Late blight on potato in Flanders, Belgium: field trials and characteristics of the *Phytophthora infestans* population.

B. Heremans[1] and G. Haesaert[2]

[1] Hogeschool Gent – Proefhoeve Bottelare, Department of Biotechnology, Landscape architecture and Agriculture, Diepestraat 1, B-9820 Merelbeke, Belgium
[2] Hogeschool Gent, Department of Biotechnology, Landscape architecture and Agriculture, Voskenslaan 270, 9000 Gent, Belgium
Betty.Heremans@hogent.be

Summary

Commercial fungicides were tested in the field for efficacy on foliar late blight caused by *Phytophthora infestans*. The fungicide treatments for late blight control were conducted at either 7- or 10-day intervals. The effect of the fungicide treatments on epidemic development, tuber rot and blight incidence and tuber yields were determined. Last summer late blight development was arrested in July and at the beginning of August due to high temperatures and lasting drought. Foliar disease severity significantly affected potato tuber yields. Lowest tuber yield was noted in plots with high late blight infection levels (nontreated control and an experimental mixture of organic acids (Vi-Care, 1 l/ha)) while highest yields were recorded in plots with low late blight infection. Late blight infection on leaf level was not significantly correlated with % tubers that showed late blight symptoms. No fungicide scheme completely arrested epidemic development under the environmental conditions of the trial. The effect of propamocarb hydrochloride + chlorothalonil (Tattoo C, 2.5 kg/ha) was less suppressive for *P. infestans* than the other fungicides tested for both interval systems. However, fenamidone + mancozeb (Sereno, 1.5 kg/ha), zoxamide + mancozeb (Unikat Pro, 1.8 kg/ha), dimethomorph + mancozeb (Acrobat, 2 kg/ha), cyazofamide + heptamethyltrisiloxane (Ranman 200 ml A/ha + 150 ml B/ha) and cymoxanil + famoxadone (Tanos, 0.6 kg/ha) controlled *P. infestans* most effectively for both interval systems. Also the other fungicides controlled foliar late blight sufficiently. Only small differences were observed between the different treatments.

A total of 51 isolates of *P. infestans* were collected from disease outbreaks in commercial potato crops and private gardens in 2003. Isolates were recovered successfully from single lesions of diseased potato foliage. Not from all isolates pure cultures were obtained due to contaminations with *Fusarium* species and bacteria. The structure of the population was analysed phenotypically. Characteristics of the isolates included *in vitro* growth rate, mating type, *in vitro* sensitivity to the phenylamide fungicide metalaxyl-M and allozyme genotype at glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) loci.

Key words: potato, late blight, *Phytophthora infestans*, fungicide efficacy, mating type, metalaxyl-resistance, *Gpi*, *Pep*

Introduction

The control of potato late blight, *P. infestans* requires repeated applications of several fungicides during the potato growing season. Fungicides sprays for late blight are a significant cost input in Belgian potato production. Normal practice is to start spraying when plant canopies closed rows and to continue at 7 to 10 days intervals until the crop is ready for harvest or desiccation. On the susceptible cultivar 'Bintje' professional growers will apply 10 to 14 sprays in most seasons that cost between 200-400 €/ha for the fungicides depending on product choice. In contrast, a restrictive government policy on the use of pesticides and an increasing public concern regarding food safety and the environment call for drastic reduction of the chemical inputs in agriculture. Decision support systems such as the Flemish warning system based on the Guntz-Divoux epidemiological model, have the potential to reduce cost inputs and the input of fungicides by increasing spray intervals when disease risk is low.
Furthermore, the 'new' populations of *P. infestans* consisting of both mating types is present in most parts of the world. In these populations sexual reproduction may be expected to occur, which would have important consequences for potato late blight control.

The purpose of this study was to compare new commercial potato fungicides commonly used to control late blight and to investigate the efficacy of these new fungicides for the control of foliar and tuber blight.

P. infestans isolates were collected from disease outbreaks in commercial potato crops and private gardens and phenotypically characterised to study the biological diversity within the *P. infestans* population in Flanders.

Material & Methods

Field trial

A field experiment was carried out on the experimental farm of the 'University College Ghent' at Bottelare during the growing season 2003. Several fungicides (Table 1) were compared in a

Table 1: Fungicides used in the field trial 2003.

Commercial product	Active matter
Tanos	150 g/ha cymoxanil + 150 g/ha famoxadone
Ranman	80 g/ha cyazofamide + 126 g/ha heptamethyltrisiloxane
Shirlan	200 g/ha fluazinam
Unikat Pro	1.2 kg/ha mancozeb + 149.3 g/ha zoxamide
Sereno	0.125 kg/ha fenamidone + 0.625 kg/ha mancozeb
Tattoo C	0.938 kg/ha propamocarb + 0.938 kg/ha chlorothalonil
Acrobat extra WG	0.12 kg/ha dimethomorph + 1.07 kg/ha mancozeb
Stamina + Unikat Pro	1.5 kg/ha potassium phosphite + 1.2 kg/ha mancozeb + 149.3 g/ha zoxamide
Stamina + chlorothalonil	1.5 kg/ha potassium phosphite + 1 kg/ha chlorothalonil
Stamina + Shirlan	1.5 kg/ha potassium phosphite + 160 g/ha fluazinam
Vi-Care	experimental mixture based on organic acids

spray system based on 7- and 10-day intervals. The experiment was set up with the variety 'Bintje'. Treatments were carried out with a AKZO sprayer to 3 m wide and 8 m long plots. The spray boom was equipped with TJet nozzles (XR Tjet 8003) spaced 50 cm apart. The water volume was always 300 l/ha. The experimental design was a split block design with the spray intervals as sub-blocks. The fungicide treatments were randomised within the blocks.

Following crop husbandry measures were taken: planting date of certified seed potatoes: 9 April 2003; row distance: 0.68 m; fertilisation: in autumn 35 ton digested dung, in spring 104

kg/ha N, 31 kg/ha P_2O_5 and 260 kg/ha K_2O; herbicide treatment: prosulphocarb + metobromuron: 3,6 kg + 1,0 kg/ha (Defi + Patoran: 4,5 l).

Inoculum production and foliage inoculation

A mixture of 8 isolates of *P. infestans* was used for artificial infection. Inoculum was produced by the following procedure: sporangia were washed from sporulating lesions on detached leaflets of the susceptible potato cultivar 'Bintje' by rinsing the lesions with chilled distilled water + 0.01 % Tween and adjusted to 10^4 sporangia per ml using a Bürker counting chamber. To release zoospores, the resulting sporangial suspension was chilled for 1.5 h at 6 °C prior to inoculation. Plants of the border rows across the experimental plots were inoculated by spraying 8.700 sporangia/plant on 18 June in the late afternoon. In total 72 plants were infected with *P. infestans*. Before inoculation and 15 h after inoculation, the plants were sprayed with water to create optimal humidity conditions for infection. Between 23 June and 4 July the mean temperature fluctuated between 14.9 and 20 °C. And between 30 June and 3 July 52.5 mm of rain was fallen. Those weather conditions favoured the development of *Phytophthora* infections all over the plots.

Fungicides and concentration

The fungicides used in this field experiment were commercial formulations of systemic and protectant fungicides. Eleven active matters were studied (Table 1). Fungicide applications began after plant canopies closed rows and are summarized in table 2.

Table 2: Summary of treatments of the field trial 2003.

Treatments	Date of treatment																												
	9/05	29/05	5/06	12/06	18/06	19/06	26/06	2/07	4/07	10/07	15/07	17/07	22/07	24/07	29/07	31/07	5/08	6/08	7/08	11/08	14/08	19/08	23/08	25/08	27/08	29/08	30/08	16/09	18/09	25/09
Planting																														
Herbicide treatment																														
Block I (disease-free)																														
~~Infection (7 + 10 days)~~																														
Block II: 7 days						II	II	II																						
10 days						II		II			II																			
Block III: 7 days										III		III																		
10 days													III		III															
Block IV: 7 days														IV		IV		IV												
10 days																	IV				IV			IV						
Harvest: 7 days																														
10 days																														

All plots were sprayed with 1.6 kg/ha mancozeb (Dithane 2 kg/ha, Protex) on a weekly basis to protect foliage from natural infection by *P. infestans* (block I). Thereafter, the plots were 3 times treated with the different fungicides (Table 1) at either 7-day or 10-day intervals (block II). After the last application the experimental fields were sprayed twice on a 7-day basis with 1.3 kg/ha mancozeb + 90 g/ha cymoxanil (Curzate M 2 kg/ha, Du Pont) (block III). After the last treatment, the different fungicides were applied 3 times at either 7-day or 10-day intervals (block IV).

Diquat 600 g/ha (3 l/ha Reglone, Zeneca) was used to dessicate leaves and stems. During the growing season foliage destructions were also carried out in the infected border rows and plots which were infected for 50 % and more to limit the epidemic pressure.

Disease estimates

To measure the intensity of foliage blight caused by *P. infestans* the assessment key of Cox & Large (1960) was used: 0.0 % blight: no disease observed; 0.1 %: a few scattered plants blighted, no more than 1 or 2 spots in 10-m radius; 1 %: up to 10 spots per plant, or general light infection; 5 %: about 50 spots per plant, up to 1 in 10 leaflets infected; 25 %: nearly every leaflet infected, but plants retain normal form, plants may smell of blight, field looks green although every plant is affected; 50 %: every plant affected and about 50 % of leaf area destroyed, field appears green, flecked with brown; 75 %: about 75 % of leaf area destroyed, field appears neither predominantly brown nor green; 95 %: only a few leaves on plants, but stems green; 100 %: all leaves dead, stems dead or dying.

The overall amount of percentage blight per plot was assessed at regular intervals.

Data were analysed by performing analysis of variance (SPSS10.0). The Duncan- and paired t-test were used to compare treatment means.

Harvest

Tubers were harvested by hand to minimise wounding. Two rows over a distance of 5 m were harvested from the centre of each plot. All tubers were washed, weighed after grading and assessed for blight within 8 days after harvest. Washed tubers were examined visually for the presence or absence of lesions symptomatic of late blight. Furthermore, infected tubers were cut longitudinally to confirm the presence of dry brown corky rot in the tuber beneath the lesion, a symptom typical of late blight tuber infection. The diagnosis of tuber blight was further confirmed by observing sporangia production after incubating tubers with characteristic lesions in plastic containers containing moist paper towels. The amount of blighted tubers was defined as the rotten tubers (but due to the bacterial rot no characteristic blight symptoms could be observed) plus the tubers visually clearly infected by *P. infestans*.

Purification and characterization of *P. infestans* isolates

The isolates were recovered successfully from single lesions of diseased potato foliage. Not from all isolates pure cultures were obtained due to contaminations with *Fusarium* species and bacteria. A selective pea broth agar amended with 2 ppm griseofulvine, 19 ppm nystatine, 10 ppm Benlate (50 % benomyl), 30 ppm rifampicine50 ppm nalidic acid, 40 ppm 8-azaguanine and 30 ppm neomycine was used. After isolation into pure culture, isolates were kept on pea broth agar at 15 °C in the dark and routinely maintained.

Mating type was determined by pairing the isolates individually with isolates of known mating type on pea broth agar. After 3 to 4 weeks of incubation at 18 °C in darkness, the plates were microscopically examined for oospores at the hyphal interfaces between the isolates.

The response to metalaxyl-M was determined by inoculating a hyphal plug of *P. infestans* isolates onto pea broth agar amended with 0, 5 and 100 ppm metalaxyl-M. Plates were incubated at 18 °C in the dark and after 7 days colony diameters were measured.

Genotypes at the polymorphic loci *Gpi* (EC 5.3.1.9.) and *Pep* (EC 3.4.3.1.) were determined using the protocol of Goodwin et al. (1995). The genotypes of unknown isolates were determined by compairing their banding patterns with those of proper controls.

Results & Discussion

The incidence of foliage blight was scored 77, 85, 92, 99, 106, 113 and 119 days after planting. The field experiment in 2003 indicated that all the tested fungicides had a strong suppressive effect on established epidemics at 7-day and 10-day application intervals compared to untreated plots (Fig. 1, Fig. 2). Only the experimental mixture Vi-Care presented a bad foliage protection against late blight at both spray intervals used. In 7-day spray intervals the control plots and plots sprayed with Vi-Care were rapidly infected for 50 %. The plots sprayed with Vi-Care were treated already with diquat on 6 August to decrease the disease pressure in the surrounding plots. The differences in control efficiency for the other fungicides tested were rather small and statistically not significant. In 10-day spray intervals

significant differences between the fungicides were observed: propamocarb + chlorothalonil, mancozeb + potassium phosphite + zoxamide and fluazinam gave a significant lower foliage protection than the other fungicides tested. In the plots sprayed with Vi-Care the percentage of foliar blight was after 5 weeks as high as in the control plots.

A lower tuber yield was observed for the untreated plots and plots sprayed with Vi-Care at the two spray intervals tested (Fig. 3). No significant differences were observed for the other fungicides tested for the 7-day spray interval. The average tuber yield fluctuated between 48.6 and 52.2 ton/ha and the mean yield of all treatments was 50.0 ton/ha. In the 7-day application interval the percent tuber rot was significantly higher for cymoxanil + famoxadone and potassium phosphite + chlorothalonil, respectively 3.5 and 3.2 % (Fig. 4). The amount of diseased tubers was significantly lower with fenamidone + mancozeb and fluazinam, respectively 0.1 and 0.3 %. At 10-day application schemes cymoxanil + famoxadone had the highest tuber yield: 55.8 ton/ha. For the other fungicides tested the yield fluctuated between 43.4 and 55.1 ton/ha and the mean yield for all treatments was 49.9 ton/ha. In the 10-day spray interval the plots treated with propamocarb + chlorothalonil, mancozeb + zoxamide, mancozeb + potassium phosphite + zoxamide and potassium phosphite + chlorothalonil had the highest number of tuber blight, 2.9, 2.8, 2.7 and 2.6 % respectively. For the other fungicides the amount of infected tubers fluctuated between 0.7 and 1.6 %. For the amount of infected tubers there were no significant differences between the different treatments at the 10-day spray interval. At both application intervals mancozeb + fenamidone and fluazinam showed the best protection against tuber blight.

Taking into account all the parameters evaluated (disease incidence, tuber yield, tuber blight) a 7-day application interval protects the foliage better against late blight then a 10-day-spray

interval. Mancozeb + fenamidone, mancozeb + dimethomorph and potassium phosphite + chlorothalonil protected the potato crop slightly better then the other fungicides tested. But the differences were small and statistically not different.

Significant differences in growth rate were observed among the 41 isolates grown on pea medium by comparing the main radial growth of the isolates after 7 days.
All the isolates tested were of the A1 mating type.
Isolates with sensitive, intermediate and resistant responses to metalaxyl-M were detected in the population. Thirty isolates had a growth of less then 40 % at 5 µg metalaxyl-M per ml. Two isolates had a growth of less then 40 % at 100 µg metalaxyl-M per ml. Eight isolates had a growth of more then 40 % at 5 and 100 µg metalaxyl-M per ml.
Cellulose acetate electrophoresis was used to examine *Gpi* and *Pep* banding pattern of the population of *P. infestans* attacking potato in Flanders. All the isolates tested produced the 100/100 *Gpi* isozyme electromorph. This *Gpi* isozyme type is characteristic for the new population of *P. infestans*. Five different allozyme genotypes of the *Pep* loci were identified: 92/92, 96/96, 100/100, 92/100, 83/100. The dominating banding pattern for *Pep* was 100/100 (10 isolates). Eight isolates produced the 96/96 *Pep* isozyme type and 8 isolates had the 92/100 *Pep* genotype. The *Pep* allozyme type 92/100 is characteristic for the old population of *P. infestans*.
Notwithstanding the fact that no A2 mating type was isolated, a high level of biological diversity was detected within the population of isolates analysed. This diversity may have evolved from local processes including sexual recombination and selection rather than through long-distance migration.

Acknowledgements

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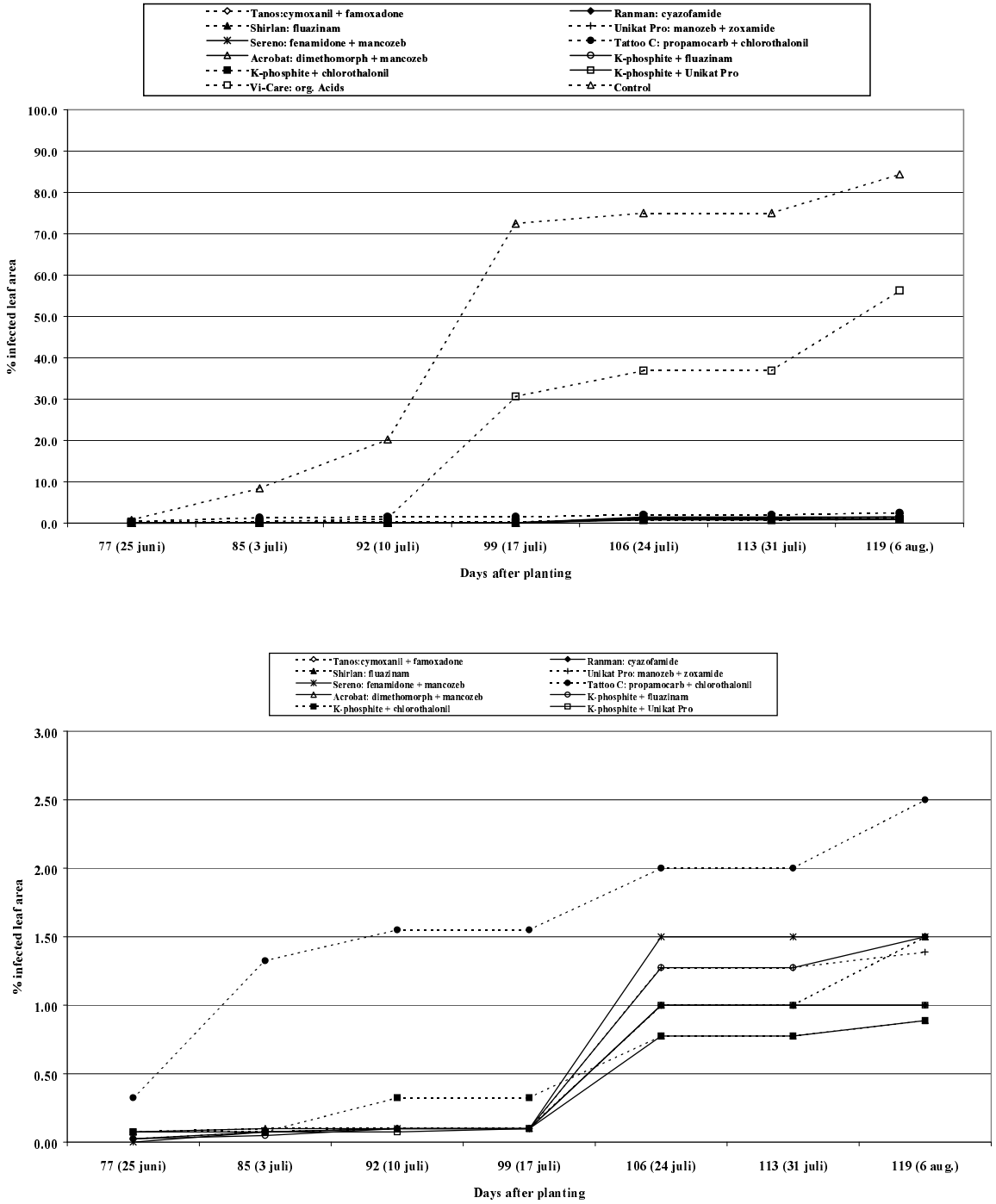

Figure 1: Influence of the fungicides applied in 7-day intervals on the infection level of late blight of 'Bintje' during the growing season 2003.

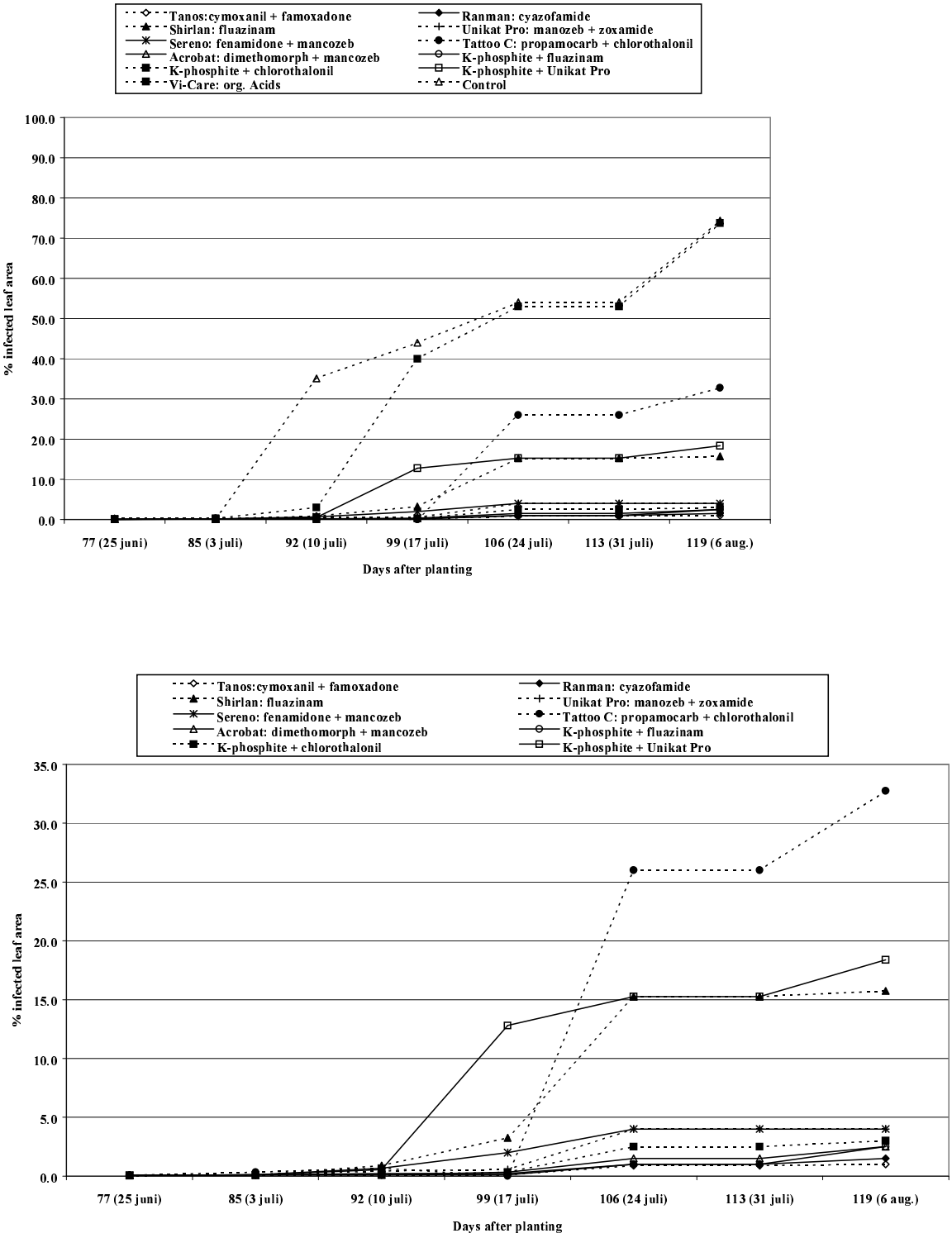

Figure 2: Influence of the fungicides applied in 10-day intervals on the infection level of late blight of 'Bintje' during the growing season 2003.

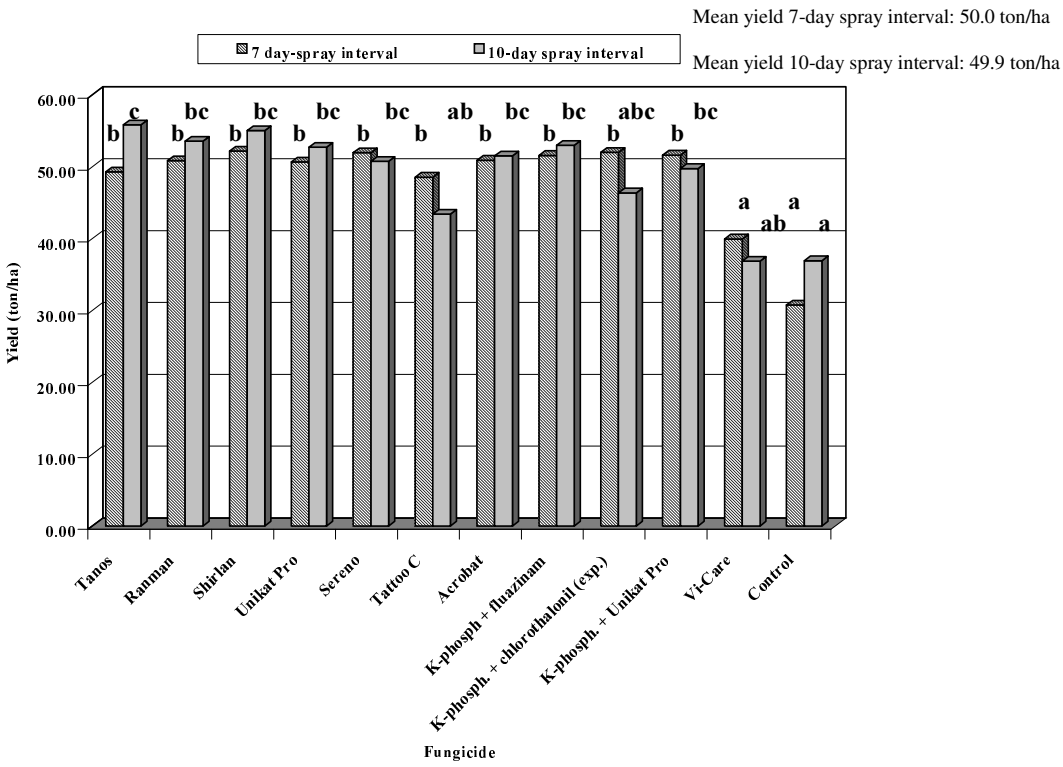

Figure 3: Influence of fungicides applied at 7-day and 10-day intervals on tuber yield in 'Bintje' during the growing season 2003.

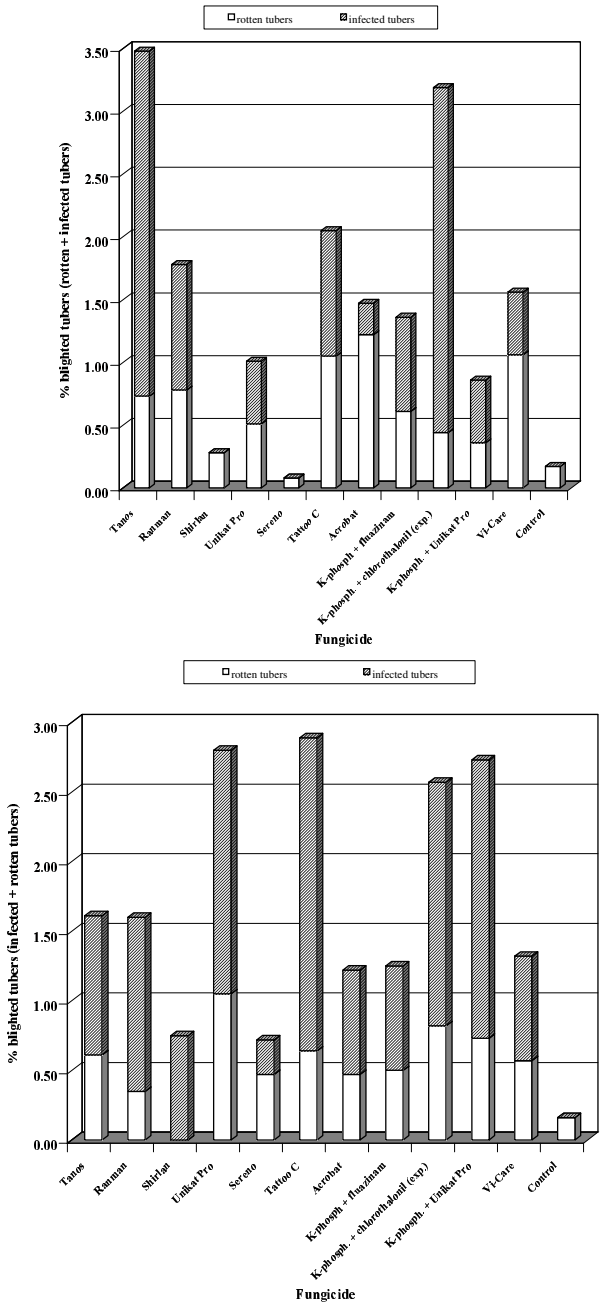

Figure 4: Influence of fungicides applied at 7-day (A) and 10-day (B) intervals on tuber blight in 'Bintje' during the growing season 2003.