Review

Fire blight (*Erwinia amylovora*) disease in Morocco: Current status and action for its management

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Abstract

Fire Blight, caused by *Erwinia amylovora*, is one of major economic threats to pear and apple growing areas worldwide. In Morocco, this devastating bacterium was first detected in 2006 on pear plantations in Ain Orma district of the Meknes region. Since then, the pathogen was progressively spreading to different regions of pear, apple and quince production, resulting in important losses. As a result, over 2312 Ha of pear, quince and apple orchards were eradicated in 2013. The situation was described as an economic disaster never seen before in the country. Currently, the middle Atlas region, the stronghold of the Moroccan rosaceous production, was entirely affected by this disease. Following the appearance of the bacterium, several approaches including morphological, physiological, biochemical, serological and molecular traits, were undertaken for the early detection and identification of *E. amylovora*. In this review, we discuss rigorous actions taken by Moroccan authorities to overcome the fire blight outbreak, which includes entire eradication of infected commercial orchards and individual trees. Therefore, the disease incidence rate was reduced, and the infected zone was restricted. In addition, during the last 10 years, studies on bacterium strains diversity and origin were performed using different methods; RFLP, RAPD, rep-PCR, fAFLP, VNTR and biochemical tests. In recent years, with the use of tolerant cultivars, research is oriented to develop an effective and reliable alternative control strategy for fire blight disease using an integrated approach.

Keywords: *Erwinia amylovora*, Morocco, detection, diversity, economic losses

INTRODUCTION

Erwinia amylovora, which causes fire blight in pear (*Pyrus communis*), apple (*Malus domestica*) and quince (*Cydonia oblonga*) and some other members of the Rosaceae family, is one of the most challenging pathogens of pome fruit production worldwide (Johnson, 2000; Momol and Aldwinckle, 2000; van der Zwet, 2002). The pome fruit is an important agricultural sector in Morocco, it plays an important agronomical and socio-economical role by allowing more than 18 million working days a year. Pome fruit occupy an area of more than 40,000 ha and the major apple-growing areas are in Middle Atlas, Saïs, Haouz and Moulouya. The production depends on the growing conditions and weather conditions of the season. The average production of pome fruit is estimated at 800,000 tons/year. However, during last decade, apple and pears yields were hindered by a very devastating disease called fire blight. Fire blight is a disease that causes considerable damage to host plants (pome fruit rosaceous) by destroying flowering twigs and resulting in the loss of yield as well as a significant constraint for the exports. Furthermore, the presence of the bacterium in areas with favorable environmental conditions (warm and humid), increases the risk of the disease. This is the case of Mediterranean regions, where fire blight has caused significant damage.

Since the first symptoms of fire blight, detected in 2006 in 'Ain Orma' district of Meknes (Fatmi *et al.*, 2008), the epidemic spread quickly to other producing areas causing important economic losses. Therefore, the establishment of an integrated pest management strategy for managing this disease is highly recommended and must be based on the adoption of all sanitation measures in order to limit the incidence and spread of the disease. However, the key element in the management of fire blight is the prevention of blossom infections because a large part of the inoculum is provided by cankers and bacterial exudates from floral infections. Accordingly, this review is dealing with actions undertaken by the Moroccan authorities when fire blight was first detected in the country and the control measures carried out during the years 2006-2017, providing more information on monitoring and detection procedures as well as management strategies to minimize its the impact.

Fire blight: the disease, biology, host range and distribution

Erwinia amylovora, responsible for fire blight, was first reported in the United States in 1793 in the Hudson Valley, New York State (Billing, 2000). The symptoms of the disease on the host plants vary according to the affected part. The infected flower fades, dries up and dies, taking on a dark color (Figure 1). Infected shoots and twigs wither, blacken and die, curling into a shepherd's staff. The infected leaves curl up and seem burned by fire (Wilcox, 1994). The infected fruit does not reach maturity and goes from brown to black and shrivels as if they mummified; usually stays attached to the branch. The main branches and the trunk can also present cankers, which can cause the death of the tree if the disease progresses rapidly (Steiner, 2000).

The most serious form of primary blight infection is the formation of cankers that persist during the winter. The bacterium overwinters exclusively on infected host plants. In the spring, in mild and humid weather, the cycle of the disease starts: the cankers of infected plants produce a bacterial exudate, or primary inoculum. It is carried out by wind, rain, insects or birds, infecting the open flowers of the same plant or other host plants. The bacterium multiplies on the outside, on the pistils of the flowers and penetrates the plant via stomata (orifices through which the plant breathes), nectaries (glands which secrete the juice) or by wounds. It can spread inside the host plant, infecting flowers, twigs, branches or leaves. New cankers can develop on infected branches or twigs, forming lesions that persist throughout the winter (Figure 1). Cankers usually stop producing exudate during the summer and remain inactive until the following spring, when they can become active again, starting the cycle of the disease again (Solymár *et al.*, 2002). The Secondary infection might occur during the growing season. The source of the secondary inoculum is basically the bacterial exudate that oozes out from the lesions of the shoots, leaves, fruits or branches and is disseminated by wind, rain, insects or birds. Immature fruits may be infected with *E. amylovora* through natural lenticels and diseased branches. Fruit infection usually occurs during summer following hailstorms. The infected fruit produces a bacterial exudate, then dries and shrivels, remaining attached to the branch (van der Zwet and Beer, 1999; Solymár *et al.*, 2002).

Fire blight affects many plants of the Rosaceae family, both cultivated and wild. Among the fruit trees, literature mentioned apple (*Malus*), pear (*Pyrus*), quince (*Cydonia*) and medlar (*Eriobotrya*) trees. Hedge and garden plants that are important hosts include *Cotoneaster*, *Crataegus* (hawthorn), *Pyracantha* (burning bush) and *Sorbus* (mountain ash), but some species may not be host plants (Bonn and van der Zwet, 2000). Fire blight bacteria (*E. amylovora*) are thought to be native to North America. In the early 1900s, it was reported in Canada from Ontario to British Columbia, in northern Mexico and in the United States from the east coast to California and the northwest Pacific coast (Bonn and van der Zwet, 2000). It was reported in New Zealand in 1919, in Great Britain in 1957, and in Egypt in 1964. The disease has spread throughout Northern and Western Europe, although Portugal and Finland are still exempt; it remains located in France and Switzerland, and is limited to a few homes in Spain, Italy and Austria. Norway said it has eradicated the disease. Fire blight has spread to the Mediterranean region, including Greece, Turkey, Israel, Lebanon, Iran and several Central European countries. Latin America and much of Africa and Asia are apparently free of the

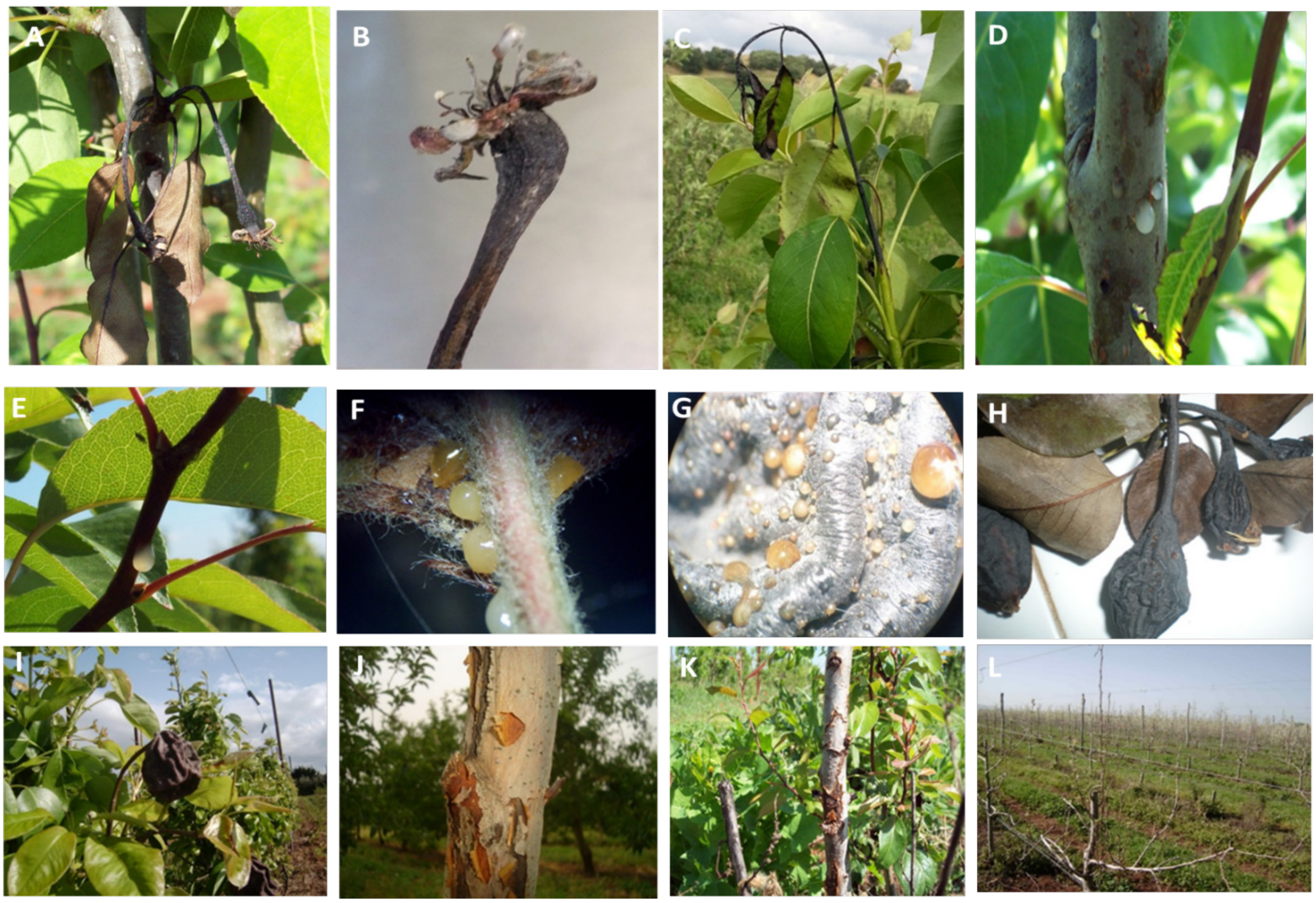

Figure 1: Fire blight Symptoms: A & B - necrotic flowers and leaves; C - necrotic shoot; D, E, F and G - drops of bacterial ooze on pear trunk, leaves and fruits; H and I - mummified immature fruits; J and K - canker of trunk and necrotic tissue; L - field damages

disease (Solymár, 2005). In 1997, Australia reported fire blight in the Adelaide and Melbourne Botanic Gardens, but eradication measures were effective, and no further outbreaks were reported. According to EPPO (2016), *E. amylovora* is currently present in more than 50 countries in the world (Solymár, 2005).

Occurrence of fire blight and its current status in Morocco

The disease was first detected, from a pear orchard in the Meknes region, on May during the growing season 2006 (Fatmi, unpublished data). In an effort to eradicate the disease, 42 ha of pears were dug up and burned in October 2006. In the spring of 2007, fire blight reappeared in the same orchard and was encountered in five other orchards with disease incidences from 1 to 60%. Three hectares of pears were removed and burned. In May and June 2007, samples from other pear orchards showing symptoms of fire blight were collected and tested. As a result, *E. amylovora* was detected on 4 farms in the communes of Aïn Orma, Aït Ouallal and Dar Oum Soltane, all located in the prefecture of Meknes (Achbani, 2007).

During surveys carried out by the National Plant Protection Organization (NPPO), new infections have been detected in the region of El Hajeb, 30 km south of Meknes. The disease appeared in pear (*Pyrus communis*) orchards in the following 8 communes: Ait Harzallah, Ait Yaazem, Laqsir, Ait Naâman, Tamchachat, Ait Bourazouine, Sbaae Ayoune and Ait Boubidane (NPPO, 2008). Surveillance has been intensified in the nearby regions of Ifrane, Fes and Khénifra. Finally, it can be recalled that in the region of Meknes since May 2006, 56 ha (corresponding to 5 orchards) have now been destroyed and that 52 ha have been sanitized (by pruning of affected shoots). In 2009, fire blight was observed in 71 farms in different counties (Meknes, El Hajeb, Sefrou, Ifrane, Taounate, Khenifra), covering more than 720 ha. More than 215 ha of pear, apple and quince have been destroyed (Yaich *et al.*, 2010). In 2010, the disease was diagnosed in the Middle Atlas (Ifrane province.) Nearly 1264 ha are heavily affected. They represent 90% of the cultivated area of pear and 100% of quince. However, other outbreaks have been discovered in Beni Mellal, Larache and Midelt. Hannou *et al.* (2013) reported that the disease affects the orchards of the entire region of the Middle Atlas, which is the stronghold of the national production of apple and pear in Morocco.

The Plant Protection services reported that, until 2013, the provinces declared infected by the disease are Ifrane, El Hajeb, Midelt, Fès, Sefrou, Taounate, Guercif, Taourirt, Taza, Boulemane, Béni-Mellal, Kénitra, Khémisset, Larache and Marrakech. The area of uprooted and incinerated orchards in the various affected provinces was in the order of 2312 Ha. Ameur *et al.* (2014) reported that fire blight has progressed most in rosaceous region affecting a total area of about 4000 ha.

Monitoring and diagnosis processes for fire blight in Morocco

Different methods were used for the detection and characterization of the bacterium *E. Amylovora*. Because of its harmfulness, *E. amylovora* has been placed on the quarantine list. After its first detection in Morocco, the decision process depends on the diagnosis protocol for *E. amylovora* guidelines of the European Plant Protection Organization (EPPO). Several approaches for the detection and identification of *E. amylovora* have been used. These methods are based on morphological, physiological, biochemical, serological and molecular properties of the bacteria.

Physiological and biochemical identification, in different studies was based on the color, the characteristic aspect of the colonies of *E. amylovora* on semi-selective mediums such as CCT, YDC, MM2Cu mediums and NBY (no growth at 36°C) or differential like King'B medium (Figure 2). Other tests were used such as Hugh and Leifson, levan production and oxydase test. The API 20E system was used for biochemical tests. Other tests

A

B

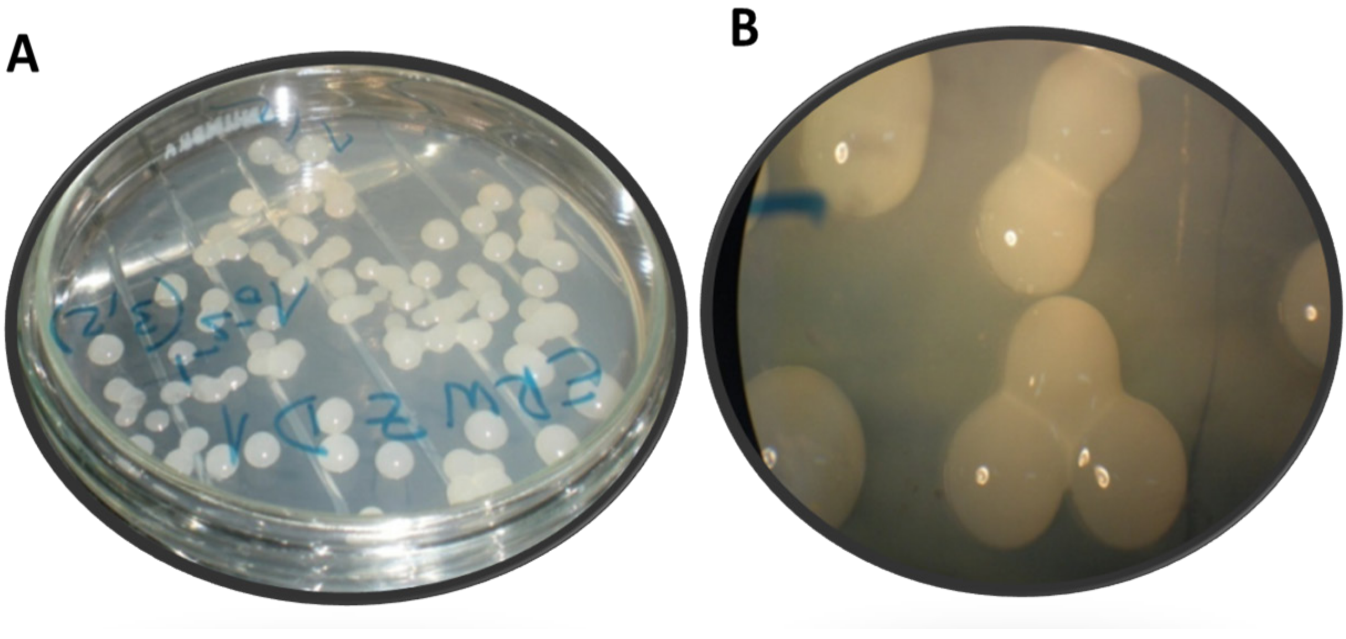

Figure 2: Appearance, color and shape of colonies of E. amylovora isolated on Levane medium after an incubation period of 48 h at 25 °C

were also used like gelatin hydrolysis; levan production; nitrate reduction; citrate; urease; indole production; growth at 36°C and at 39°C; acid production from salicin, inositol, L-arabinose and sorbitol were performed according to Jones and Geider (2001). In addition, the isolated strains were tested for hypersensitive response (HR) on tobacco (Ameur *et al.*, 2014) and for pathogenicity as described by EPPO (EPPO, 2004).

Serological test using indirect immunofluorescence according to the method described by Van de Bilt *et al.* (2008) was used by Yaich *et al.*, (2011). However, molecular techniques, such as PCR and Real time - PCR, are commonly used for detection of *E. amylovora* in preference to serological and cultural diagnoses given their efficiencies and speed. Polymerase Chain Reaction (PCR) was usually performed with extracted DNA (Llop *et al.*, 1999) using primers based on sequences of the plasmid pEA29 (primers: A and B, AJ75 and AJ76 or Peant 1 and 2), and the plasmid pEA71(primers F and R) and also from the chromosomic ams-region using primers AMSbL and AMSbR. Real time - PCR Primer pair Ea-lscF (5'-CGCTAACAGCAGATCGCA) and Ea-lscR (5'- AAATACGCGCACGACCAT), producing a 105 bp amplicons, and the TaqMan probe Ea-lscP (5'-CTGATAATCCGCAATTCCAGGATG) used were based on the sequence of the levan sucrose gene (lsc, Genbank fi le X75079) (Lehman *et al.*, 2008).

Diversity and origin of *Erwinia amylovora* in Morocco

The morphological and molecular characterization of *E. amylovora* strains and its host range is primarily important in plant quarantine, selection for disease resistance in breeding and genetic engineering programs, tracking long- or short-range pathogen dispersal, identifying possible sources of infection, distinguishing strain groups and studying the relatedness among strains (Momol and Aldwinckle, 2000). During the last decade, the study of strain diversity of *E. amylovora* was made using different methods. The host origin used in different works corresponds to different area of Morocco. Nevertheless, the main origin areas are Meknes zone and the Middle Atlas Mountains. Several tests were performed to discriminate *E. amylovora* strains (Table 1). Using RFLP, RAPD and biochemical tests. Ait Bahadou (2009) reported that *E. amylovora* strains are grouped in a homogeneous group, assuming that Morocco had probably only one introduction. However, Yaich *et al.*, (2011), with Plant material collected from 2006 to 2009 performing biochemical, pathogenicity, rep-PCR and fAFLP test, found that Moroccan *E. amylovora* strains were clearly distinguished by geographical origin, host or year of isolation. This result suggests that Moroccan strains have multiple geographical origins. Similar finding was reported by Ait Bahadou *et al.*, (2018b) using RAPD analysis and the sensitivity test to streptomycin. This study demonstrated clearly that fire blight outbreaks might be caused by the introduction of infected plant material or other sources of inoculum from different countries where streptomycin was not authorized for fire blight control. Moreover, Hannou *et al.*, (2013) performed a comparative analysis of Moroccan strains with three reference strains from Spain, France and England and their results revealed some polymorphisms compared to England and the French strains, but, notable similarities were observed with a Spanish strain obtained from plants imported from Belgium.

Management strategies for fire blight in Morocco

The fire blight outbreak was a real economic disaster that Moroccan horticulture sector suddenly faced and in order to control the worrying situation, the Department of Agriculture shortly acted by taking different measures as the disease is rapidly spreading. The current control strategy of fire blight relies on an integrated approach that combines horticultural practices, sanitation, use of predictive models and application of well-timed bactericides sprays (Solymar, 2005). The application of antibiotics likes streptomycin, oxytetracycline, kasugamycin, and oxolinic acid was very effective for the fire blight control (Haidar, 2014). These substances can completely block the multiplication of *E. amylovora* by acting on protein synthesis and to date there is no resistance to these products in local populations of this bacterium. Unfortunately, their use in Morocco to fight bacterial diseases is not allowed.

Table 1: The most relevant research studies on Fire blight bacterium in Morocco

Areas	Years	Number of strains	Used method	References
Meknès and Sefrou	Spring of 2009	8 strains	RFLP, RAPD and biochemical tests	(Ait Bahadou, 2009)
El Hajeb, Meknes and Sefrou	2006-2009	48 strains	Biochemical, pathogenicity, rep-PCR and fAFLP	(Yaich *et al.*, 2011)
The Middle Atlas Mountains in Morocco	2009-2010	39 strains	Sequences from tandem repeat of variable numbers (VNTR)	(Hannou *et al.*, 2013)
Kenitra,Meknes and Middle Atlas Mountains	2006-2011	402 strains	Phenotypical, biochemical and PCR	(Ameur *et al.*, 2014)
Azrou, Aïn Leuh, Beni mellal, El Hajeb, Ifrane, Immouzer, Larache,Meknès, Oulmes, Sefrou-El Menzel and Tifelt	Spring of 2013	37 strains	RAPD and the sensitivity to streptomycin	(Ait Bahadou *et al.*, 2018b)

After the fire blight being declared in the country in the early spring of the 2006 growing season, the Ministry of Agriculture and Maritime Fisheries has put in place an emergency plan to deal with this worrying situation. The main actions of this urgent plan were summarized as follows:

• Promulgation of the Gubernatorial Orders that make the fight against the disease mandatory and quarantine the infected areas;

• Prospecting and demarcation of infected zones in most regions producing rosaceous;

• Planning debate and awareness meetings with professionals and local authorities;

• Arrangement of sanitation campaigns for infected orchards (elimination of the sources of infection from leaves, twigs and fruits: 30-50 cm for the apple tree and 1 meter for the pear and quince in below the infected area, disinfection of pruning tools, elimination of second flowering before opening, etc.);

• Prohibit the circulation of beehives during flowering from infected areas;

• Promulgation of a ministerial decree N° 2241 of August 18th concerning the prevention and the fight against the Bacterial Fire disease, published in the official bulletin, on November 18th, 2010.

This strategy was based on three axes: i) investigate the situation of disease spread in Meknes region and other rosaceous growing areas, ii) training and educating the producers and technicians and executives of the Ministry of Agriculture about the fire blight disease, and iii) setting up an integrated pest management program against fire blight disease. Therefore, rigorous actions were taken after the disease being declared. Among them, a national eradication program of infected commercial orchards and individual trees was conducted. As a result, over 2312 Ha of pear, quince and apple orchards in Morocco were eradicated in 2013 (Figure 3). In this period, 1629 Ha in Ifrane, 248 Ha in El Hajeb, 298 Ha in Khémisset, 56 Ha in Larache, 16 Ha in Fès, 14 Ha in Khénifra and 8.5 Ha in Taza were eradicated. Losses caused by fire Blight, 7 years after being detected, were estimated to more than $ 2 million, including the cost of removal and replanting. The fight against the disease continues and surveys were performed yearly by the relevant department to determine the progress of the disease. In 2015, an area of 9144 hectares was prospected and 1716 hectares were sanitized by pruning the affected organs.

At the orchard level, the most commonly used control strategy for the management of fire blight was the use of tolerant cultivars to the disease and pruning of diseased twigs and branches combined with application of copper-based formulations and /or preventive spraying of regulating plant growth and inducing plant defence products such as fosetyl-Al (Aliette™, Bayer) prohexadione-calcium (Regalis , BASF), or BHT (1,2,3-benzothiadiazole-7-carbothioic acid S-methyl ester) (sold as Bion™ by Syngenta). After the establishment of fire blight in Morocco, farmers gave up the susceptible cultivars of pear and are using the ones considered resistant such as Harrow cultivars.

CONCLUSIONS AND PROSPECTS

Since the detected of fire blight in Morocco, government directive and practical measures were applied for managing the disease. As mentioned above, the general disease incidence rate was reduced and the infected zone was narrowed. Now, the development of biocontrol agents for the control of fire blight in Morocco have attracted considerable attention as possible alternative in an integrated control strategy (Ait Bahadou *et al.*, 2017; Ameur et al., 2017; Ait Bahadou *et al.*, 2018a) and with the use of plant defense activators (Ait Bahadou *et al.*, 2017). Furthermore, other studies underscored the potential of natural compounds like plant extracts and essential oils to reduce the incidence rate of the disease. However, some alternatives require further attention such the probing of apple defense immunity, which might help to discover some new resistance genes and the evaluation and development of fire blight risk assessment systems based on forecast modeling.

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Figure 3: Complete eradication of commercial orchards (A) and individual trees (B)

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