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PREVALENCE OF APPLE SCAB IN DIFFERENT LEBANESE REGIONS AND THE STUDY OF ISOLATE RESISTANCE TO TWO FUNGICIDES

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Abstract

The following study was undertaken in order to determine the incidence of apple scab in different Lebanese regions and to evaluate the resistance of Venturia inaequalis, pathogen of apple scab disease, to two kinds of fungicides: Kresoxym-methyl and Difenoconazole.

A survey was carried out to locate the regions in Lebanon where the apple scab was expanding rapidly due to bad techniques and excessive treatments put ahead by farmers in an attempt to counter the disease more than ever resistant to fungicides sold in the market.

The Bekaa, South Lebanon, North Lebanon and Mount Lebanon were visited, and 27 samples were collected from villages across the regions. Isolates obtained from these samples were grown on culture media amended with three different concentration of each of Kresoxym-methyl and Difenoconazole in order to test their resistance to these two fungicides.

Résumé

L'étude suivante a été effectuée afin de déterminer l'incidence et la sévérité de la tavelure du pommier dans certaines régions libanaises et d'évaluer la résistance de Venturia inaequalis à deux types de fongicides: Kresoxym-methyl et Difenoconazole.

Une prospection a été faite pour situer les régions libanaises où la tavelure du pommier se répandait rapidement du fait des mauvaises techniques et des traitements excessifs appliqués, ce qui la rendait plus que jamais résistante aux fongicides les plus efficaces vendus sur le marché local.

La Békaa, le Liban Sud, le Liban Nord et le Mont Liban ont été visités et 27 échantillons ont été prélevés à travers le pays.

Les isolats obtenus à partir des échantillons ont été traités par trois différentes concentrations de Kresoxym-methyl et trois autres de Difenoconazole pour tester leur résistance à ces deux fongicides.

L'étude a permis de prouver qu'un traitement minimal de chacun des deux fongicides était en mesure de stopper la maladie dans certaines régions libanaises.

INTRODUCTION

Lebanon is a small mountainous country with an area of 10 452 km^2 and a population estimated at about 3.6 million (Latham, 2000). It comprises six administrative Muhafazats (governorates, namely Mount Lebanon, North Lebanon, South Lebanon, Nabatiyeh, the Bekaa Valley and Beirut), subdivided into 24 Cazas or districts (Latham, 2000).

Until the mid-1970s, Lebanon was a prosperous middle-income country driven mainly by the service sector, which contributed more than 70 percent of the GDP. From 1975 to 1992, the country, its economy and its population, suffered the destructive effects of a severe civil war. Since then, the progressive recovery of normal economic activities has led the public sector to concentrate its efforts on reconstructing infrastructure and improving population welfare, primarily focusing on the most vulnerable groups (Latham, 2000).

Many crops are cultivated in Lebanon, among them apple which is considered one of the most important crops (Ministry of Agriculture, 1999). But its cultivation is constrained by many diseases and mainly by apple scab (Saad, 2004).

Apple scab is considered a major disease in some Lebanese regions such as Akkar, Fneidek, Keserwan (Saad, 2004), and Lebanese farmers use fungicides to control it. However, the incidence and severity of the disease are not well determined and farmers complain from the inefficacity of some used fungicides.

The aim of this study is to improve the efficiency of the struggle against *Venturia inaequalis*. Therefore, we propose to:

- Evaluate the incidence of apple scab in different Lebanese apple growing regions;
- Monitor the level of resistance of apple scab isolates to two types of fungicides: Kresoxym methyl and Difenoconazole, commonly used for the control of apple scab disease.

MATERIALS AND METHODS

Field work and laboratory experiments were undertaken in order to

evaluate the apple scab incidence in different apple growing regions and monitor the level of resistance of apple scab isolates to two types of fungicides: Kresoxym methyl and Difenoconazole.

Field Visits: Lebanese growing apple regions were identified and regions located in South Lebanon and the Bekaa valley were visited during the growing season of 2004. Whereas those located in Mount Lebanon and North Lebanon were visited during the growing season of 2005.

Evaluation of severity and incidence: Severity of the disease was determined on attacked fruits based on a scale from 0 to 5. Incidence of the disease was evaluated based on the percentage of trees in an orchard which were infected with apple scab.

Samples collection: Each sample consisted of several damaged fruits, around ten, taken randomly from different trees of the same orchard.

After their collection, samples were kept in the refrigerator at 4°C until their use for laboratory studies. From these regions samples of *Venturia inaequalis* isolates were randomly collected from 27 different apple orchards.

Laboratory studies: One damaged fruit was selected from each sample collected and thus representing one orchard.

Then, the fungus from this diseased fruit was isolated in order to study the efficiency of two fungicides commonly used by Lebanese growers.

Isolation of *Venturia inaequalis*: From the selected damaged fruit, the diseased part or the fungus spot was cut and placed in an empty Petri dish.

Then, using a pipette, a droplet of sterile water (0.5 ml) was poured on the diseased spot in order to facilitate the harvest of conidia after rubbing gently the pipette tip onto the spot.

The droplet containing the spores was then sucked through the pipette, and directly poured in a test tube containing 4.5 ml of sterile water from which a dilution was performed on four test tubes, containing each 4.5 ml, in order to isolate the pathogen for each orchard. From the last test tube, 75 µl of the solution were spread on a Petri dish containing a PDA medium, by using a loop.

The Petri dish was then sealed with "parafilm" and stocked in an incubator at 18°C.

After two to three weeks, spores contained in the 75 µl of the solution, started to grow.

Three single spore colonies were isolated by cutting the spot they formed on the PDA medium, and placed each in another Petri dish containing a PDA medium.

Resistance of *Venturia inaequalis* isolates: From these isolated single spore colonies, only the clean, fully grown ones were used for the extraction of new single spore colonies which were placed on Petri dishes containing the two fungicides at different concentrations. Knowing that the rates of use recommended by the manufacturers are as follows (Manual

of Phytosanitary Products, Lebanon, 2003):

- Kresoxym methyl (Stroby®, BASF Agro): 4-6 grams/20 liters;
- Difenoconazole (Score®, Syngenta): 2-3 cc/20 liters.

- **Kresoxym methyl:** Kresoxym methyl (Stroby®, BASF Agro) was used at three different concentrations **T1** (0.07 mg), **T2** (0.007 mg) and **T3** (0.0007 mg) on Petri dishes containing 15 ml of autoclaved solution, prepared by using 13 g of Bacteriological Agar (Liofilchem) medium/liter of distilled water.
  T4 representing the control autoclaved solution at the rate of 15 ml of Bacteriological agar/Petri dish, prepared using 13 g of Bacteriological Agar (Liofilchem) medium/liter of distilled water, without the fungicide, in order to compare **T4** to the resistance of the isolates against the fungicides tested through three different treatments **T1, T2 and T3**.
- **Difenoconazole:** Difenoconazole (Score®, Syngenta), was used at three different concentrations **T5** (3.6 ml), **T6** (0.36 ml) and **T7** (0.036 ml) on Petri dishes containing 20 ml of autoclaved solution, prepared by using 39g of dehydrated PDA (HiMedia) medium/liter of distilled water.
  T8 representing the control autoclaved solution at the rate of 20 ml of PDA medium/Petri dish, prepared using 39 g of PDA/liter of distilled water, without the fungicide, in order to compare **T8** to the resistance of the isolates against the fungicides tested through three different treatments **T5, T6 and T7**.

RESULTS AND DISCUSSION

During field visits, apple scab incidence was evaluated in different Lebanese apple growing regions. In parallel, laboratory experiments undertaken allowed us to monitor the level of resistance of *Venturia inaequalis* isolates to two types of fungicides: Kresoxym methyl and Difenoconazole.

Field visits: Twenty seven apple orchards located in different Lebanese regions were visited during the growing season of 2004 (3 orchards in Southern Lebanon and 3 orchards in the Bekaa valley) and during the growing season of 2005 (7 orchards in Mount Lebanon and 14 orchards in Northern Lebanon).

Visual oservations: In most visited orchards, symptoms of apple scab were evident on fruits and leaves: light olive-colored, irregular spots on leaves and fruits. The lesions were olive green to gray with a velvety surface. Lesions on older leaves appeared on the upper surface of the

leaves. Infected young leaves remained small and curled. Infected fruit developed circular scab lesions, velvety and olive green at first but later becoming darker, scab-by, and sometimes cracked. The cuticle of the fruit was ruptured at the margin of the lesions.

Severity and incidence of apple scab: Out of the 27 visited orchards, 3 in South Lebanon, and 3 in the Bekaa valley were found healthy. Whereas, 8 orchards in Mount Lebanon and 14 orchards in North Lebanon were found attacked by apple scab.

Severity: Severity of fruits attack was estimated based on the scale from 0 to 5. In Jezzine, Kfarhoun, Kfarmelki as well as in Ainata, no fruits were attacked in all visited orchards. In other localities as Wata El Joz (1 orchard), Hrajel (1 orchard), Akkar El Atika (2 orchards), Al Haffa (5 orchards) and one orchard in Fneidek, fruits were slightly attacked as only 5% of the external surface showed symptoms of apple scab. In parallel, moderate to extensive scabby fruits were found in Mairouba (1 orchard), Kfardebian (1 orchard), Al Haffa (2 orchards), Fneidek (4 orchards). Fruits severity was the highest in two orchards: Mairouba and Kfertay in Mount Lebanon, where 75 to 80% of the fruit surface attacked was noticed.

Incidence: Six apple orchards (Jezzine, Kfarhoun, Kfarmelki as well as 3 in Ainata) out of the 27 visited were found healthy where no infection was found and incidence of the disease was zero. It is important to know that the climate in these regions is dry because the Bekaa Valley and the Anti-Lebanon Mountains are shielded from the influence of the sea by the Lebanon Mountains. The result is considerably less precipitation, lower humidity and a wider variation in daily and yearly temperatures (Atlas climatique du Liban, 1977). One hundred percent of tree infection was found in Mairouba, Kfertay and Akkar al Atika. Some localities of Mount Lebanon as Hrajel, Kfardebian and Baatouta were slightly attacked (incidence varied from 1 to 10%). In parallel, incidence going from 10% to 60% was recorded in most localities visited in North Lebanon. The relatively high incidence in these orchards could be explained by the fact that these regions are humid or because of rivers passing through the orchards creating a humid microclimate (Mairouba and Kfertay), apple scab being a disease favored by high relative humidity as stated by Agrios (1997).

Laboratory experiments:
On fruits collected from the diseased orchards, the pathogen was isolated from scab spots in order to confirm visual observations in the field. All observed symptoms were due to *Venturia inaequalis*.

Resistance of *Venturia inaequalis* isolates:
Difenoconazole and Kresoxym-methyl are the most used fungicides. Both of them are with unisite mode

of action and should not be used frequently in a growing season in order to avoid the appearance of resistant isolates (Geagea, 2005).

Results of apple scab samples resistance to Kresoxym-methyl:

In conclusion, we can say that for the two localities, Mairouba and Al Haffa, where the samples were collected, a **T1** (0.07 mg/l) concentration, the highest, should be used in order to eradicate efficiently the apple scab as isolates showed resistance to lower concentrations. This could be due to the fact that Kresoxym-methyl is frequently used in these two localities leading thus to the appearance of resistant isolates.

Regarding the remaining localities such as Kfardebian, Al Haffa, Fneidek, Akkar al Atika where the samples were collected, a **T3** (0.0007 mg/l) concentration is advised to be used in order to eradicate efficiently the Apple Scab, avoiding the use of an excess of fungicides which will eventually increase the resistance of the fungus to fungicide treatments.

Results of apple scab samples resistance to Difenoconazole:

In conclusion, we can say that for the localities Akkar al Atika, Fneidek and Al Haffa where samples collected, a **T7** (0.036 ml/l) concentration, the lowest, should be used in order to eradicate efficiently the apple Scab, avoiding the use of an excess of fungicides quantities which will eventually increase the resistance of the fungus to fungicide treatments.

Regarding the localities Kfardebian, Baatouta, Kfertay, Al Haffa where the samples were collected, a **T6** (0.36 ml/l) concentration is advised to be used in order to eradicate efficiently the apple scab.

Whereas for the localities Mairouba, Hrajel, Fneidek where the samples were collected, a **T6** (0.36 ml/l) concentration is also advised to be used in order to eradicate efficiently the apple scab. But in addition, further experiments should be undertaken in order to be sure of whether a **T 7** (0.036 ml/l) concentration is truly efficient on the samples of these localities.

Based on the above results, it is obvious that isolates from different localities reacted differently versus different concentrations of Difenoconazole. In fact, most isolates collected from Mount Lebanon needed higher concentrations of Difenoconazole to stop their growth than in North Lebanon. This could be related to the frequent use of Difenoconazole which led to the appearance of resistant isolates.

CONCLUSION

This study was undertaken in order to determine the incidence of apple scab in different Lebanese regions and evaluate the resistance of *Venturia inaequalis*, causal agent of Apple scab disease, to two kinds of fungicides: Kresoxym-methyl and Difenoconazole.

A survey was carried out to locate the regions in Lebanon where the apple scab was expanding rapidly due to bad techniques and excessive treatments put ahead by farmers in an attempt to counter the disastrous consequences of such a disease more than ever resistant to the most efficient fungicides sold in the market.

The Bekaa, South Lebanon, North Lebanon and Mount Lebanon were visited, and 27 samples were collected from villages across the regions. Incidence was 100% in Mairouba, Kfertay, Akkar El Atika whereas 50 to 60% of attacks were recorded in Fneidek and Al Haffa. Incidence was nul in Ainata, Jezzine and Kfermelki.

Laboratory identification was done in order to confirm the visual observations. Samples were brought to the laboratories of the Holy Spirit University of Kaslik and the American University of Beirut, where they were isolated and identified. After that, different isolates of *Venturia inaequalis* were selected in order to test their resistance to two of the most used fungicides in Lebanon: Kresoxym-methyl and Difenoconazole.

Nineteen isolates out of twenty-one showed susceptibility to the three concentrations of Kresoxym-methyl, even the lowest one (0.0007 mg/l). Two isolates showed moderate resistance to the two concentrations 0.007 mg/l and 0.0007 mg/l and susceptibility to the highest concentration 0.07 mg/l.

Isolates of six samples collected from North Lebanon were susceptible to the three concentrations of Difenoconazole whereas five isolates from Mount Lebanon and five isolates from North Lebanon were found susceptible to the two concentrations of 36 ml/l and 0.36 ml/l, but resistant to the lowest concentration of 0.036 ml/l.

Results of the study showed that a minimum concentration of Kresoxym-methyl could be used in Mount Lebanon, North Lebanon and is more beneficial for an efficient eradication to diminish the incidence of apple scab on apple orchards in Lebanon. Moreover, conclusions were also made regarding the susceptibility of the isolates to Difenoconazole, which showed that higher concentration of this fungicide was needed in the region of Mount Lebanon in comparison to the region of North Lebanon, in order to stop apple scab disease.

Apple scab has disastrous economic consequences to the majority of the farmers and the excess of treatments actually applied in trying to hinder the advance of the disease is very harmful to the environment and worse, increase the resistance of *Venturia inaequalis* to all kinds of fungicides. Therefore, more studies alike this one should be undertaken every year in each region in order to collect the biggest amount of data which will help the experts in limiting the disease spread as a first step, then to eradicate it as a second step.

And to reach this objective, forecasting models would be the most effective way to counter the disease by essentially limiting the use of fungicides and should be implemented in Lebanon to cover all its affected regions.

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