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

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Keywords: *Pyrus communis*, *Alternaria alternata*, *Pseudomonas syringae*, fungicides

Abstract

Dead (dormant) flower buds of pear are an important phenomenon in pear production in the Netherlands. Vigourous or unbalanced tree growth and *Pseudomonas syringae* pv. *syringae* (P.s.s.) are mentioned as likely causes of dead flower buds. *Pseudomonas syringae* pv. *syringae* was occasionally isolated from diseased flower buds. However, *Alternaria alternata* was nearly always isolated from diseased buds and also often in symptomless flower buds. By identifying the causal agent of dead flower buds disease, an effective control strategy can be developed. In field trials it was proven that fungicide treatments can reduce disease incidences significantly.

INTRODUCTION

Dead flower buds are a common phenomenon in pear culture in the Netherlands, Belgium and Mediterranean countries (Deckers and Schoofs, 2001; Montesinos and Vilardell, 1991, 2001; Wenneker et al., 2004, 2006). Disease cases are also reported from South America; e.g., Uruguay and Brasil (E. Leoni, pers. commun.). 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%. First reports about the disease in the Netherlands date from the 1960's. The problem is mostly found in the main pear cultivar 'Conference', but cultivars such as 'Doyenne du Comice', 'Verdi' and 'Gieser Wildeman' are also affected. Adequate control strategies are not available.

Until now, the bacterium *Pseudomonas syringae* pv. *syringae* (P.s.s.) was commonly regarded as the causal agent of dead flower buds in pear. However, the relation between P.s.s. and dead flower buds in orchards has never been proven in the Netherlands. It was 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, 2001). Other possible causes mentioned are unbalanced (vigourous) tree growth, abiotic stresses, incompatibility between scion and cultivar, and other plant pathogens and pests.

The effect of tree growth regulation has been examined. However, no positive effect of tree growth regulation; i.e. applications of Luxan ethephon (a.i. ethephon 48%; 100–250 ml ha^{-1}; 4 applications: starting 10–14 days after bloom with one week interval), Regalis (a.i. prohexadione-Ca, 10%; 1 kg ha^{-1}; 3 applications: starting in 3-leaf stage with three week interval), or root pruning (one side of the tree in May and other side in June)

was observed in the trials (Wenneker et al., 2004, 2006). From these results it was concluded that tree growth control is not an effective way to reduce dead flower buds incidence on pear.

In our 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. However, the fungus *A. alternata* was (nearly in all samples) found in diseased flower buds and also in symptomless flower buds. 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, (ii) to isolate possible pathogens and (iii) to develop possible control strategies.

MATERIALS AND METHODS

Alternaria alternata (Pathogen) Assessment in Commercial Orchards

In 2004 in 8 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).

Fungicide Trial

Trials were performed in 2005 and 2006, in a pear orchard located at the experimental station at Randwijk, the Netherlands. The orchard was of spindle shaped pear trees (cultivar Conference) on Quince MC rootstock. Trees were planted in 1999 in a single row planting system (3.5 m x 1.5 m). The efficacy of Rovral aquaflow (a.i. iprodione 50%) was tested at a dosage of 1500 ml per 1000 liter (0.15% v/v).

Spray applications were carried out with a handheld spray gun (manufacturer EMPAS) with a 1.2 mm ceramic hollow cone nozzle at 1.1–1.2 Mpa and a spraying volume of 1000 L ha^{-1}. The experiment was done in a randomised 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) Fourteen spray applications: starting end of May 2005 – till harvest, after harvest and before bloom 2006.
3) Nine spray applications: starting end of May 2005 – till harvest.
4) Three spray applications: starting end of May 2005 with two weeks interval.
5) Three spray applications: starting early July 2005 with two weeks interval.
6) Three spray applications: starting early August 2005 with two weeks interval.
7) Two spray applications: October 2005 with two weeks interval.
8) Three spray applications: March and April 2006 with two weeks interval.

Control of *Alternaria alternata* and Disease Assessment

Before bloom (February 2006) per treatment 50 dormant flower buds (randomly taken over replicates) were individually assessed for infection with *Alternaria*. The buds used for determination of infections were surface sterilized and cut into two pieces. The flower primordia of each bud were plated onto PDA. Disease incidence was assessed at the beginning of bloom (April 2006). 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. The relation between infection rate and dead flower buds was determined.

RESULTS

Alternaria Assessment in Ccommercial Orchards

In the commercial orchards the internal visual symptoms (i.e. necrotic spots and dead flower primordia) ranged from 2–50%. The infection rate ranged from 10–85%. The fungus *Alternaria alternata* was found in nearly all diseased flower buds and also often in symptomless flower buds. There was a very good correlation between the occurrence of visible symptoms and infection with *Alternaria alternata* (Fig. 1).

Fungicide Trial

1. Control of *Alternaria* Infection. *Alternaria* infection of the dormant flower buds ranged from 4–64% (Fig. 2). Lowest infection rates were observed in the most frequently sprayed treatments (14–9 spray applications; treatments 2 and 3, respectively). Less spray applications resulted in higher infections rates. Infection rates of late spray applications (treatments 7 and 8) were comparable to the untreated control.

2. Control of Dead Flower Buds. Dead dormant flower bud incidences ranged from 14% - 48% (Fig. 3). Lowest dead flower bud incidences were observed in the most frequently sprayed treatments (9–14 applications; treatments 2 and 3, respectively). Less spray applications resulted in higher dead flower bud incidences. Late spray applications (treatments 7 and 8, with 2 or 3 applications, respectively) were comparable to the untreated control.

3. Relation *Alternaria* Infection and Dead Flower Buds Incidence. Figure 4 shows the correlation between the infection rate of flower buds with *Alternaria* and the occurrence of dead flower buds. This figure shows that control of *Alternaria* reduced dead flower buds incidences significantly.

DISCUSSION AND CONCLUSIONS

Until recently, it was commonly accepted that the bacterium *Pseudomonas syringae* pv. s*yringae* 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. 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 budbreak.

Extensive research in Spain (Montesinos and Vilardell, 2001) did not reveal a significant relation between dead flower bud incidence and *Pseudomonas* levels. In addition, antibacterial treatments control (copper and kasugamycin) did not prevent the occurrence of dead flower buds. Also, in the Netherlands a relation could not be proven (Wenneker et al., in prep.). However, the fungus *A. alternata* was (nearly in all samples) found in diseased flower buds and also in symtomless flower buds. In laboratory tests the pathogenicity of *A. alternata* was proven on flower buds of detached pear twigs (Wenneker et al., in prep.). Therefore, it is assumed that *A. alternata* is the causal agent of dead flower buds of pear in the Netherlands.

The genus *Alternaria* encompasses both nonpathogenic and pathogenic species. Most *Alternaria* species are saprophytes (Thomma, 2003). Some species are (opportunistic) plant pathogens that cause a range of diseases on crops as cereals, ornamentals, vegetables and fruits. *A. alternata* is known to cause late blight in pistachio (Pryor and Michailides, 2002; Evans et al., 1999) and several diseases in fruit crops such as moldy-core in apple (Reuveni et al., 2002) and brown rot in citrus (Timmer et al., 1998). Pathotypes per species are found with a distinct and limited host range, characterized by the production of host-specific toxins essential for pathogenesis

(Johnson et al., 2000).

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 commercial orchard revealed that the infection rate of dormant flower buds with *A. alternata* can be over 80%. This shows the potential (financial) risk for individual growers in years with favourable conditions for infection and disease expression. Important issues for further research are the differences in infection rates and disease expression between orchards; e.g., effect of inoculation pressure, spraying scheme, and cultural practices.

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. 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.

Multiple spray applications with iprodione reduced Alternaria infections in the present study. Also, a significant correlation with dead flower buds disease was proven. Surveys in commercial pear orchards in the Netherlands revealed high infection rates in dormant flower buds. 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. The registration of effective fungicides against *Alternaria*, such as Rovral (a.i. iprodione; this study), Sygnum (a premix fungicide containing pyraclostrobin + nicobifen (BASF); Reuveni, 2005) or Switch (a premix fungicide containing cyprodinil + fludioxinil (Syngenta); M. Wenneker, unpublished data) would be useful to control dead flower bud disease of pears in the Netherlands.

ACKNOWLEDGEMENTS

This research is funded by the Dutch Product Board for Horticulture. The authors wish to thank Ron Anbergen, Marian van Dieren and Nina Joosten for their excellent co-operation in this research.

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Figures

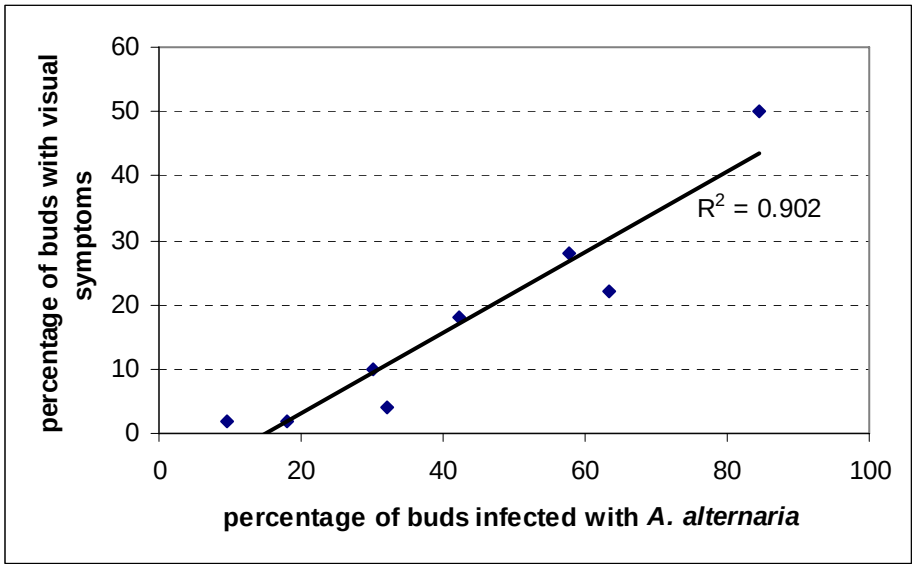

Fig. 1. Relation between visual symptoms and infection of *A. alternata*.

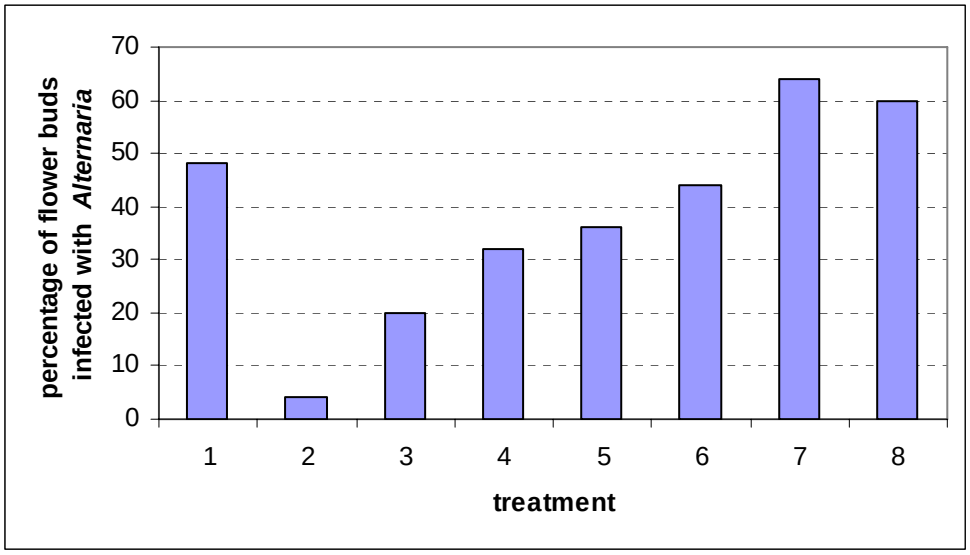

Fig. 2. Efficacy of treatments against *A. alternata* infections.

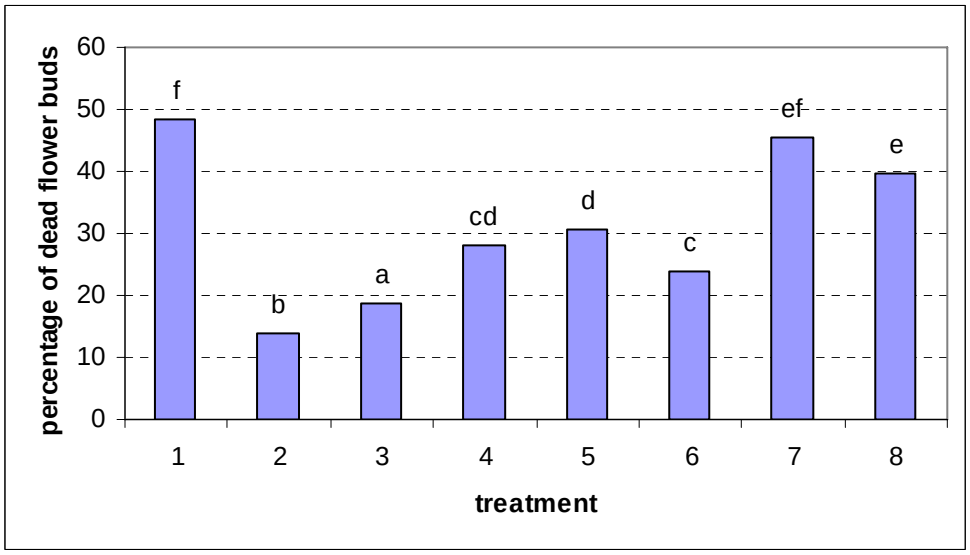

Fig. 3. Efficacy of treatments against dead dormant flower bud disease.

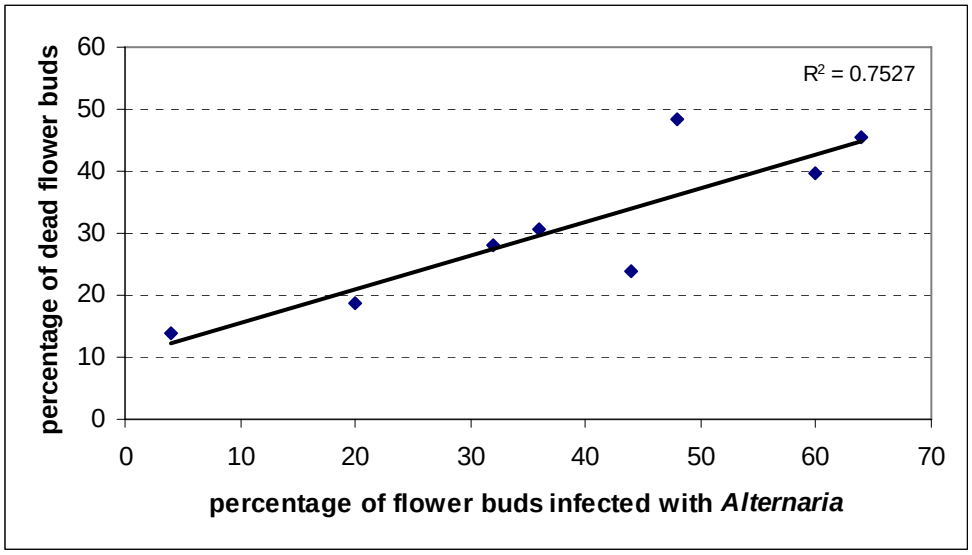

Fig. 4. Correlation between infection rate with *Alternaria* and dead flower buds.