Survey on Symptom Expression and Damage Caused by Pepino Mosaic Virus in Commercial Tomato Production in Belgium

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Abstract

Pepino Mosaic Virus (PepMV) was first reported on greenhouse tomato (*Solanum lycopersicum*) in the Netherlands and the UK in 1999. Since then PepMV is worldwide established in the tomato industry and has become a significant problem. An extensive survey among Belgian tomato growers was conducted, assessing their experiences with PepMV in the 2005 and 2006 growing season, and their strategies to cope with this relatively new viral threat. Symptom expression and the economical damage caused by PepMV were investigated. An increase in PepMV infected tomato greenhouses was seen during the 2004 and 2005 growing seasons. The infection risk appeared to be significantly higher in dense tomato cultivation areas. It was shown that a PepMV infection early in the growing season does not guarantee fewer symptoms as compared to a late infection. The number of tomato growers that deliberately inoculated their crops early in the growing season as an immunization strategy decreased significantly after 2005, as results were not satisfying.

INTRODUCTION

Pepino mosaic virus (PepMV), a Potexvirus originally isolated from pepino (*Solanum muricatum*) in Peru in 1980, was first reported on greenhouse tomato (*Solanum lycopersicum*) in the Netherlands in 1999 (Jones et al., 1980; van der Vlugt et al., 2000). Since then, this viral disease has been established in the tomato industry worldwide. At present, five PepMV genotypes (EU, LP, CH2, US1 and US2) have been described, four of which (EU, LP, US2 and CH2) have been reported to occur in Europe (Mumford and Metcalfe, 2001; Aguilar et al., 2002; Cotillon et al., 2002; Verhoeven et al., 2003; Lopez et al., 2005; Pagan et al., 2006; Maroon-Lango et al., 2005; Ling, 2007; Hanssen et al., 2007). A survey conducted in commercial tomato production facilities in Belgium revealed that the PepMV population in Belgium in 2006 was dominated by the CH2 genotype, as this genotype occurred in almost 90% of the infected tomato crops. In addition to the CH2 genotype also the EU genotype was present, with both genotypes often occurring in mixed infections (Hanssen et al., 2007). A wide range of symptoms has been associated with PepMV infection in tomatoes, including leaf mosaic, leaf distortions, yellow rectangular leaf spots, nettle heads, stunting, fruit deformation, browning of fruit petals and fruit discoloration. The latter is generally considered as the most damaging PepMV symptom as it reduces the commercial value of the fruit (Soler et al., 2000; Mumford and Metcalfe, 2001; Spence et al., 2006).

To reduce economic losses caused by PepMV infection, some tomato growers in the Netherlands and Belgium deliberately inoculate their plants with PepMV at the start of the growing season, as it has been suggested that early PepMV infections are less damaging than infections that occur later in the growing season. In addition, it is believed that inoculation of tomato plants with a weakly aggressive isolate of PepMV offers protection against subsequent infection with a more aggressive isolate (immunization). This effect is based on cross-protection, a principle that was successfully used in the seventies to protect tomato plants against *Tobacco mosaic virus* (TMV) (Rast, 1972). However, in the case of PepMV the efficiency of this strategy has not yet been proven. In contrast, it was recently shown that a tomato crop infected with the EU genotype was subsequently invaded by the CH2 genotype, or vice-versa (Hanssen et al., 2007).

Proc. II[nd] Intl. Symposium on Tomato Diseases
Eds.: H. Saygili et al.
Acta Hort. 808, ISHS 2009

Moreover, this secondary infection coincided with an increase in PepMV symptom expression (Hanssen et al., 2007). An elaborate survey was conducted in the Belgian tomato production industry to assess the PepMV damage as perceived by the growers and to study the results obtained by applying the immunization strategy in commercial tomato greenhouses.

MATERIALS AND METHODS

Study Site

The tomato industry in Belgium is located in the Northern, Flemish district of the country. As tomatoes in Belgium are exclusively produced for the fresh market, fruit quality and appearance is of major importance. Since quality standards during production are very high, the majority of the tomatoes have to be sold in the highest or first quality class in order to remain profitable. The major tomato growing areas in Flanders are covered in this study.

Survey 2005

A questionnaire was designed to conduct a survey on the experience of the Belgian tomato growers with PepMV in 2005, aiming to reveal grower's perception on damage caused by and problems associated with PepMV in the Belgian tomato production during the 2005 growing season. Special attention was given to the results obtained by the immunization strategy that was applied by part of the interviewed growers. PepMV infected tomato production sites were divided into two groups: the production sites that were deliberately inoculated with PepMV at the start of the growth season, further referred to as the 'inoculated sites', and the ones that suffered from a natural infection further referred to as the 'natural infections'. An Access database was designed to manage and process the acquired dataset.

Survey 2006

A second questionnaire was designed to assess growers' experiences during the 2006 growing season, composed of multiple choice questions to facilitate the written completion of the questionnaire by the growers. The tomato production sites infected with PepMV during the 2006 growing season were divided into three groups: a group of production sites that were deliberately inoculated at the start of the growing season, further referred to as the 'inoculated sites', the ones that suffered from a natural infection in the beginning of the growing season (January to March), further referred to as the 'early natural infections' and finally the ones that became infected later in the growing season (from April onwards), further referred to as 'late natural infections'. In this classification a production site was considered infected as soon as the grower recognized typical PepMV symptoms, as the exact time of infection was not known. An Access database was designed to manage and process the acquired dataset.

RESULTS

PepMV Incidence

Until 2003, the incidence of PepMV in the Belgian tomato production was low. However from 2004 onwards, a gradual increase in the number of PepMV infected tomato greenhouses was seen (data not shown). As may be expected, the infection risk appears to be related to the distance between the tomato production sites (Fig. 1). When the distance between the production sites was less than 50 meters, 75% of the production sites were infected with PepMV at least once in the period between 1999 and 2005. This percentage was only 23% when the distance was more than five kilometres.

The immunization strategy, in which tomato plants are deliberately inoculated with PepMV in a young stage to minimize the damage, was originally introduced in the Netherlands. In Belgium, this strategy gained acceptance in 2004, and a peak was reached

in 2005 when 14% of the growers "immunized" their tomato plants. This number significantly decreased in 2006 to 6% of the growers.

Assessment of PepMV Damage in the 2005 Growing Season

About 40 % of the growers considered fruit appearance as the most damaging problem associated with PepMV. However, 21% and 20% named production losses and a reduction in tomato shelf life, respectively, as the major PepMV related problem.

In inoculated production sites, the first symptoms were seen in January, February and March, shortly after inoculation. The main symptoms in these greenhouses were fruit marbling and nettle-head, which were reported by 22% and 21% of the growers respectively, followed by fruit deformation (13%), bubbling of the leaf surface (11%) and yellow rectangular leaf spots (10%). In natural infections, two peaks were seen with regard to the first display of PepMV symptoms, the first one coinciding with the start of the season (January, February and March) and a second, more pronounced, peak in summer (Fig. 2).

The reported symptoms differ between naturally infected tomato plants and inoculated plants (Fig. 3). With regard to fruit symptoms, the percentage of growers reporting fruit marbling as an important symptom was 34% in the case of a natural infection, and only 22% upon inoculation. Some differences in PepMV symptoms were also seen on vegetative plant parts (head and lower leaves) with a higher incidence of nettle-head (21% versus 14%) and bubbling of the leaf surface (11% versus 6%) in inoculated crops when compared to naturally infected tomato plants.

As tomatoes showing the typical PepMV-related marbling or discoloration are generally downgraded from the first or highest quality to the second quality, the percentage of second class tomatoes can be considered as an indicator for quality loss caused by PepMV infection. Prices paid for second class tomatoes are approximately 30% lower than the first class prices. Growers were asked to assess the percentage of class one tomatoes produced in the 2005 growing season as a consequence of PepMV infection. In general, this loss was considered rather limited. In the group of growers that applied the immunization strategy, 79,5% assessed the damage as less than 5%. This percentage was somewhat lower (71,4%) in the group of growers with natural infections. In inoculated production sites, the percentage of class one tomatoes was estimated at 5 to 10% by 5% of the growers, at 10 to 20% by another 5% of the growers, and at more than 20% by 10% of the growers, while these percentages were higher in the group of growers with natural infections, coming down to 7%, 7% and 14% respectively. These results indicate that fruit marbling or discoloration was somewhat less common upon immunization, although the differences were rather small.

Assessment of PepMV Damage in the 2006 Growing Season

In 2006 Belgian tomato growers experienced a significant loss in production due to PepMV infection. In about 50% of the PepMV infected production sites the production loss was estimated at more than 5%, and in one fifth of the cases even more than 10%. The quality loss, measured by the percentage of class one tomatoes, was generally seen as limited; 68% of the growers estimated the percentage of class one tomatoes lower than 5%. However, these results reflect that also PepMV related fruit quality problems were somewhat more pronounced in 2006 than in 2005.

In 2006 a remarkably high portion of growers reported the first symptoms (35%) in the start of the growing season, more specifically in January and February (Fig. 2). When growers were asked for the putative source of infection, the same 35% of the growers suspected the nursery as the source. Like in 2005, a second peak in display of the first symptoms was seen in the summer months.

Only minor differences between inoculation and natural infection were seen in the assessment of the PepMV related damage in 2006 (Fig. 3b). The number of growers that evaluated the PepMV damage as moderate to severe was 58% and 59% in the case of inoculation and natural infection, respectively. However, the PepMV damage was

assessed as severe to very severe by 29% of the growers that inoculated their crop, as compared to 23% for natural infections. Production losses due to PepMV were estimated as more than 10% by 21% of the growers that inoculated their plants, which was comparable to the percentage for naturally infected production sites. However, production losses are estimated lower for natural infections late in the growing season. In this group, only 15% of the growers estimated the production loss at more than 10%. The same trend can be seen for the estimation of fruit quality losses, as 23% of this group estimated the percentage of class one tomatoes due to PepMV infection at more than 5%, compared to 29% en 28% in the groups of inoculated crops and natural early infections, respectively.

DISCUSSION

It was shown that the PepMV infection risk increases with the density of greenhouses in the tomato production area, suggesting that PepMV transmission by birds and insects like bumblebees and biological control agents can attribute substantially to the spread of the virus. Transmission of PepMV by bumblebees has been reported by Lacasa et al., in 2003.

Two peaks were seen with regards to the first display of symptoms in naturally infected production sites, one coinciding with the start of the growing season and the other one in the summer months. In 2005, the first peak can probably be attributed to the transfer of last year's infection to the new crop, with symptoms showing up shortly after infection. In 2006, the situation was somewhat different as a high number of tomato production sites was infected already early in the growing season, with the putative time of infection in the nursery stage. This situation is reflected by a high incidence of first PepMV symptoms in January and February 2006. To explain the second peak in the summer months, two hypotheses can be postulated. Since all results presented are based on the growers' perceptions and not on sample analyses, it is not possible to determine the exact time of PepMV introduction in the crop. Therefore, the moment the growers first noticed the PepMV symptoms does not necessarily coincide with the point of introduction. It is speculated that in the case of a natural PepMV infection in the late spring or early summer months, a latent period of several weeks, and in some cases even of several months, can be seen (Hanssen et al., unpublished data). As PepMV symptom expression is known to increase with decreasing light intensity, the shortening of the days after July could explain the appearance of the first PepMV symptoms in this period, even if the production sites were already infected for several weeks or months. An alternative hypothesis is that hygienic measures are becoming less strict in the summer months due to the high work pressure, thus increasing the infection risk.

When applying the immunization strategy, inoculation of the plants is performed early in the growing season in young tomato plants. Leaf symptoms, especially nettle-head and yellow leaf spots, tend to be more pronounced in young plants (Spence et al., 2006). Therefore, the higher incidence of these symptoms in inoculated plants can be explained by the fact that plants were infected in a young stage. In 2005, the impact on fruit quality was somewhat lower in this group when compared to the natural infections. However, a large part of the natural infections in 2005 was considered as late infections, as the first symptoms were noted after the first quarter (Fig. 2). Therefore, the higher incidence of fruit marbling in this group can be expected based on former studies reporting that the impact on fruit quality is higher when the PepMV infection is introduced at a later stage (Jones and Lammers, 2005; Spence et al., 2006). This observation is confirmed by the assessment of the percentage of class one tomatoes as a result of PepMV infection.

The production losses due to PepMV infection were generally considered as more pronounced in 2006 as compared to 2005, which might be related to the high incidence of early natural infections in 2006. As PepMV infection in young plants generally results in more pronounced symptoms on the leaves and in the head of the plants, a growth reduction is usually seen in plants that are infected in a very young stage, which can lead to production losses.

In the overall assessment of damage caused by PepMV in 2006, only minor differences could be seen between the two groups of growers (immunization vs. natural infection). However, when subdividing the natural infections into early infections and late infections, the production loss and the loss in fruit quality were the lowest in the case of a late natural infection. As more fruit marbling is usually seen in the summer months in the case of a late infection, this result is rather unexpected. However, a first display of PepMV symptoms in the summer months implies a PepMV-free cultivation period during the first production months. This might result in a higher yield as compared to inoculated crops, since growth reduction is usually seen after inoculation, thus compensating for putative losses later in the season.

It can be concluded that, when considering the yield and fruit quality over the entire production season, inoculation of tomato plants early in the growing season was not considered to result in less damage as compared to naturally infected tomato crops. From 2006 on, the immunization strategy was therefore generally abandoned in Belgian tomato cultivation. This survey shows that the results obtained by Belgian tomato growers applying the immunization strategy were generally not satisfying. As a consequence, the majority of the Belgian tomato growers now choose a preventive approach, applying very strict hygienic measures both during the winter stop, to clean and disinfect the greenhouse, and in the course of the growing season, to prevent the introduction of PepMV in the tomato crop.

ACKNOWLEDGEMENTS

The authors thank IWT Vlaanderen (IWT 040718), LAVA cvba (Boortmeerbeek, Belgium) and Greenpartners (Sint-Katelijne-Waver, Belgium) for financial support. B.P.H.J.T. is supported by a VIDI grant of the Research Council for Earth and Life sciences (ALW) of the Netherlands Organization for Scientific Research (NWO), and funding from the Centre for BioSystems Genomics (CBSG).

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Figures

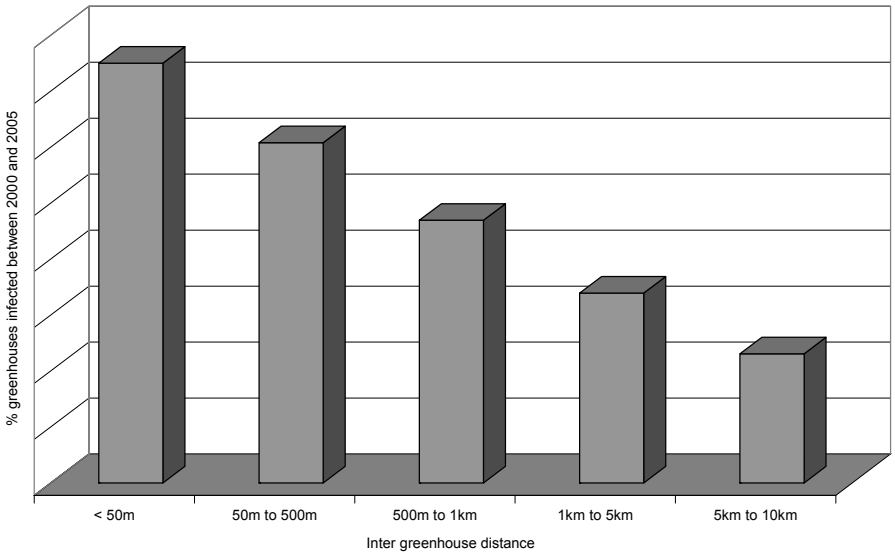

Fig. 1. Percentage of tomato production sites (as part of the total number of interviewed growers) infected with PepMV at least once between 1999 and 2005, related to the minimal distance to adjacent greenhouses.

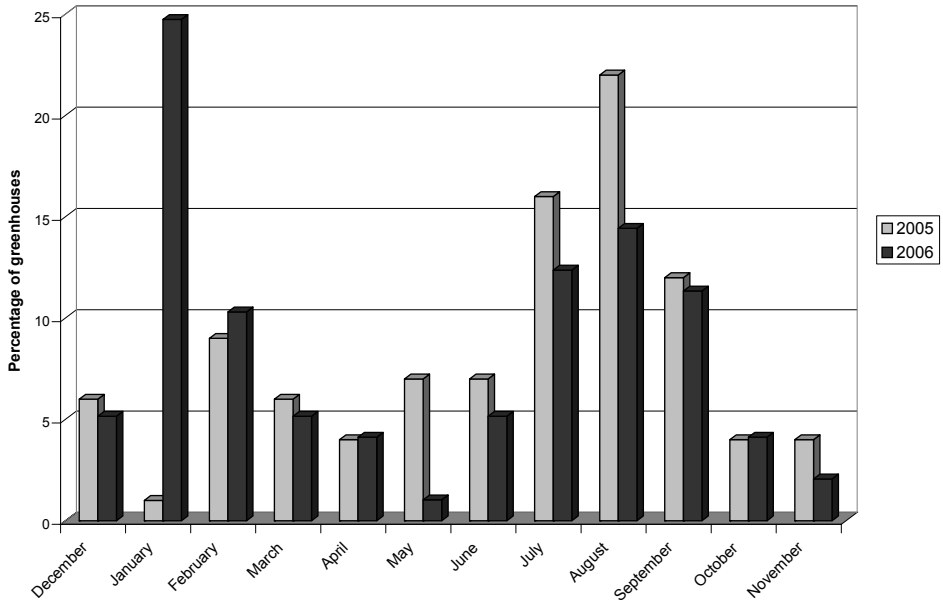

Fig. 2. Time of first PepMV-symptom display in greenhouses with a natural infection in 2005 and 2006 as reported by the growers, presented as the percentage of growers reporting the first symptoms in a particular month. Percentages are calculated on the total number of natural infections in 2005 and 2006. Two peaks are seen, one in February – March, and one in summer.

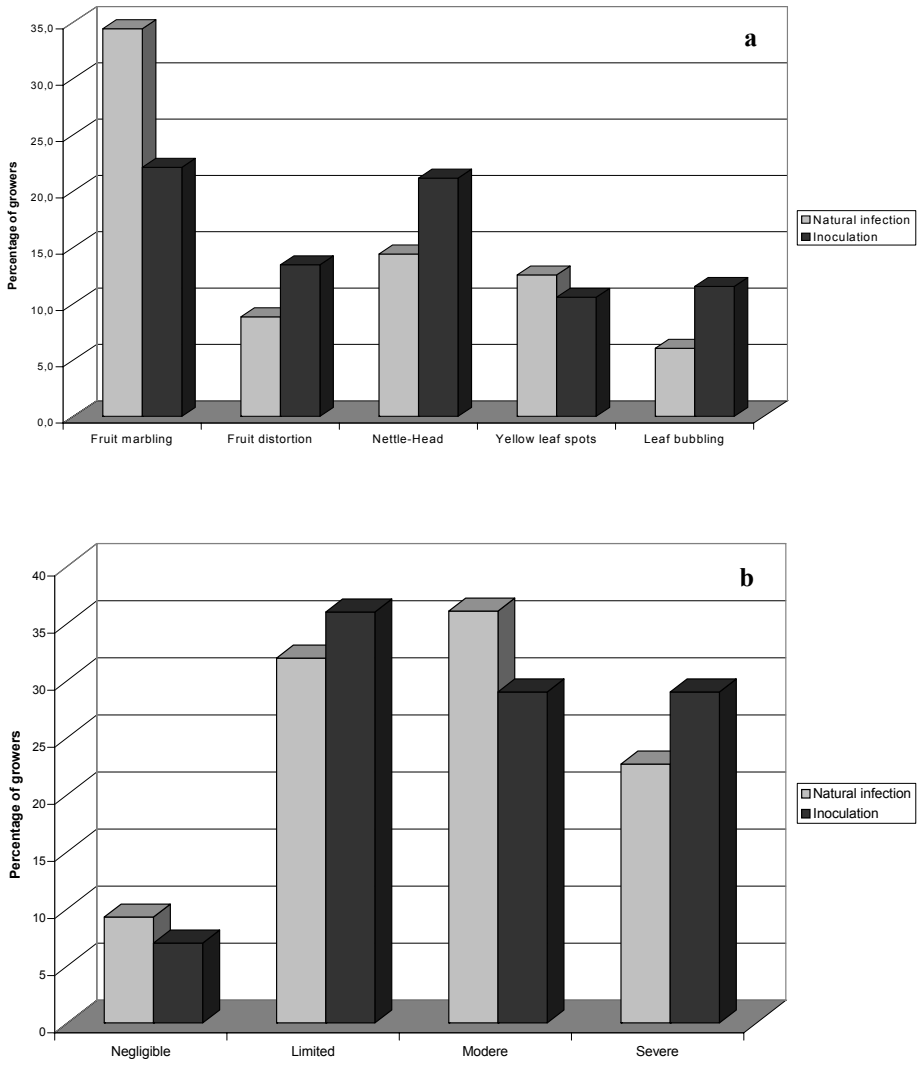

Fig. 3. Comparison between naturally infected tomato production sites and inoculated production sites in 2006: (a) percentage of growers in each group reporting a particular PepMV symptom as important and (b) percentage of growers in each group assessing the damage caused by PepMV as negligible, limited, moderate and severe.