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### **SPATIAL DISTRIBUTION, SPREAD AND SURVIVAL OF *XANTHOMONAS FRAGARIAE* IN STRAWBERRY**

**J.M. VAN DER WOLF<sup>1</sup>, P. KASTELEIN<sup>1</sup>, M. KRIJGER<sup>1</sup>,  
R. CZAJKOWSKI<sup>1</sup> & T. BEEN<sup>1</sup>  
J. VANDROEMME<sup>2</sup> & M. MAES<sup>2</sup>**

<sup>1</sup> Plant Research International, Wageningen, The Netherlands

<sup>2</sup> ILVO, Merelbeke, Belgium

*Xanthomonas fragariae* is the causative agent of angular leaf spot disease of strawberry. For propagation material it is considered as a quarantine disease in Europe. To improve control strategies, we studied the spatial distribution, plant colonization and survival of this pathogen in different research projects.

For surveys on fields with symptomless propagation plants, there is a need for a reliable sampling protocol. Two naturally infected fields (A and B) with propagation plants were sampled containing a low number of foci of diseased plants (respectively 3 and 12). Per infected field one plot of ca. 100 m<sup>2</sup> was sampled with a symptomatic plant in the centre. Per plot, one plant per m<sup>2</sup> was sampled. From each plant, all compound leaves were analyzed individually using immunofluorescence staining (IF). Field A showed an aggregated distribution of infected plants. Only 70 out of 621 compound leaves were infected. In field B all but two plants were infected, and 434 out of 732 compound leaves. Currently, in the EU, 10 compound leaves per 2000 m<sup>2</sup> are sampled. Using this protocol, the probability of detecting the infection would have been 59% in field B and 11% in field A, if we presume the same distribution pattern to apply for at least 200 m<sup>2</sup>. We therefore suggest to increase the sample size in fields with symptomless plants.

To study dissemination and plant colonization in detail, in greenhouse experiments GFP-tagged transformants of *X. fragariae* are being used. Methods were fine-tuned for detection of GFP-tagged strains, which included confocal laser scanning microscopy, TaqMan PCR and FPI, a non-invasive monitoring system. Leaf inoculation of plants in a greenhouse experiment resulted in systemic infections of entire plants within 4 weeks.

If multiple infections occur and entire fields have to be destroyed, plant material often is sprayed with a herbicide and ploughed under. We investigated which herbicide is most appropriate for haulm destruction and how long *X. fragariae* can survive in plant debris in soil. Inoculated, symptomatic plants were treated with Round up, Grammoxone, Duplosan or water. After haulm destruction, leaves and the remaining plant parts were separately put in net bags and buried in crates with a humus-rich sandy soil. Crates were stored at temperatures prevailing in the Netherlands during recent mild winters (6-11 °C). Grammoxone most effectively killed the plants and enhanced decomposition in soil. *X. fragariae* could be detected with PCR-amplification but not with IF in leaves and crop debris after storage for 6 months. One out of 16 strawberry bait plants planted on soil containing infected plant material which had been stored for 11 weeks was PCR positive. Survival of *X. fragariae* in crop debris in soil during winter seasons and infection of new strawberry crops from soil-borne inoculum cannot be excluded.

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