Epidemiology and Effective Control of *Alternaria alternata*, Causal Agent of Dead (Dormant) Flower Bud Disease of Pear

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Abstract

Dead flower buds are a common phenomenon in pear culture in The Netherlands, Belgium and Mediterranean countries. Disease cases are also reported from South America. The disease is characterized by a partial or complete necrosis of flower buds during tree dormancy. The disease progresses during winter and spring, eventually resulting in the death of most flowers and decay of buds at flowering. In The Netherlands the problem is mostly found in the main pear cultivar 'Conference', but cultivars such as 'Doyenne du Comice' and 'Gieser Wildeman' are also affected. Disease incidence may be as high as 80-90%. Possible causes mentioned are abiotic stresses, incompatibility between scion and cultivar, and plant pathogens and pests. Research in recent years revealed that pear growth regulation does not prevent the occurrence of dead flower buds. Also, the bacterium *Pseudomonas syringae* pv. *syringae* (P.s.s.) was commonly regarded as the causal agent of dead flower buds in pear, although the relation between P.s.s. and dead flower buds in orchards has never been proven in The Netherlands. However, the fungus *Alternaria alternata* was found in diseased flower buds and also often in symptomless flower buds. A linear relationship between infection rate and dead flower bud disease incidence was found. Pathogenicity tests and Koch's postulates were carried out. It was concluded that *A. alternata* is the causal agent of dead (dormant) flower bud disease. *A. alternata* is known to cause late blight in pistachio and several diseases in fruit crops such as moldy-core in apple and brown rot in citrus. By identifying the causal agent of dead flower bud disease, an effective control strategy could be developed. In field trials it was proven that fungicide treatments can reduce disease incidence significantly.

INTRODUCTION

Dead flower buds are a common phenomenon in pear culture in The Netherlands, Belgium and Mediterranean countries (Deckers and Schoofs, 2001, 2008; Montesinos and Vilardell, 1991, 2001; Wenneker et al., 2004, 2006). Disease cases are also reported from South America; e.g., Uruguay and Brasil, and South Africa. The disease is characterized by partial or complete necrosis of flower buds during dormancy or budbreak. Depending on disease severity, symptoms vary from reduced number of flowers per bud to buds completely killed.

The disease is present in most years but does not cause problems, due to the abundance of flower buds in normal years. However, in years with low bud numbers per tree, the disease causes significant (financial) losses, which was the case in 2001 in The Netherlands. Disease incidences may be as high as 80-90%. The problem is mostly found in the main pear cultivars 'Conference' and 'Doyenne du Comice'.

Until recently, it was commonly accepted that the bacterium *Pseudomonas syringae* pv. s*yringae* (P.s.s.) was the causal agent of dead flower buds of pear. This was partly due to the fact that *Pseudomonas syringae* is proven to be the causal agent of blossom blast (Mansvelt and Hattingh, 1986; Whitesides and Spotts, 1991). The symptoms of blossom blast are characterized by blast of blossom and leaves which occur in periods of cool wet weather during bloom and post-bloom stages. However, these symptoms differ from the symptoms of dead flower bud disease, which are characterized by partial or complete necrosis of flower buds during dormancy or bud break.

The relation between P.s.s. and dead flower buds in orchards has never been proven in the Netherlands. It was also concluded that population levels of P.s.s. were not significantly correlated to the amount of disease, in an extensive study over ten years in Spain (Montesinos and Vilardell, 1991, 2001). Other possible causes mentioned are unbalanced (vigourous) tree growth, abiotic stresses, incompatibility between scion and cultivar, and other plant pathogens and pests. However, dead flower buds caused by the pear bud weevil (*Anthonomus pyri*) are easily distinguished from dead flower bud disease.

Extensive research in The Netherlands (Wenneker et al., 2004, 2006) showed that the fungus *A. alternata* is always present in diseased flower buds and also often in symptomless flower buds. In laboratory tests the pathogenicity of *A. alternata* was proven on flower buds of detached pear twigs. Therefore, it is assumed that *A. alternata* is the causal agent of dead flower buds of pear in The Netherlands. In this study P.s.s. was only isolated sporadically from bulk samples and individually diseased flower buds. This indicates that the bacterium plays a minor role in dead dormant flower bud disease in The Netherlands. By identifying the causal agent of dead flower bud disease of pear, an effective control strategy can be developed, e.g., fungicide schemes with *Alternaria* specific fungicides as Rovral (a.i. iprodione).

The objectives of the project are (i) to monitor disease development and (ii) to develop control strategies.

MATERIALS AND METHODS

Alternaria alternata (Pathogen) Assessment in Commercial Orchards in 2007 and 2008

In 2007 and 2008 in 13 commercial pear orchards (cultivar 'Conference') random samples of 100 flower buds per orchard were taken. In the laboratory 50 buds were individually assessed for the presence of symptoms, and 50 buds were individually tested for infection with *Alternaria*. The buds used for determination of infections were surface sterilized (30 minutes in 2.5% formaldehyde-solution (a.i. 40%) and thoroughly washed in sterile demineralized water to remove sterilizing agent) and cut into two pieces. The flower primordia of each bud were plated onto potato dextrose agar (PDA), and assessed for the presence of *A. alternata*.

Fungicide Trial

Trials were performed in 2006 (spray applications) and 2007 (disease assessments), in a pear orchard located at the experimental station at Randwijk, The Netherlands. The orchard consisted of pear trees of the cultivar 'Conference' on Quince MC rootstock. Trees were planted in 1997 in a single row planting system (3.5×1.5 m).

Spray applications were carried out with a cross flow sprayer (Homeco Urgent) with Albuz lilac hollow cone nozzle at 5 bar spraying pressure, and a volume of 320 L ha^{-1}. The experiment was done in a randomized block design with four replicates. Each replicate consisted of 7 trees. Observations were made on the middle 5 trees.

The experiment consisted of the following treatments:

1) Untreated control.
2) Thiram (a.i. thiram - 80%): weekly spray applications (twelve applications): starting end of May 2006 till harvest. Dose: 2 kg/ha.
3) Switch (a.i. 37.5% cyprodinil+25% fludioxonil): weekly spray applications (twelve

applications): starting end of May 2006 till harvest. Dose: 0.8 kg/ha.
4) Switch (a.i. 37.5% cyprodinil+25% fludioxonil): three spray applications: starting end of May 2006 with two weeks interval. Dose: 0.8 kg/ha.
5) Switch (a.i. 37.5% cyprodinil+25% fludioxonil): three spray applications: starting end of May 2006 with four weeks interval. Dose: 0.8 kg/ha.
6) Switch (a.i. 37.5% cyprodinil+25% fludioxonil): two spray applications: end of June 2006 and end of July 2006. Dose: 0.8 kg/ha.
7) Euparen (a.i. tolylfluanid): weekly spray applications (twelve applications): starting end of May 2006 till harvest. Dose: 2.25 kg/ha.
8) Saponin (a.i. saponin): weekly spray applications (twelve applications): starting end of May 2006 - till harvest. Dose: 7.5 L/ha.
9) Malvin (a.i. captan - 80%): weekly spray applications (twelve applications): starting end of May 2006 till harvest. Dose: 2.25 kg/ha.

Control of *Alternaria alternata* and Disease Assessment

Disease incidence was assessed at the beginning of bloom (April 2007). All flower buds per tree were counted and the disease incidence per tree was calculated from the overall count (and expressed as percentage dead flower buds).

Mean disease incidence of all trees for each replicate was used for statistical analysis. Effect of treatments was determined with ANOVA at a 0.05 probability level (Genstat^{TM} version 8.11). The relation between infection rate and dead flower buds was determined.

RESULTS

***Alternaria* Assessment in Commercial Orchards in 2007 and 2008**

In 2007 the commercial orchards the infection rate with *A. alternata* ranged from 66-100%. The disease incidence ranged from 33-93%. In 2008 disease pressure appeared to be lower than in 2007. In 2008 the infection rate ranged from 6-90%. The disease rate ranged from 4-57%. The fungus *Alternaria alternata* was isolated from nearly all diseased flower buds and also often from symptomless flower buds. There was a very good correlation between the occurrence of visible symptoms and infection with *Alternaria alternata* in 2007 and 2008 (Figs. 1 and 2).

Fungicide Trial

Dead dormant flower bud incidence ranged from 27-79% (Fig. 3). Lowest dead flower bud incidences were observed in the Switch (a.i. cyprodinil+fludioxonil) sprayed treatments. There was no statistical significant difference between the number of applications or the moment of application. No effect was observed from thiram, saponin and tolylfluanid applications. The captan applications appeared to increase the disease incidence.

DISCUSSION AND CONCLUSIONS

Until now, it was commonly accepted that the bacterium *Pseudomonas syringae* pv. s*yringae* (P.s.s.) was the causal agent of dead flower buds of pear. This was partly due to the fact that *Pseudomonas syringae* is proven to be the causal agent of blossom blast. However, the symptoms of blossom blast are characterized by blast of blossom and leaves which occur in periods of cool wet weather during bloom and post-bloom stages. These symptoms differ from the symptoms of dead flower bud disease; which are characterized by partial or complete necrosis of flower buds during dormany or bud break. Mainly due to ice-nucleation activity (INA) of *Pseudomonas syringae* and often large resident populations of this bacterium in orchards a relation with dead flower buds is assumed, and Koch's postulates have been completed (Montesinos and Vilardell, 1991).

In The Netherlands a relation between P.s.s. and dead dormant flower buds could not be proven. However, the fungus *A. alternata* was (nearly in all samples) found in

diseased flower buds and also in symptomless flower buds. In laboratory tests the pathogenicity of *A. alternata* was proven on flower buds of detached pear twigs. Therefore, it is assumed that *A. alternata* is the causal agent of dead flower buds of pear in The Netherlands.

The results in this paper indicate a significant relationship between disease symptoms in dormant flower buds and infection rate of *A. alternata*. Apparantely, *A. alternata* is capable of penetrating and infecting pear flower buds. The survey in the commercial orchard revealed that the infection rate of dormant flower buds with *A. alternata* can be 100%. However, the disease incidence varies strongly between orchards and years.

Apparantely, choice of fungicides is important in achieving good control of dead flower bud disease. Timmer et al. (2000) noted that, though, some cultural measures can help to control *Alternaria* brown spot in citrus, fungicide applications are essential to produce blemish free fruit. However, choice of fungicides is important (Yogev et al., 2006). According to Reuveni (2006) attempts to control *Alternaria* and moldy-core in apple by using foliar sprays of several fungicides, e.g., benomyl, captan, dodine, iprodione, mancozeb, or some of their combinations have been unsuccessful in the past, probably due to low efficacy. This might also explain the high infection rates of pear flower buds with *Alternaria* in The Netherlands.

Deckers et al. (2008) found an effective control of dead flower buds disease and a clear improvement of the flower bud quality with fosetyl-Al treatments. The best application window of the treatments with fosetyl-Al to solve the problems with dead flower buds on pears seems to be the post bloom period. This confirms our observations (Wenneker et al., 2008).

In previous trials it was proven that multiple spray applications with iprodione reduced *Alternaria* infections in pear flower buds significantly (Wenneker et al., 2008). Also, a significant correlation with dead flower buds disease was shown. In surveys in commercial pear orchards over a number of years in The Netherlands, high infection rates in dormant flower buds were found. It is possible that standard registered fungicides in pear growing control most fungi, with the exception of *Alternaria alternata*, and thereby creating conditions for massive growth of *Alternaria* on pear buds. We expect that the (recent) registration of effective fungicides against *Alternaria*, such as Bellis (a premix fungicide containing pyraclostrobin+boscalid - BASF) or Switch (a premix fungicide containing cyprodinil+fludioxonil - Syngenta), will be very useful to control dead flower bud disease of pears in The Netherlands.

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Figures

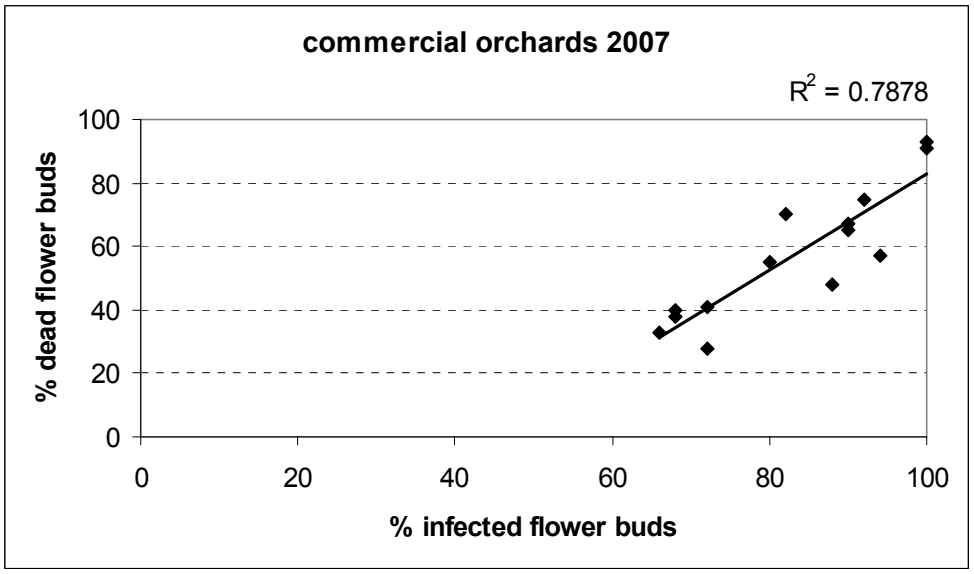

Fig. 1. Relation between visual symptoms and infection of *A. alternata* in 2007.

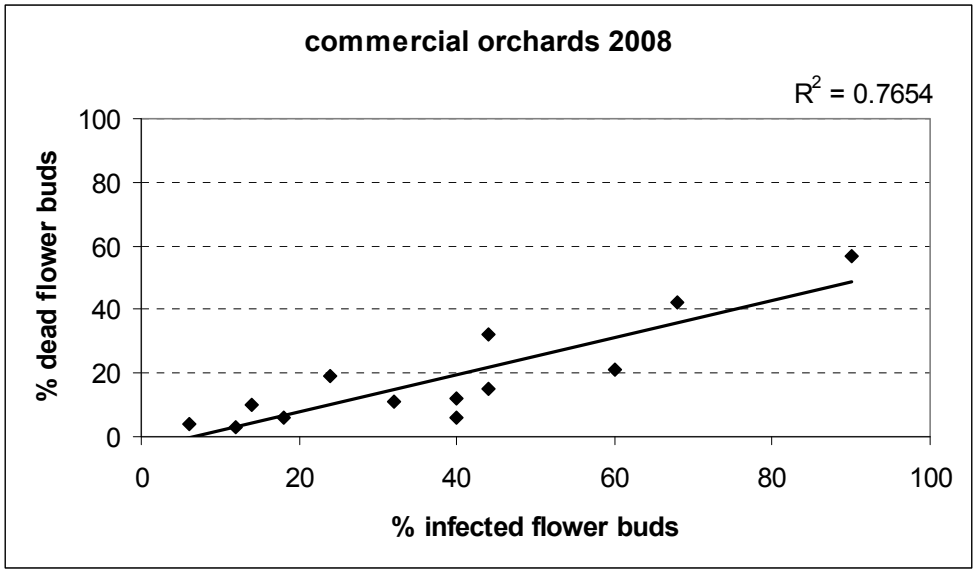

Fig. 2. Relation between visual symptoms and infection of *A. alternata* in 2008.

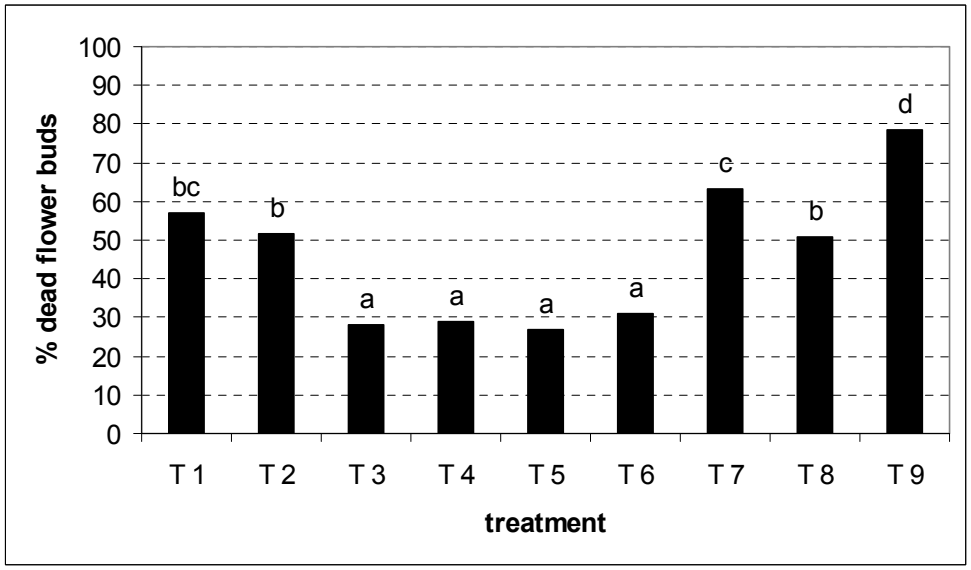

Fig. 3. Efficacy of fungicide treatments against dead dormant flower buds of pear (effect of treatments was determined with ANOVA at a 0.05 probability level).