétit!*

Apples, like other agricultural commodities, are subject to important diseases that, if not managed, can limit production and profitability. In France, there are several research and educational institutions that provide research-based plant disease information and advice to the apple industry. Perhaps the most prominent and best known of these is INRA.

INRA—"From the Molecule to the Marketplace"

What is INRA? INRA is the national institute for agricultural research in France; INRA is an acronym for *Institut National de la Recherche Agronomique*. It is placed under the joint supervision of the Ministry of Higher Education and Research and the Ministry of Agriculture and Fisheries. INRA's efforts fall under five scientific directorates: Environment, Forests, and Agriculture; Plant and Plant Products;

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Animal and Animal Products; Human Nutrition and Food Safety; and Society, Economics, and Decision-Making. Within the five scientific directorates, there are 17 scientific departments; most plant pathologists work within the Department of Plant Health and Environment.

Mission. Working in both a European and worldwide context, INRA's mission is to provide solutions to current and potential (agricultural) problems of major significance (2). This mission includes the following: To ensure better nutrition for people and preserve their health. To sensibly develop and manage land and the environment. To promote scientific and technological innovation, particularly in the life sciences, while remaining vigilant and responsible. To understand and control the complexity of our biological, economic, and social systems.

Research themes. Many research themes emerge from this broad mission, several of which pertain to plant pathology. Research themes include land management, sustained production, improvement of human nutrition, responses to consumer needs, diversification of products and improvement of their competitiveness, understanding the genome and physiology of living organisms and of process engineering, adaptation of plant and animal species and production methods, aid in economic decision-making, and providing public policy information.

With 21 research centers throughout France employing 3,800 scientists, INRA is prepared to address the major issues facing agriculture. INRA personnel are civil servants and are recruited on the basis of annual competitive entry examinations. INRA has been a major source of agricultural innovations, including the widely adapted French Axe apple growing system. For more information about INRA, consult the INRA Internet site.

INRA at Angers

Angers, a city of about 200,000, is located between Tours and Nantes in the western part of the Loire Valley. The INRA center at Angers is situated in a "research park" adjacent to the campus of the University of Angers. The research park also includes high technology enterprises, research laboratories, and agricultural service agencies. The University of Angers accommodates a plant physiology and microbiology section. In addition, the *Institut National d'Horticulture* (INH), a horticulture and landscape architecture school, is located on the same campus. Thus, researchers at INRA in Angers have the opportunity to collaborate with University biologists, horticulturists, and various researchers at the enterprises and agencies in the research park. The INRA center in Angers began as an enology station in 1902. It joined the national Institute for

Agricultural Research in 1924, then INRA, created in 1946. With an initial effort in fruit breeding, the Angers research center quickly diversified into research in other fields: plant pathology, ornamental horti-

culture, seeds, plant breeding, and horticultural crop science.

Research activities. Research at INRA-Angers is mainly concentrated in the area of fruit and ornamental horticulture, viti-

Fig. 1. (A) Massive wall of Angers château. This historic castle is a major landmark in downtown Angers. (B) Château of Chenonceaux, built over a river. Construction of Chenonceaux was supervised by Catherine Briçonnet, and later it was home to Catherine de Medici and Diane de Poitiers. (C) Chambord, a grand château. Once a royal hunting lodge, the château eventually became a series of royal apartments. (D) Château of Azay le Rideau, a historic sixteenth century castle home. (E) Château of Villandry and its formal gardens. (F) Part of the kitchen garden at Villandry showing apple trees located in the garden.

Fig. 2. Loire Valley apples. (A) Braeburn apples grown in the French axe high hedge-row style. (B) Young apple orchard in bloom showing dense planting and trellis. (C) Netting for protection from hail. (D) Apples and other produce displayed at outdoor market in Angers.

cultural lands, and seeds. Multi-disciplinary teams of researchers are investigating the following subjects: characterization and modification of the genome of fruit trees and ornamentals—pome fruits, ornamental shrubs, and pelargonium; plant-parasite relations—the interaction between scab and apple trees and resistance selection, *Erwinia amylovora-Maloidea* cellular interactions, fire blight management, plant and seed bacterial diseases, phyllosphere microbiology, and phytobacterial taxonomy and diversity; production and biology of woody ornamentals grown in containers and in the urban environment; and vineyard soils and wine—determination of soil-climate effects on grape yield and wine quality.

Plant pathology at INRA-Angers. The plant pathology unit at INRA-Angers has had an especially robust program in phyto-bacteriology over several decades. There is now, in addition, a solid research program on pome fruit fungal diseases, especially apple scab. The unit is housed in its own research building, but the scientists collaborate with personnel in other units housed in nearby laboratories on the site. For example, the fruit and ornamental species breeding station nearby maintains germ plasm collections useful for disease resistance breeding. Their collection includes 2,000 dessert and 180 cider apple varieties in addition to hundreds of varieties of pears, currants, and raspberries, and

scores of quince, gooseberries, and blueberries. Numerous ornamentals such as crabapple (*Malus*) (Fig. 3A and B), *Berberis*, *Crataegus*, *Forsythia*, *Pyracantha*, and *Weigela* are also maintained.

The plant pathology unit is housed in laboratories and offices (Fig. 3C) equipped with excellent facilities for doing basic molecular plant pathology and applied and practical research. In addition to ample on-

site field orchards, there are greenhouses (Fig. 3D), plant and microbial containment facilities, growth chambers, phytotrons, sophisticated laboratory equipment, computer facilities, and a plant pathology library. The official French collection of plant-pathogenic bacteria (CFBP), with more than 3,000 strains, is housed and managed in this unit, where it was created 30 years ago. Perhaps most importantly,

Fig. 3. Venues at INRA-Angers. (A) Part of flowering crabapple collection in spring. (B) Crabapple collection in fall. (C) Building housing plant pathology (*pathologie végétale*) unit. (D) Plant pathology greenhouses.

Table 1. Examples of some dessert apple varieties^a and their percentage of total production in the Loire Valley, France

Varietal grouping	% crop	Apple varieties (% total production where indicated)
Early varieties	Less than 1%	Akane, Arkcharm, Cybelle, Delbarestivale, and Sunrise
Yellow “Golden” types	41+%	Delblush, Earligold, Golden Delicious (41%), Golden Supreme, Newgold, Ozark Gold, and Primgold
Bicolored varieties	30.9+%	Arlet, belle de Boskoop, Braeburn (6.5%), Cox’s Orange Pippen, Delbard Jubilee, Elstar (4.2%), Falstaff, Fiesta, Fuji (1.1%), Gala (7.7%), Harmonie, Idared (4.9%), Jonagold (4.7%), Melrose (1.8%), Meran, Pinova, Querina, Regali, and Suntan
Red varieties	5.5+%	Delbartardive, Delicious Rouge (Red Delicious) (5.5%), Gloster, Redwinter, and Starkrimson
Green varieties	10+%	Baujade and Granny Smith (10%)
Reinette type varieties	8.1+%	Belchard, Reine des Reinettes (3.5%), Reinettes Blanche, Reinettes Grise du Canada (4.6%), Rubinette

^a There are, in addition to the fresh market apples, hundreds of different cider apple varieties grown for *cidre*, a fermented, low-alcohol beverage. Most cider production, however, occurs in the Brittany and Normandy regions.

Table 2. Apple characteristics and use suggestions found on an apple bag label in France

Variety	Fruit characteristics	How to use the fruit
Belle de Boskoop	Firm, acidic, aromatic	Grill, in pies, in salads
Elstar	Crisp, fairly sweet, refreshing	Eat raw, in salads, sauce
Gala	Crisp, sweet, refreshing	Bake, toast without skins
Golden Delicious	Sweet, juicy, delicate aroma	Bake quartered, in beignets, eat raw (when slightly green), sauce
Granny Smith	Crisp, juicy, sour	Eat raw with lemon, microwave whole, in mousse, in apple surprise, toast quartered
Idared	Crisp, lightly sour	Eat raw, fry or toast slices, in salad
Jonagold	Crisp, sour, sweet, aromFatic	Eat whole or quartered raw, in sauce
Melrose	Sweet, juicy, aromatic	In beignets, in vegetables
Reinette Canada	Sour, melts-in-the-mouth, aromatic	Eat raw with light lemon, in pies, in vegetables, sauce
Reine des Reinettes	Crisp, juicy, sour	Bake or stuff
“les Rouges”	Crisp, sweet, juicy	In cakes, microwave without skins

the unit is staffed with competent and diligent field, greenhouse, and laboratory assistants who, as in most science research institutions, actively participate in the work of the scientists. In addition, a number of graduate and undergraduate students originating from diverse universities in France do research in this unit as part of their senior or master's theses and Ph.D. dissertations. We Americans found the plant pathology unit at INRA-Angers to be conducive to research, certainly up to the standards expected at our home institutions.

The INRA pome fruit pathologists recently hosted the 5th IOBC Workshop on Integrated Control of Pome Fruit Diseases. This international meeting, attended by 68 participants representing nearly all the European countries plus all the other continents, gave plant pathologists from all over the world a chance to share apple disease research. In addition, participants experienced the culture, history, food, and wine of the Loire region. *Bien sûr*.

Apple Scab Research at INRA-Angers

Scab, caused by the fungus *Venturia inaequalis* (Cooke) Wint., is the most common and devastating disease of apple fruit and foliage in the Loire Valley region (Fig. 4A). Growers expend considerable resources on repeated fungicide applications to manage this disease each season. The laboratory at INRA-Angers, Unité de Pathologie Végétale, headed by L. Parisi, has a major research program dealing with apple scab. This program consists of research on host and pathogen genetics, orchard planting strategies for scab resistant apples, effect of environment and conidial inoculum dose on disease, and durable apple resistance in Europe.

Pathogenicity of *V. inaequalis* and genetic relationships between host and pathogen. This work was done in collaboration with the plant breeding unit (*Unité d'Amélioration des Plantes Fruitières et Ornamentales*) of INRA-Angers. From this work, the existence of *V. inaequalis* strains (race 6) able to overcome the resistance of the *Vf* gene was first recorded (32), and their pathogenicity to many apple clones was studied (31). The *Vf* gene from *Malus floribunda* 821 has been used successfully to provide resistance to scab in apple breeding programs in North America and Europe for several decades (23). The emergence of this new race could compel apple breeders incorporating the *Vf* gene into new cultivars to modify their strategy. It enabled us to undertake studies on characteristics of the resistance of the parental clone, *M. floribunda* 821. We showed that host resistance conferred by the *Vf* gene was due to at least two major genes; the second gene, thought to be responsible for a hypersensitive reaction, was named *Vfh* (7,8). Only a few years after the discovery

of race 6, Roberts and Crute (38) identified additional strains of *V. inaequalis* able to overcome the resistance of the *M. floribunda* 821 clone. The pathogenicity of these strains, now called race 7, was studied. It was found that race 7 was avirulent to the cultivar Golden Delicious (6,11,30). Thus, this Golden Delicious resistance gene was named *Vg*. These studies demonstrate that the interactions between *V. inaequalis* and apple fit the gene for gene theory; corresponding to host resistance genes, two pathogen avirulence genes, *avrVf* and *avrVg*, have been identified (7). The laboratory is currently identifying molecular markers associated with the avirulence genes of *V. inaequalis*.

Planting strategies for the recently released *Vf*-resistant cultivars. The presence in several European countries of races 6 and 7 of *V. inaequalis* (28) can compromise the commercial success of the *Vf*-resistant cultivars, which are of great interest for scab control, especially in orchards managed by biological or integrated disease control (33). It may be necessary to establish strategies for the planting of the *Vf*-resistant cultivars in order to preserve their resistance. Cultivar mixtures, used for several years in annual crops, can be one of these strategies (16). The use of cultivar mixtures for disease control in perennial crops has not been extensively studied. We started a mixed cultivar project in 1994 with the planting of an experimental orchard. Using the susceptible Smoothee cultivar and the *Vf*-resistant Baujade cultivar, we compared the levels of scab in monoculture blocks, in blocks with alternate rows, and in blocks alternating trees within rows. The orchard was not treated for scab, and disease epidemiology was studied. In 1998, the resistant blocks remained scab free, while the susceptible blocks were diseased. Furthermore, there were significant differences in disease severity (number of scab lesions per shoot) between monocultures and mixed plantings. Disease severity in June was highest in the susceptible monoculture blocks, less in blocks with alternate cultivars row by row, and still less in blocks alternating cultivars within rows. However, fruit scab incidence and severity did not show significant differences between the plantation designs (13). A new orchard comprising three intermixed cultivars tests the efficacy

of cultivar mixtures in combination with reduced fungicide applications.

Effect of environment and conidial inoculum dose on secondary infections by *V. inaequalis*. Temperature and duration of leaf wetness have been shown by many investigators to influence infection of apple leaves by ascospores and conidia of *Venturia inaequalis* (25). A table showing the number of hours of leaf wetness needed for primary scab infections at various temperatures was developed by Mills in 1944 (27). Apple growers can now use computer-assisted Mills-based predictive systems to determine the risks of primary infection by ascospores (22). Similar tables or curves representing the relationship between hours of leaf wetness, temperature, and infection by apple scab have been developed by several researchers. Following analysis of these reports, a revision of the Mills table was proposed (26). This revision was further modified (40) when it was determined that the wetness duration required for infection is approximately the same for ascospores and conidia.

At INRA-Angers, we studied the effect of temperature and leaf wetness on secondary infection of apple leaves by conidia. Using apple seedlings, high inoculum doses (250×10^3 conidia per ml), and favorable controlled environmental conditions, three curves (C1 to C3), which describe light, medium, and heavy infection levels by conidia, were developed (29). The C3 curve (Fig. 5) represents high levels of seedling infection (more than 50 spots per plant) and is similar to a low risk curve developed in South Africa (39). Because greenhouse-grown apple seedlings exposed to high inoculum doses under ideal conditions are highly susceptible to scab, the C3 curve is thought to be equivalent to significant but low levels of infection in the orchard.

The effect of inoculum dose on scab infection was studied. We reported on the relationship between inoculum dose, temperature, and disease development for leaf wetness duration times indicated by the C3 curve. In addition, we studied the relationship between duration of leaf wetness, temperature, and disease development for a single, low inoculum dose (20).

In these studies, apple seedlings (2-month-old Idared $\times$ Golden Delicious) were inoculated with conidia of *V. inae-*

Fig. 4. (A) Apple scab lesions on foliage. (B) Fire blight canker and blighted shoot on apple.

qualis in order to study the effects of inoculum dose and leaf wetness duration on development of apple scab symptoms. For each experiment, the C3 curve (indicating heavy seedling infection levels) was used as the basis for relating infection to temperature and leaf wetness duration. In one series of experiments, seedlings were treated with inoculum doses of 1.5, 5.4, 15.6, 32.2, 81.2, and 250×10^3 conidia per ml, and leaves were kept wet during C3 infection periods at temperatures of 6, 11, 16, and 22°C. Figure 5 shows that for C3 infection periods at these temperatures, leaves would be wet for 26 h 0 min, 13 h 4 min, 9 h 52 min, and 6 h 48 min, respectively. At all four temperatures, disease incidence (scab lesions per plant) increased with increasing inoculum doses up to about 81.2×10^3 conidia per ml. In a second series of experiments, seedlings inoculated with 10×10^3 conidia per ml were kept moist for infection periods ranging from 0.6 to 2.0 times the C3 leaf wetness duration curve at 6, 11, 16, and 22°C. Disease incidence increased with increasing duration of leaf wetness and generally leveled off between 1.5 and 2.0 times the C3 curve wetness duration. At this inoculum dose (10×10^3 conidia per ml), doubling the leaf wetness duration, indicated by the C3 curve, resulted in high disease incidence, similar to levels obtained with a higher inoculum (250×10^3 conidia per ml) and shorter wetness period (1.0 C3).

The inoculum doses used in this research are consistent with inoculum doses reported in the field (25). However, the relationship between inoculum dose used in the C3 curve experiments and amount of secondary inoculum produced in the orchard, which then results in some level of new scab infection, is not completely known.

In preliminary studies, we were able to show that under ideal conditions, a single

conidium of *V. inaequalis* was capable of causing a scab lesion. In addition, we determined that young, expanding leaves of apple seedlings were most susceptible when they attained an area of 4.25 to 9.00 cm² (length $\times$ width). These studies were done using very low inoculum doses to infect highly susceptible apple leaves and incubating them under moist conditions for at least twice the C3 time duration.

Durable apple resistance in Europe (D.A.R.E). Plant pathologists and breeders at INRA-Angers are collaborating with colleagues and the nursery industry in eight European countries to develop apples with durable resistance to scab and powdery mildew (*Podosphaera leucotricha*). This program is coordinated by Y. Lespinasse, head of the plant breeding unit at INRA-Angers. The project objectives include: (i) characterization of the resistance status of a large range of apple cultivars, (ii) assessment of the pathogenicity variability of the two fungi, (iii) genetic dissection of apple resistance-susceptibility with molecular markers, (iv) incorporation of many disease resistance genes (resistance gene pyramiding), and (v) consumer preference assessment regarding scab resistant varieties (14,24). As coordinator, INRA-Angers plays an important role in this project; the plant pathologists are mainly involved in the first two objectives.

Fire Blight Research at INRA-Angers

Fire blight has been present in North America since the colonists first arrived and tried to grow susceptible pears and apples (Fig. 4B). It is endemic and widespread in all of North America and is not a quarantined disease. There are no state or federal regulations concerning the movement of host plants or the detection or eradication of fire blight outbreaks. There

are some state laws designed to aid in the removal of abandoned or poorly managed orchards that could serve as sources of inoculum for nearby commercial orchards. For the most part, fire blight control is done independently by growers without any government intervention.

The American experience is in distinct contrast to Europe and especially France, where fire blight was only discovered in 1972. Now, fire blight is considered to be widespread in most of France. Research with fire blight at INRA-Angers (Plant Pathology and Breeding Units) began in 1978, when fire blight began to decimate the Passe Crassane pears in southwestern France. This pear was extensively planted because it is a high-quality dessert pear, but unfortunately it is highly susceptible to fire blight.

The research program and objectives in France were distinct from the approaches taken in North America because of the major differences between fire blight origins and outbreaks. In addition, France was unique from other Northern European countries where fire blight had been present for nearly 20 years but was a relatively less significant disease.

France was the first "infested" country in Europe with an exporting business in both fruit and nursery production, including pear, apple, and ornamentals such as *Pyracantha* and *Cotoneaster*. The climate was warmer than in Northern European countries such as England, Belgium, Denmark, the Netherlands, Germany, and Poland, where fire blight had been present from as early as 1966. The use of antibiotics like streptomycin was likely to be forbidden by French government authorities, as happened in the early 1970s when tetracycline was not allowed for management of *Pseudomonas syringae* pv. *persicae* on peach. Under French conditions, susceptibility of a number of local varieties was largely unknown. Another important aspect was that fire blight in Europe was (and still is) a quarantined disease, because only a few northern countries had the disease in 1978.

This background explains some of the government regulations and the different strategies used for the containment and management of fire blight in France. These regulations have had pronounced effects on cultivation and movement of fire blight-susceptible plants in France. For example, fire blight is still considered a quarantined disease because it is not present in all areas of France. In some areas, each new outbreak of fire blight is considered a new introduction, and infected plants and nearby host plants are eradicated. This action was initiated in 1972 in northern France, where *Crataegus* hedges were removed, and continued through 1978 in southwestern France, where pear and occasionally apple orchards were destroyed after the discovery of fire blight.

Fig. 5. The C3 curve: the relationship of leaf wetness duration and temperature on apple foliage infection by conidia of *Venturia inaequalis*. Under the same conditions, any point on this curve should result in equivalent levels of disease (e.g., high disease levels for greenhouse-grown seedlings or low to moderate in the orchard).

Eradication is not currently conducted in all areas but is still performed in so-called *zones protégées* (protected zones). These *zones protégées* (with respect to fire blight) are designated at the European Union level and are periodically revised according to the expansion of the geographic range of the disease. They are zones where fire blight is considered absent, or at least not established. This implies that in such zones, eradication of blighted plants is performed under official control as soon as fire blight is detected. Therefore, the designation of *zone protégée* allows for inspection and eradication of diseased plants within the zones and prohibition of importation of hosts that may be carriers of *E. amylovora* into the zones. These zones are routinely inspected by the Ministry of Agriculture (Plant Protection Service) during the growing period between May and September. The primary inspection is for visual symptoms but may also require laboratory analyses using indirect immunofluorescence antibody staining, agglutination with specific antibodies, confirmation with API-20E test strips, and pathogenicity on susceptible hosts.

Outside the *zones protégées*, fire blight is considered an endemic disease. Therefore, nurseries in these areas are allowed to ship susceptible plant material only under strict regulations and following official surveys to be sure that the plants are symptomless and that no fire blight has been seen in the area surrounding the nurseries for 1 year. These plants, as well as plants from *zones protégées*, are given a specific *passport* (phytosanitary certificate) that allows their distribution within Europe and some other countries.

Certain species or cultivars of apple, pear, or ornamental plants are much more susceptible than others, and the presence of these susceptible plants creates a higher risk for fire blight outbreaks with subsequent spread to less susceptible plants. Therefore, certain plants are not allowed to be grown, marketed, or transplanted in France. Part of this prohibition is necessary because the government may subsidize the removal of infected plantings and it does not make sense to allow highly susceptible cultivars to be planted and then have to pay for their removal. The current list of prohibited plants includes: Beurré, Durondeau, Passe Crassane, Bronstar, Laxton's Superb, and Madame Ballet pear cultivars;

Abbondanza and James Grieve apple; five cider apple cultivars; all species and cultivars of *Crataegus*; several *Cotoneaster* and *Pyracantha* species; and Kumoi and Nijiseki Asian pears.

In the late 1970s, France was caught in the middle. Northern European countries had fire blight but lacked intensive fruit and ornamental production and blight-favorable conditions, while Southern European countries produced fruit under blight-favorable conditions but were not yet contaminated. This situation was the background for the preparation of a European Research Program, coordinated by INRA-Angers and subsidized by the European Community. Three successive research programs were supported from 1978 to 1994, involving partners in Italy, Greece, Spain, England, and the Netherlands. Most applied research concerning breeding, epidemiology, chemical control, and detection of the bacteria was performed within the framework of these European programs.

Diagnosis and detection. In the 1970s, the symptoms of fire blight were usually unknown by French technical officers, and diagnosis was often difficult. Therefore, our early work dealt with biochemical characterization (37) and diagnostic techniques for the bacteria. An immunofluorescence test with a specific antiserum was developed which is still widely used by the Plant Protection Services for diagnosis. In a collaboration with Spanish researchers, enzyme-linked immunosorbent assay (ELISA) techniques using monoclonal antibodies were developed (19). These techniques were very important for the official surveys and regulatory procedures performed by the Plant Protection Service.

Chemical control. Several chemicals already used internationally as well as some experimental compounds that had been used for other diseases were tested for fire blight control in France according to a procedure proposed at the European level (34). From these experiments, two chemicals were proposed: fosetyl Al (Aliette), registered in France, and flumequine, registered as Firestop in France and some European countries (10). Aliette is still the most widely used chemical against fire blight, in spite of inconsistent experimental results (36). Flumequine was shown to be a potential replacement for streptomycin in the United States but was never registered (42), while in Europe it is expensive so

growers don't use it routinely. Tests with copper compounds confirmed their role in prevention of fire blight infections, but their phytotoxicity curtailed widespread use (35).

Warning systems. It was apparent that climatic factors were important and could be used to determine favorable conditions for fire blight and its ultimate severity. Therefore, a warning system based on Billing's work (9) and climatic studies, worked out in collaboration with the French meteorological office, was developed and prepared as a computer program called *Parefeu* (Firescreens) (21). This warning system provides information both for timing of searches for early symptoms in the orchard and also for timing of preventive chemical sprays.

Varietal behavior and plant breeding. Entire orchards consisting of certain cultivars such as Passe Crassane, Alexandrine Douillard, and Madame Ballet pears, and Peau de Chien cider apples, were destroyed by fire blight despite intensive sanitation and spray measures; whereas other cultivars escaped the disease under the same soil and climatic conditions. This showed that the best long-term approach to fire blight control would be the development of new resistant genotypes with good horticultural qualities. The selection of cultivars of pear, cider apple, *Pyracantha*, and *Cotoneaster* using traditional breeding methods was pursued at INRA-Angers in cooperation with the plant breeding unit. Currently, genetic transformation is being used to develop selected pear genotypes such as Comice and Passe-Crassane. Some selections have been screened in the greenhouse and are now being evaluated in the field for resistance to fire blight and retention of the original horticultural characteristics.

Under the framework of the European Research Program and in cooperation with Italy, Spain, Greece, England, and Austria, over 500 genotypes of common apple and pear varieties, rootstocks, and the ornamentals *Sorbus*, *Cotoneaster*, *Pyracantha*, and *Crataegus* were screened for inherent susceptibility to fire blight. Inoculations were usually made on shoots in the field, but sometimes both shoots and flowers were inoculated (34). Reactions of apple genotypes varied from very resistant to very susceptible; the percentages of genotypes in fire blight resistance and susceptibility groupings are found in Table 3. Identification of these genotypes will be useful in apple breeding and fire blight quarantine efforts.

Pathogenicity of *Erwinia amylovora*. While these applied programs were carried out, fundamental aspects of the *E. amylovora*-plant interaction were examined with the aim of better understanding the infection process. This work began in INRA-Angers and continues in collaboration with another INRA laboratory in Paris (INRA-

Table 3. Summary of reactions of apple genotypes to fire blight (percent in each disease reaction class)

Reaction to fire blight	1990 (75 genotypes)	2000 (192 genotypes)
Very resistant	41	27
Resistant	20	21
Intermediate	12	19
Susceptible	24	25
Very susceptible	3	8

INA-PG). Mutagenesis of the virulent French type-strain 1430 (43) resulted in the discovery (with other research teams including Cornell University in New York) of the *hrp* system of *E. amylovora* (5), the role of the *dsp* region, and the putative functions of coded proteins secreted (harpin, HrpW, DspA, desferrioxamine) (4,12,17,18). Further knowledge is still needed to better understand the pathogen-plant interaction and to utilize this knowledge to develop innovative control approaches.

Plant response to infection. The differential responses of genotypes of *Pomoideae* to infection showed that natural defense mechanisms exist in most fire blight host plants. In the hope that this could provide a new basis for control, research on induction of these mechanisms was undertaken, using both biological and chemical inducers. The chemical inducer Acibenzolar-S-methyl (ASM, Bion or Actigard) from Novartis (Basel, Switzerland) was extensively tested on apple and pear in the greenhouse and in the field. It showed a good level of resistance induction, at least in apple. This was directly associated with the activation of several enzymatic reactions, usually linked with systemic acquired resistance (SAR) in annual plants (41).

The role of genetically defined *hrp* avirulent mutants in the induction of resistance was shown to be related to the precise site of the mutation within the *hrp* region. Regulatory mutants were shown to be far more "protective" than secretory mutants. Similarly, these regulatory mutants induce stronger defense reactions from the plant (15).

Extending Apple Disease Research Findings in France

INRA, like the Cooperative Extension Service in the United States and its counterparts in other parts of the world, has responded to the need to extend its research findings to those who will utilize them for improved crop management. INRA plant pathologists extend research findings to growers via agricultural advisors and popular magazines and by involving organizations and consortia sponsored and funded by the national, regional, and departmental governments.

One of the important partners to the efforts of INRA is the "Centre technique interprofessionnel des fruits et légumes" (Ctifl), which serves to advise fruit and vegetable growers. Ctifl does much work in the area of applied research and extension. Through management of a research farm, hosting field days, writing fact sheets, popular trade magazine articles, and field trial bulletins, Ctifl informs apple growers of best management practices, often based on research results obtained from INRA. Ctifl also performs regulatory

functions, being responsible, with INRA, for certification of apple tree nursery stock in France. Apple growers in the Loire Valley also benefit from research and education efforts at their own research farm. This farm, called *La Morinière*, is a fruit research experiment station established south of Tours in the Loire Valley using check-off funds from growers in the region as well as local and regional government funding. *La Morinière* operates on the scale of grower fruit farms with similar technical and economic constraints. Tests of disease management approaches are done to field scale on apple varieties typical of the region.

Apple growers also have access to diagnostic and other services through the *Service de la Protection des Végétaux* (SPV) (Plant Protection Service, Ministry of Agriculture). Apple scab (*la tavelure*) disease predictive software and apple scab ascospore release data are available from SPV centers throughout the region; one is located in the research park near INRA-Angers. There are also field days for apple growers sponsored by chemical companies, sprayer manufacturers, and nurseries. At these field days, personnel from SPV and Ctifl may present information useful to growers. Thus, with its many and diverse public and private partnerships, INRA is instrumental in providing Loire Valley apple growers with research-based disease management advice.

Outlook

The apple disease situation is constantly changing. *Ce qui sera, sera*. As new problems and opportunities arise, INRA-Angers will continue to be involved in apple disease research "from the molecule to the marketplace."

The American authors gained a great deal from their association with the plant pathology group at INRA-Angers, and very likely the resident scientists and technicians there benefited from their exposure to the two visitors. But this cross-cultural collaboration yielded more than just scientific advancement. While living for a time immersed in the ambiance of the Loire Valley as already described, one is surrounded by the history, language, food, and culture of France. *Vive la différence!* Living with others in their native land enhances one's appreciation of our differences and similarities and how our passion for research and extension fits into the greater scheme of things.

Is there a language barrier for scientists working in France? *Oui et non; parlez vous français?* It is a beautiful language, but difficult to master, so we had our share of setbacks, but also our share of successes. Fortunately our hosts were very tolerant of our deficiencies. One learns quickly the use of *Bonjour! Pouvez vous m'aider?* and other useful phrases. Our colleagues at work and the people where we lived were

friendly and helpful, particularly when we used the French we knew. Even then, there were the inevitable frustrations. *C'est la vie*. The unique experiences and lifelong memories made it all worthwhile. In the end, it was difficult to say good-bye. *Au revoir!*

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