Chapter 3:

Variety performance

Section 1: Assessment of variety performance in different EU regions and organic production systems

Section 2: Clonal diversity, race spectrum and aggressiveness of *P. infestans* populations found in organic production units in different areas of the EU

Chapter 3: Variety performance

General Introduction

Varieties that are resistant to pathogens are a potentially important component of an integrated disease management strategy. This was confirmed by the survey reported in Chapter 2. Nevertheless, organic potato producers were found to grow a range of varieties including some that are not highly resistant to late blight, indicating that choice in many cases is dictated by market demands. However, the suitability of many resistant varieties for organic production in different regions of the EU is unknown. Information from variety evaluation trials in conventional agriculture may be misleading because expression of resistance can be influenced by the production system (e.g. differences in mineral nutrient availability affect the overall resistance of plants to fungal pathogens). Other characteristics such as the size of the canopy, foliage longevity, yield, tuber size grading, quality (including skin finish, dry matter content or specific gravity and reducing sugar content) and the ranking order of the performance of different varieties may differ between conventional and organic cropping systems. Therefore, there is a need to quantify the yield, quality and late blight incidence in resistant varieties grown within the context of organic rotations in different regions of the EU to; assist choice of varieties with suitable and ideally durable resistance; formulate appropriate within field diversification strategies involving mixtures or alternate rows of varieties with different types/ levels of resistance.
In addition, the effect of *P. infestans* race spectrum on the resistance of varieties should be determined, to assess the suitability of varieties to be grown for use in different areas of the EU. The potential for resistance breakdown can be assessed by analysing the virulence spectrum, race complexity and aggressiveness of *P. infestans*- isolate populations collected from infected leaves of the same reference cultivars grown in organic production units in different areas of the EU.

Section 1: Assessment of variety performance in different EU regions and organic production systems

Summary

Resistance of selected varieties to foliar and tuber blight and their yield under organic growing conditions were assessed in Switzerland, France, England and Norway in 2001 and 2002. The objective was to identify new, more resistant varieties with potential to substitute for some of the more susceptible varieties currently grown in organic cropping systems. In each country, five ‘novel’ test varieties were compared with the same two reference varieties and with two other varieties popular in organic production in that specific country. In addition, the effect of copper-based fungicides was assessed for all varieties in the United Kingdom.

Several new potato varieties (Appell, Derby, Innovator and Naturella in Switzerland; Derby, Eden, Escort and Naturella in France; 87418 (Axona), 874120 (Sarpo Mira), 8740133 (Tominia), Eve Balfour, Lady Balfour in England; N89-1756 and N92-15138 in Norway) were less susceptible to foliar and tuber blight than most of the currently grown varieties. However, their introduction into practice could be constrained by market requirements for particular characteristics of the tubers. The copper fungicide treatment reduced foliar blight severity in all varieties tested in the UK and in both years, by 27% on average, and increased yield by 20% on average, but did not affect tuber blight.

A trend towards using more blight resistant varieties in organic cropping systems should be encouraged, but this strategy alone is unlikely to eliminate the need to use copper-based fungicides.

Introduction

The aim of this study was to identify varieties with the potential to replace varieties currently used in organic farming that have better resistance to late blight with similar or higher yield, similar or lower tuber blight incidence and are acceptable for the market. Field resistance to late blight and yield of new varieties with high levels of resistance to foliar and tuber blight was assessed under organic growing conditions in Switzerland, France, England and Norway in 2001 and 2002. The new varieties were compared with those currently grown in organic agriculture in each country but are generally susceptible to the disease. In England, the effect of copper-based fungicides on late blight development and yield in a number of new and established varieties ranging from moderately resistant to highly susceptible was also examined. The introduction of more resistant varieties (and minimized use of copper fungicides) are considered to be potentially important components of an integrated blight management strategy, in combination with other approaches such as cultural practices including diversification and agronomic strategies and alternative, copper-free control treatments.

General Materials and Methods

Experiments were conducted in all four countries according to a common set of procedures developed at the outset of the programme in commercial potato fields of certified organic farms, or in organic experimental stations. At every site, two reference varieties were grown: Santé (moderately resistant) and Bintje (susceptible); two varieties already popular with organic growers in that country/region and five new varieties. The 'locally popular' varieties and 'new' varieties differed from country to country and varieties that showed limited promise in 2001 were replaced with other suitable candidates. It was assumed that before a variety was introduced into practice, it would also be tested in the context of national varietal evaluation programmes. The reasons for all replacements of varieties are given in the results section.

The varieties were planted in 3 m wide plots of four rows, 75 cm apart and 15 m long in a randomized block design with four replicates, except where stated otherwise. Agronomic management was according to local practice in commercial organic cropping systems. Late blight infections were natural in all experiments except in England in 2002 which was artificially inoculated because the likelihood of natural infection at the site was unknown and few potatoes are grown in the area. Isolate PD4 (R1, R2, R3, R4, R6, R7, R10, R11) provided by the Scottish Agricultural Science Agency was used for inoculation on 18 and 26 July.

Data recording

The "standardized area under the disease progress curve" (stAUDPC; Campbell & Madden, 1990) was calculated from the time course of foliar blight epidemics. The number of diseased or rotten tubers found during harvest and grading is expressed as a percentage of the total number of tubers harvested. stAUDPC values and percentage diseased tubers were arcsine-transformed prior to statistical analysis. The performance of varieties (foliar blight, yield and diseased tubers) was analysed separately for each year and each site with one-way analyses of variance for complete randomized block designs, followed by the Tukey-B test, with the SPSS 10.0 computer programme. Data from Pickering and Stocksfield were analysed with two-way analyses of variance adjusted to split-plot designs, followed by the Tukey-B test.

At all sites in France, Norway and Switzerland and in all seasons, late blight and yield assessments were made on 13 m of the central two rows of each plot. The outer rows and the first and last metre of each plot were discarded. In England however, the assessments at Pickering and Stocksfield were made on 6 m of the central two rows due to the split plot design, and at Close House, where the plots were much smaller, on 1.8 m.
During the growing season, plots were regularly inspected for late blight. After the first symptoms of foliar blight were seen, disease severity was assessed 1-2 times per week, using the key of James

(James, 1971). The plots were harvested with a lifter, and the potatoes collected manually. Total tuber yield was recorded and diseased tubers were counted during harvest and grading.

FRANCE (F)

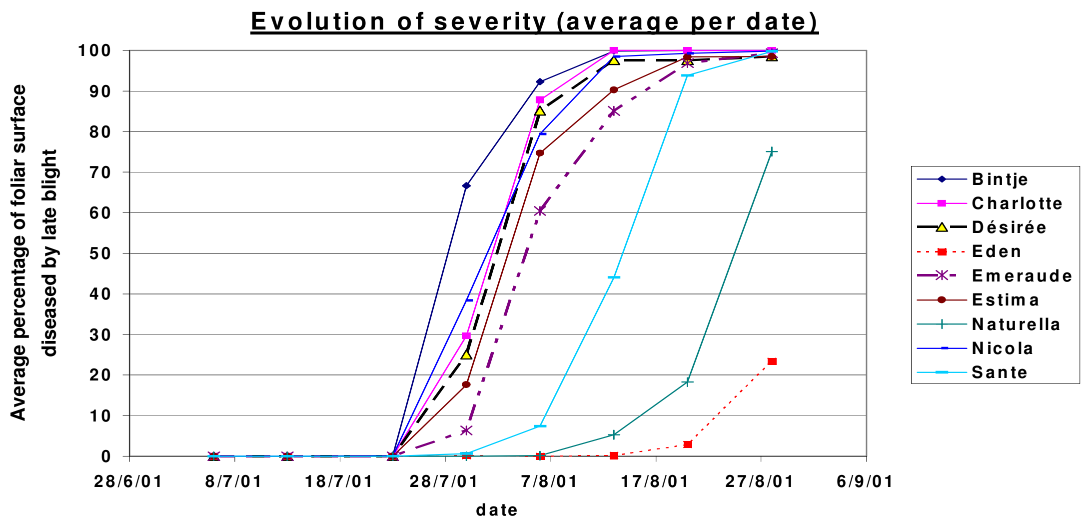
Materials and Methods

Both Experiments took place in Brittany.
Nine varieties were tested in 2001: Bintje (susceptible control), Santé (resistant control) ; Charlotte, Désirée, Eden, Emeraude, Estima, Naturella, Nicola.

Ten varieties were tested in 2002: Bintje (susceptible control), Santé (resistant control), Charlotte, Naturella, Lizen, Escort, Santé, Emeraude, Eden, Derby.

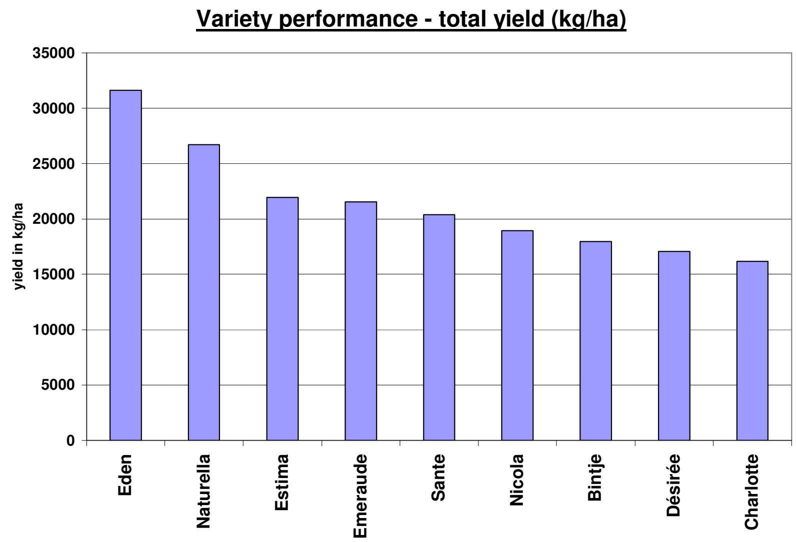
Results and Discussion
2001 Growing Season

Figure 3.1 Foliar resistance to late blight



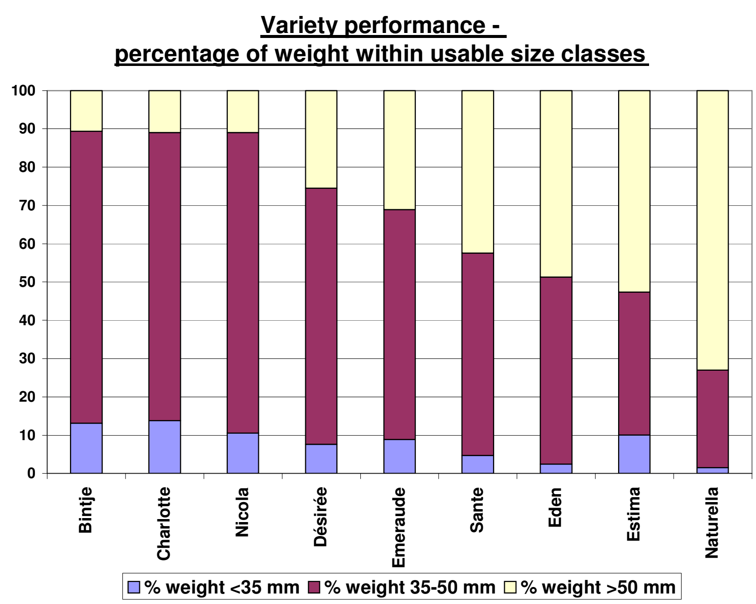
In 2001, late blight appeared later than it normally does in Brittany. Bintje (susceptible control) reached 90% in 2 weeks between 13 and 30 July. Rapid infection of Charlotte, Nicola, Désirée, Estima, Emeraude began slightly later but by 6 August was not significantly different from Bintje and all exceeded 85% by 13 August. Therefore, their foliar resistance to late blight was similar to Bintje, the susceptible control. Santé showed an intermediate level of resistance whilst Eden and Naturella were significantly the most resistant varieties to foliar blight and reached 10% infection about 3-4 weeks after the susceptible varieties, (Figure 3.1).

Figure 3.2 Yield



Total tuber yields were related to late blight resistance. The susceptible varieties Charlotte, Désirée, Bintje, Nicola gave less than 20t/ha and Estima, Emeraude about 22t/ha. Sante, although moderately resistant also gave about 20t/ha i.e. similar to the susceptible varieties, but Eden and Naturella yielded about 30 t/ha, (Figure 3.2).

Figure 3.3 Size classes

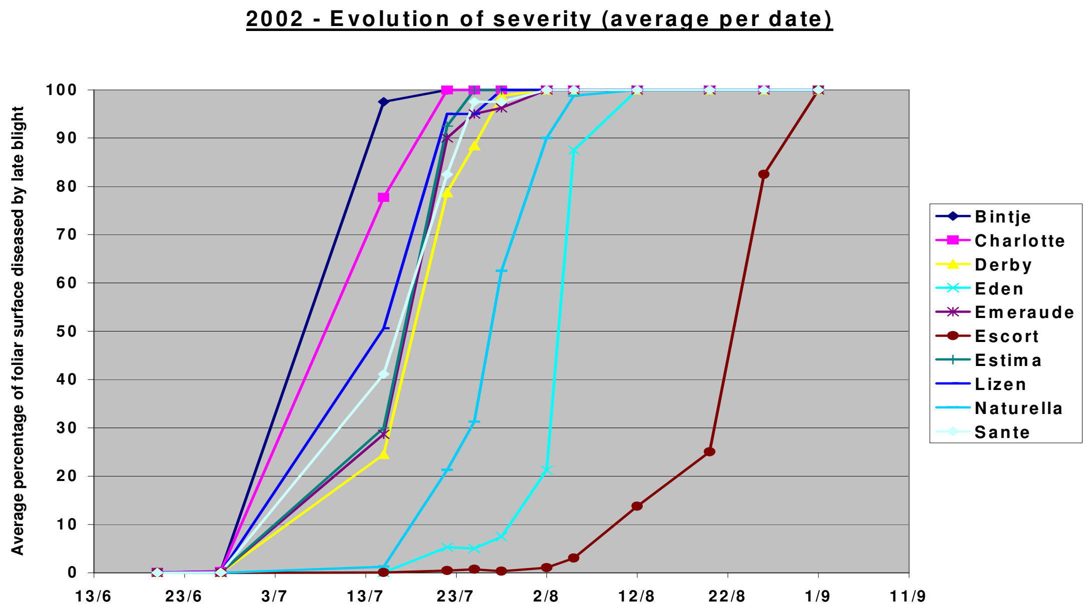


Tuber size grading of the different varieties was as expected on the basis of their known morphological characteristics and the proportion of large tubers >50mm did not seem to be affected by late blight, probably because it arrived later than normal. However, Bintje and Désirée were exceptions and gave a larger proportion of smaller tubers than expected, (Figure 3.3).

In 2001, Eden and Naturella showed good resistance and high yield (30 t/ha) and Santé, an intermediate level of resistance although yield was lower than expected (20 t/ha.) Estima and Emerald were susceptible varieties and gave a medium yield (22 t/ha) but the most susceptible varieties, Nicola, Bintje, Désirée, Charlotte yielded less than 20 t/ha

2002 Growing Season

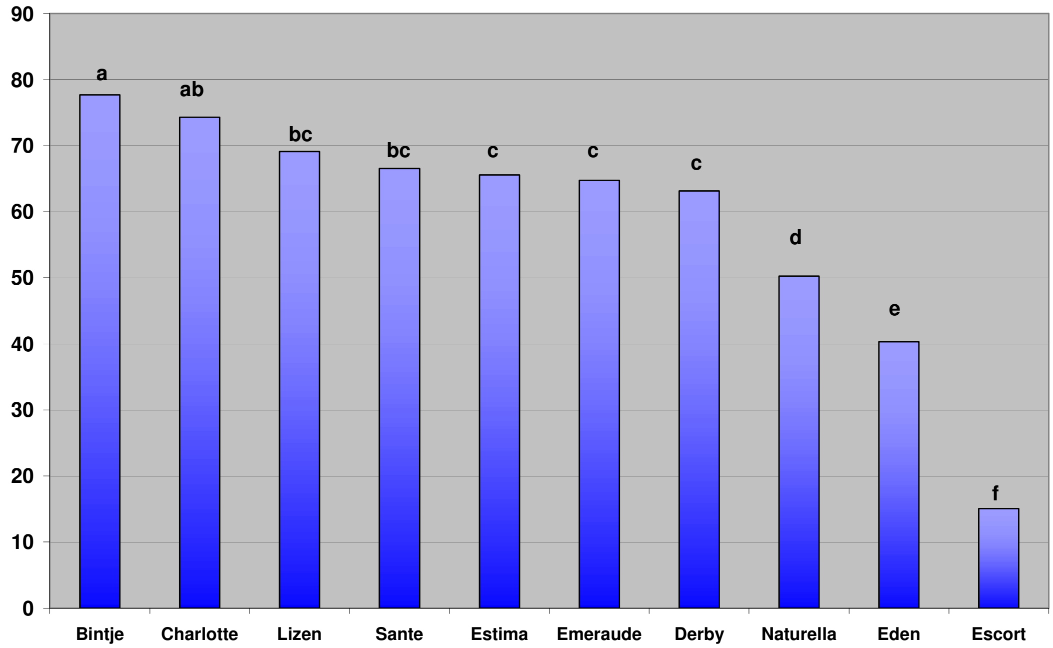
Figure 3.4 Foliar resistance to late blight



In 2002 in Brittany, late blight occurred one month sooner and the epidemic was more severe than in 2001. Consequently yields were seriously depressed and remained below 10 t/ha. In 2001, yields ranged from 15 t/ha to 30 t/ha.

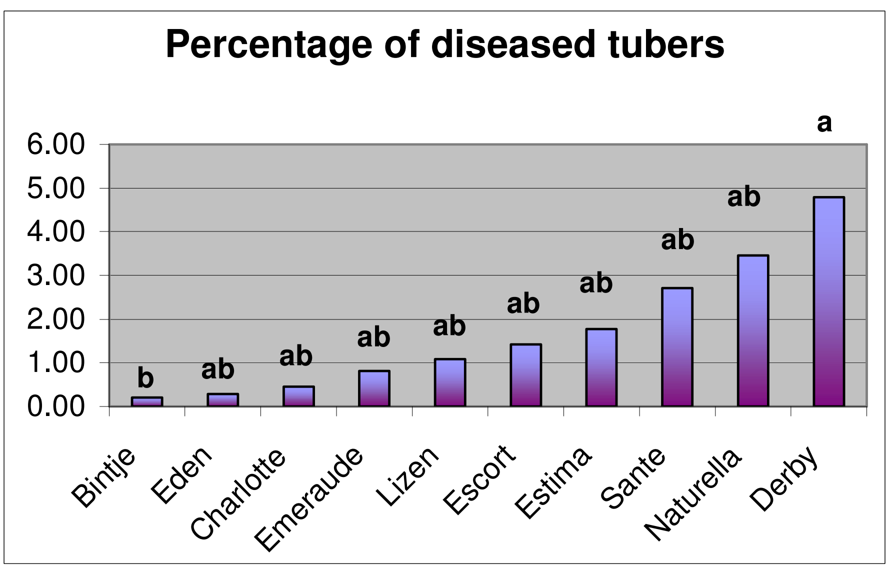
Late blight in Bintje (the susceptible control) and Charlotte developed very quickly and exceeded 75% by 13 July while most other varieties remained below 45%. Bintje and Charlotte reached 100% by 23 July (2 weeks after the outbreak of late blight) whilst Lizen, Santé, Estima, Emeraude, Derby were similar to each other and exceeded 80% by this date. Naturella, Eden and Escort were the most promising varieties in terms of late blight resistance: on 13 July infection was virtually zero. Escort seemed to be more resistant than Eden which in turn was more resistant than Naturella (severity reached 20% on 23 July for Naturella; 2 August for Eden and 16 August for Escort), (Figure 3.4).

Figure 3.5 2002 - st AUDPC



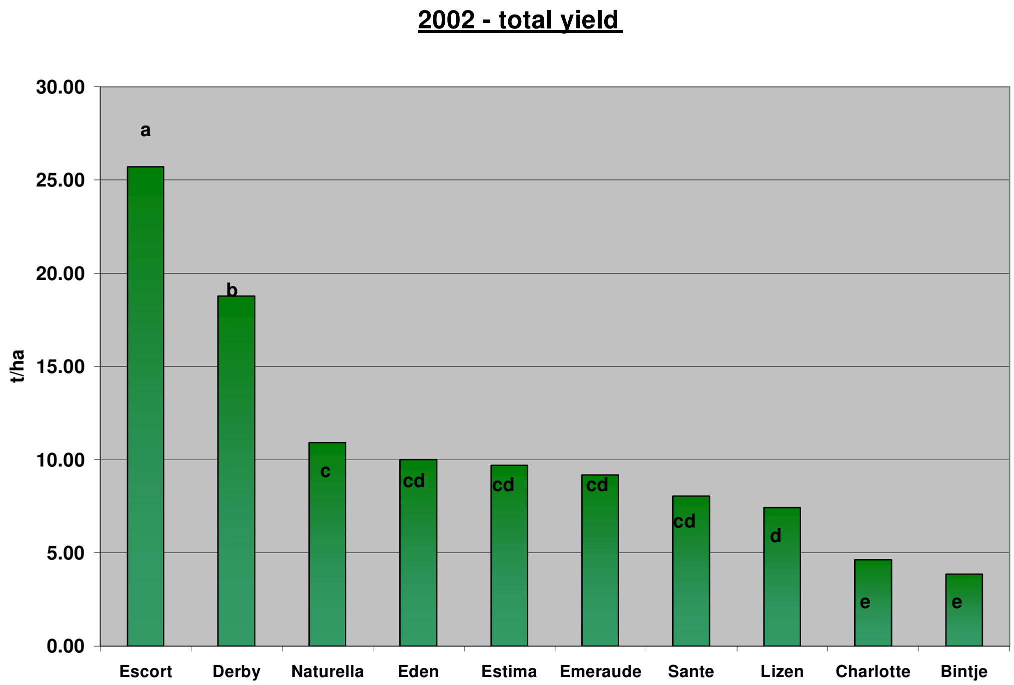
stAUDPC was strongly related to the development of disease infection. Comparing stAUDPCs, Bintje and Charlotte were highly susceptible to foliar blight (AUDPC>70) ; Lizen, Santé, Estima, Emeraude, Derby were moderately susceptible (50<AUDPC<70) ; the significantly most resistant varieties were by order, Escort (AUDPC=15), Eden (AUDPC=40) and Naturella (AUDPC=50), (Figure 3.5).

Figure 3.6 Tuber resistance to late blight



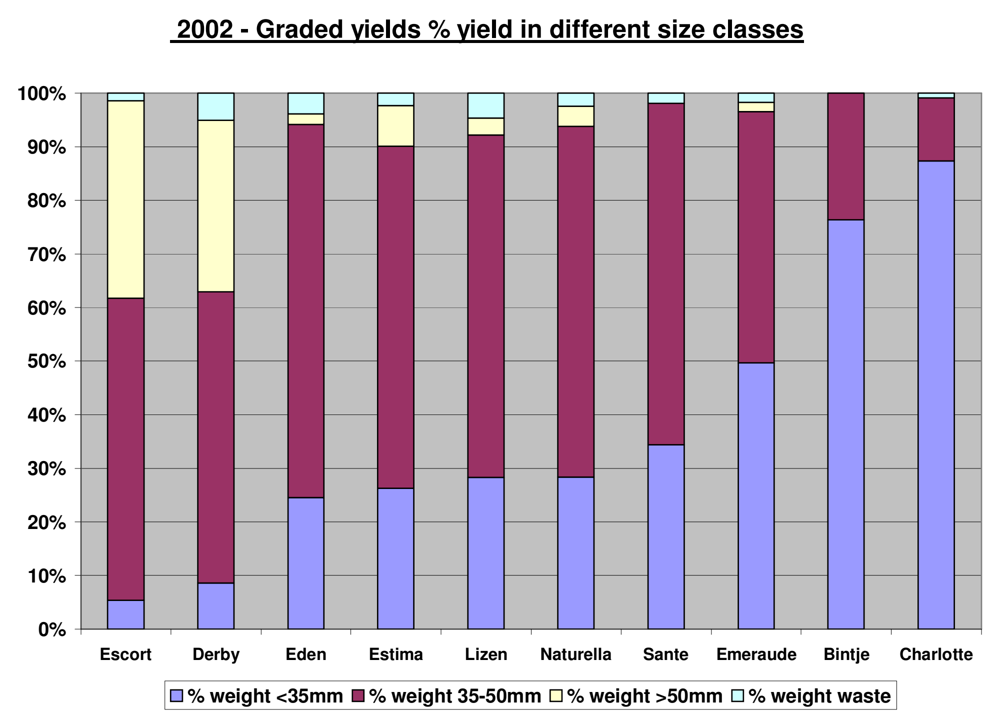
Levels of late blight were quite low and no statistically significant difference was found between varieties in terms of tuber blight. However, Derby seemed to be the most susceptible (about 5% tuber blight), (Figure 3.6).

Figure 3.7 Yield



Total yield was related to blight resistance : Charlotte and Bintje had the lowest yield (<5t/ha) and Escort and Derby the highest yield (>20t/ha). Yields of the other varieties were between 7 and 11 t/ha. These yields are very low because late blight developed very quickly in 2002 in Brittany. Total yield for Derby was quite high despite its susceptibility to foliar blight, (Figure 3.7).

Figure 3.8 Graded Yields



Escort and Derby produced large tubers (40% of yield was accounted for by tubers >50 mm) whilst Bintje and Charlotte produced the smallest tubers (about 80% of yield was accounted for by tubers <35mm), (Figure 3.8).

Conclusions

Some of the varieties tested in France were interesting in term of resistance to late blight. In 2001 Eden and Naturella showed good resistance to foliar blight and good yield and this was also confirmed in 2002. Escort, which was only tested in 2002, was very interesting with foliar resistance even better than Eden and Naturella coupled with a high yield. On the other hand, although Derby was quite susceptible to foliar late blight yield was acceptable. As expected, Bintje and Charlotte proved to be highly susceptible. Estima, Lizen, Emeraude, Santé, are popular varieties for the market and much appreciated by consumers, but because their resistance to foliage blight is low, they are unlikely to be grown successfully without the protection afforded by copper-based fungicides unless alternative treatments are available.

NORWAY (N)

Materials and Methods

In both 2001 and 2002, experiments were located at Særheim research station in Southwest Norway with a relatively cool and moist northern Atlantic coast climate. The field was managed organically and consisted of a moraine soil. Four new, blight resistant potato cultivars / breeding lines (Grom, N84-422, N89-1756, and N92-15138) were compared with three cultivars which are frequently grown in Norwegian organic agriculture (Troll, Beate, and Peik), and two reference cultivars (Bintje, Santé). Seed tubers were not chitted and planted on 18 May 2001 and 16 May 2002.

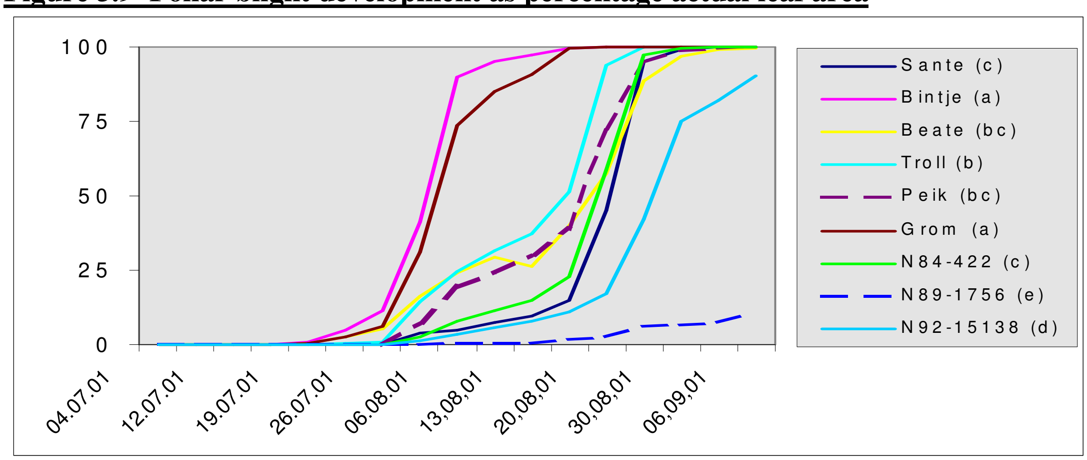
Results

2001 Growing Season

1) Foliar blight

Disease development is shown in Figure 3.9. The very first infection was observed on 12 July. There were significant differences between varieties, but no significant differences between blocks. As indicated by different letters in the legends of Figure 3.9, and based on Tukey's test the most susceptible cultivars were Bintje and Grom (stAUDPC = 35-33), intermediate susceptible varieties were Troll, Peik, Beate, N84-422, and Santé (stAUDPC = 26-17). N89-1756 and N92-15138 showed the slowest development of late blight (stAUDPC = 11-1).

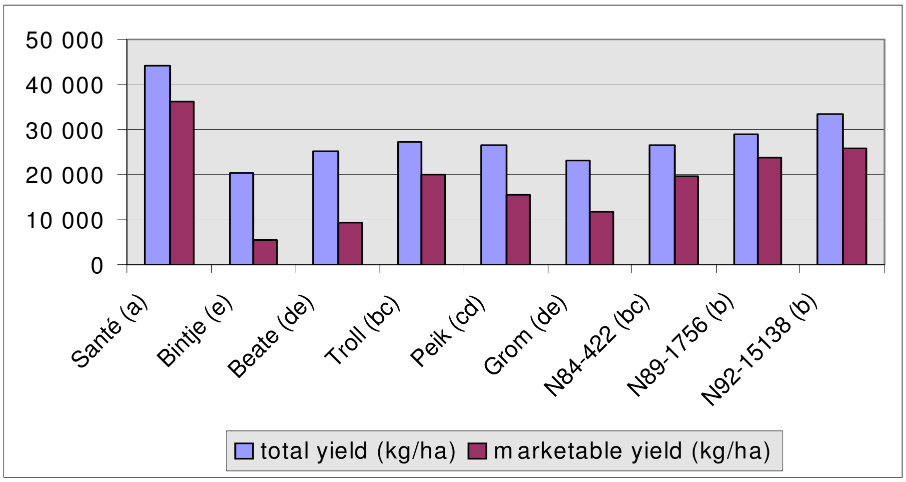
<u>Figure 3.9 Foliar blight development as percentage actual leaf area</u>



2) Yield

Yields are shown in Figure 3.10. There were significant differences between cultivars for marketable yield and the % of total yield that was marketable varied considerably between varieties

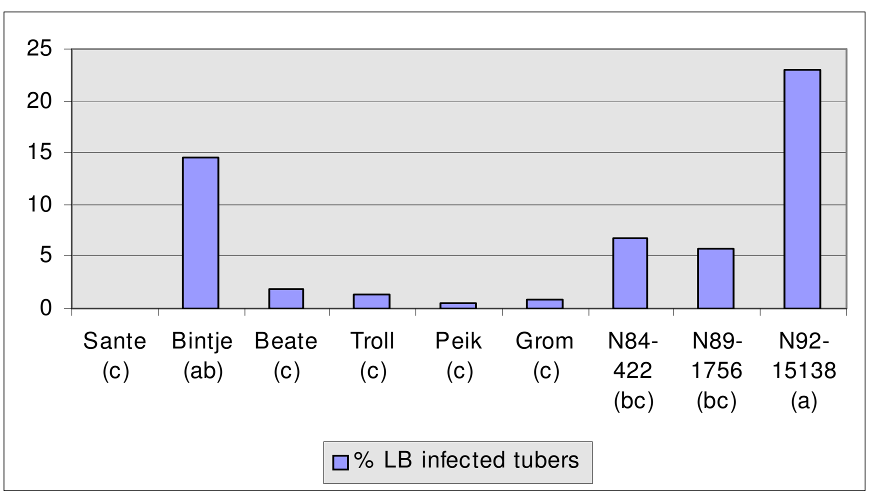
Figure 3.10 Yields



3) Tuber blight

Incidence of tuber blight is shown in Figure 3.11. No infected tubers were found in Santé. In the group of intermediate resistant cultivars the established cultivars performed reasonably well. However the new breeding line N92-15138 which showed a slow foliar late blight development in this group showed a high tuber blight incidence. The breeding line N89-1756 appears to be interesting based on both good foliar and tuber resistance.

Figure 3.11 Tuber blight



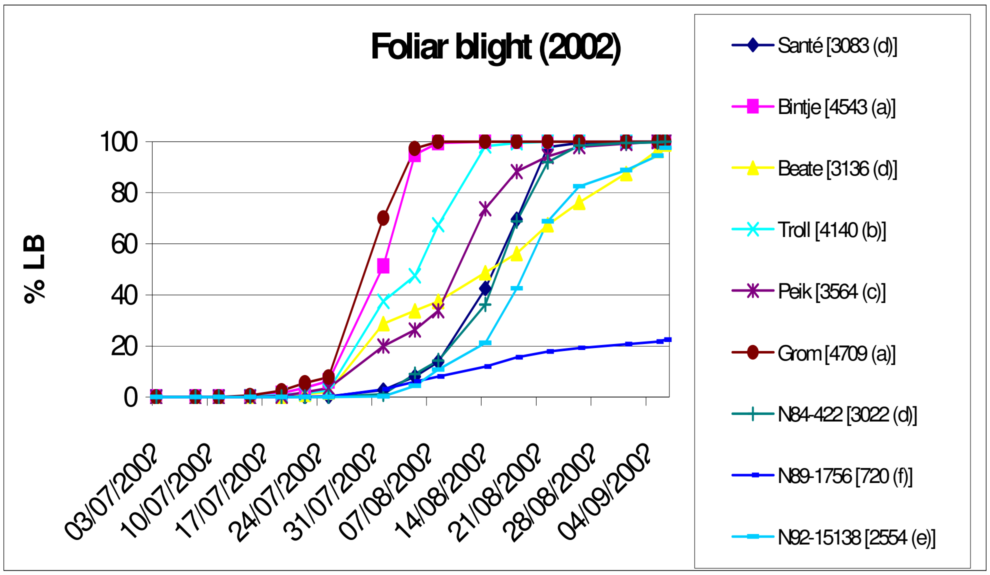
2002 Growing Season

1) Foliar blight

Disease development is shown in Figure 3.12. The very first infection was observed on 12 July. There were significant differences between varieties, but no significant differences between blocks. As indicated by different letters in the legends of Figure 3.12, and based on Tukey's test the most susceptible cultivars were Grom and Bintje (stAUDPC = 47-45), intermediate susceptible varieties were Troll, Peik, Beate, N84-422, N92-15138 and Santé (stAUDPC = 37-26). N89-1756 showed the slowest development of late blight (stAUDPC = 7).

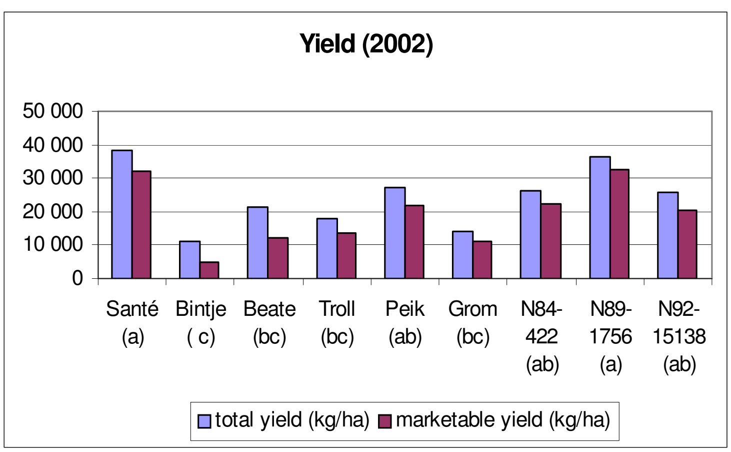
Figure 3.12 Foliar blight development as percentage actual leaf area

2) Yield



Yields are shown in Figure 3.13. There were significant differences between cultivars for marketable yield. In 2002 cultivar Santé and the breeding line N89-1756 gave the best yield performance. For Santé the yield was comparable to season 2001, for N89-1756 the 2002 season was significantly better, since this late bulking variety had great advantage of the favourable weather conditions in the second half of the growing season.

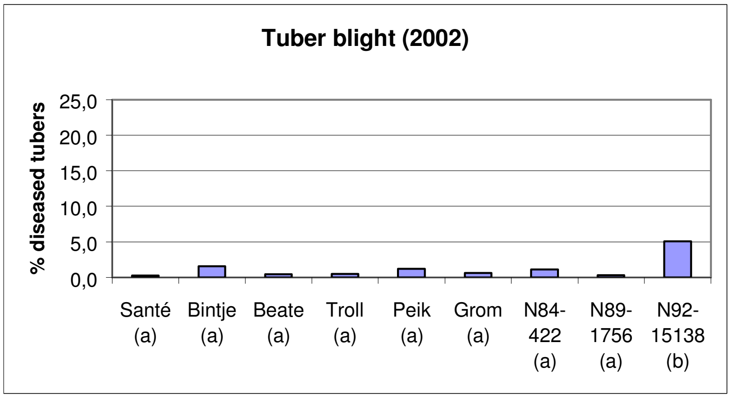
Figure 3.13 Yields



3) Tuber blight

Incidence of tuber blight is shown in Figure 3.14. In general the number of infected tubers was lower in 2002 than in 2001. All tested cultivars showed some infection with only N92-15138 at a significantly higher level. Even the very susceptible cultivar Bintje showed little tuber infection, indicating unfavourable conditions for tuber blight in 2002. The significantly higher level of tuber blight indicates again the susceptibility of breeding line N92-15138 for tuber blight.

<u>Figure 3.14 Tuber blight</u>



Discussion and Conclusions

In 2001, cultivar (breeding line) N89-1756 showed a marked low late blight severity at the end of the growing season. The cultivar Grom performed similar to the susceptible reference cultivar Bintje. The other cultivars showed an intermediate behaviour with regard to foliar blight development. These cultivars differed in the early development of the epidemic, but were defoliated at a comparable date. The reference cultivar Santé gave a surprisingly high yield notwithstanding the intermediate foliar late blight development.
There were significant differences in tuber blight incidence between the cultivars. Santé was the most resistant and N92-15138 the most susceptible cultivar. Santé was the most interesting cultivar with regard to marketable yield and tuber blight. N89-1756 was a good second with a better foliage performance, but lower yield and with more tuber blight.

In 2002, cultivar (breeding line) N89-1756 showed the lowest low late blight severity at the end of the growing season as in 2001. Although the final late blight severity in this breeding line was higher in 2002 this was not reflected in the tuber yield. The cultivar Grom performed similar to the susceptible reference cultivar Bintje. The other cultivars that showed a clear intermediate performance in 2001, showed more variation in 2002 with regard to foliar blight development. In 2002 the reference cultivar Santé gave a similar high yield notwithstanding the intermediate foliar blight development. Breeding line N89-1756, which is a late bulking cultivar, could realise its high yield potential in 2002 better than in 2001, due to good weather conditions in the late summer and autumn. Under these conditions it yielded similar to the reference cultivar Santé. The same favourable weather conditions were probably responsible for the lower tuber blight incidence in 2002 than in 2001.The difference in weather conditions between growing season 2001 and 2002 indicates two important principles. Firstly, that importance of potato late blight resistance is reduced when it leads to a cultivar choice that increases instability in yield performance. Secondly, early bulking cultivars can contribute to achievement of reasonable yields in cases of early late blight development.

Considering the data from both seasons 2001 and 2002 the results can be regarded as consistent. The observed differences between years can be explained fairly well on the basis of the differences in weather conditions between the two seasons. This also indicates what the impact can be of different environmental conditions for the performance of each potato cultivar. Both potato producers and retailers favour predictability of the amount of produce i.e. yield and hence supply and hence seek to avoid factors increasing instability. Cultivars sensitive to unpredictable unfavourable conditions such as weather are thus less attractive. The breeding line N89-1756 showed to be such a cultivar, despite its high level of both foliar and tuber resistance and may be attractive in areas with a natural longer growing season.

Clearly, there is a need for information on cultivar performances under the full range of environmental conditions that are likely in a production area of interest. Not only is the weather subject to annual variation but also late blight severity. For organic potato production under conditions where it is impossible to evade the disease, other eco-physiological properties should be considered such as the onset and rate of tuber bulking. In that respect, cultivar Santé was interesting because it gave a high yield even where there was significant late blight development later in the growing season. The interval between the onset of bulking and the epidemic development of late blight is crucial in this respect – the longer, the better.

UNITED KINGDOM (UK)

Materials and Methods

The 2001 trial took place at Stanfield Hall Organic Farm, Pickering, North Yorkshire. The 2002 trial was at Nafferton Farm, Stocksfield, Northumberland, Newcastle-upon-Tyne. In both cases, no irrigation was applied. In 2001, the varieties included Lady Balfour, Eve Balfour, Cara, Claret, Nicola, Sante, Bintje, Princess, Donella and 874120. In 2002, they were Lady Balfour, Eve Balfour, Cara, Claret, Nicola, Sante and Bintje and the un-named varieties 87418, 874120 and 8740133. The standard reference varieties used were Bintje (susceptible) and Sante (moderately resistant). In 2001, the planting was very late (25 May) because of very wet spring conditions. In order to examine the relative influence of varietal resistance and Cu-fungicides on blight infection, copper oxychloride sprays were applied to half of every plot. ('Headland Inorganic Liquid Copper', Headland Agrochemicals Ltd., U.K.; active ingredient: copper oxychloride at 435 g l^{-1} equivalent to 256 g l^{-1} elemental copper). A total of 7.68 kg ha^{-1} elemental copper was applied to the treated half-plots in three sprays made between 26 July and 29 August. When 95% of the foliage had been destroyed by blight, the stems of plants were cut off at ground level to facilitate harvesting.

<u>Note:</u> un-named ‘varieties’ 874120, 87418 and 8740133 were subsequently named Sarpo Mira, Axona and Tominia respectively and Sarpo Mira and Axona have been National Listed.

Results

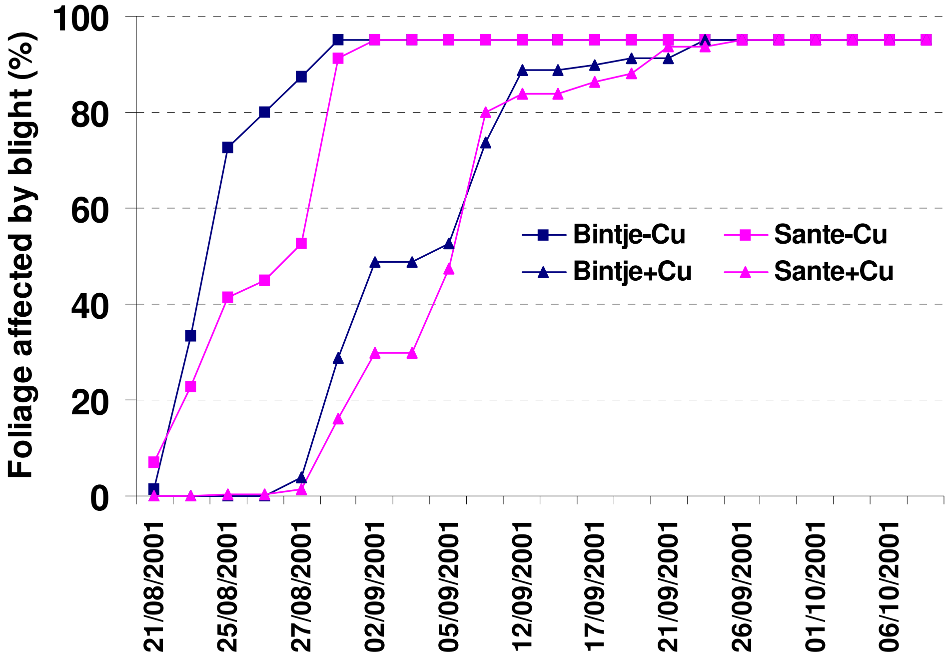
<u>Effect of variety choice and copper fungicide use on foliar blight of potato in organic potato production systems</u>

Resistance of the foliage to late blight varied considerably ($P<0.01$) between varieties of potato and between seasons. The observed levels of resistance to late blight varied from very susceptible to moderately and highly resistant in terms of Area Under the Disease Progress Curve (AUDPC). Late blight infection was more severe in 2001 than 2002 (Figure 3.17). Copper delayed foliage infection by approximately 10 to 14 days on average (Figure 3.15 and 3.16). There was also a significant interaction between variety and copper treatment.

In 2001, there was a highly significant difference in disease development ($P<0.01$) between copper treated and untreated crops in most of varieties, except for the 2 resistant varieties, Eve Balfour and 874120 (Figure. 3.15) which showed very low levels of blight until late in the growing season, even when Copper was not applied.

Figure 3.15 Effect of copper fungicide treatment on late blight development in selected potato varieties in 2001

(a)



(b)

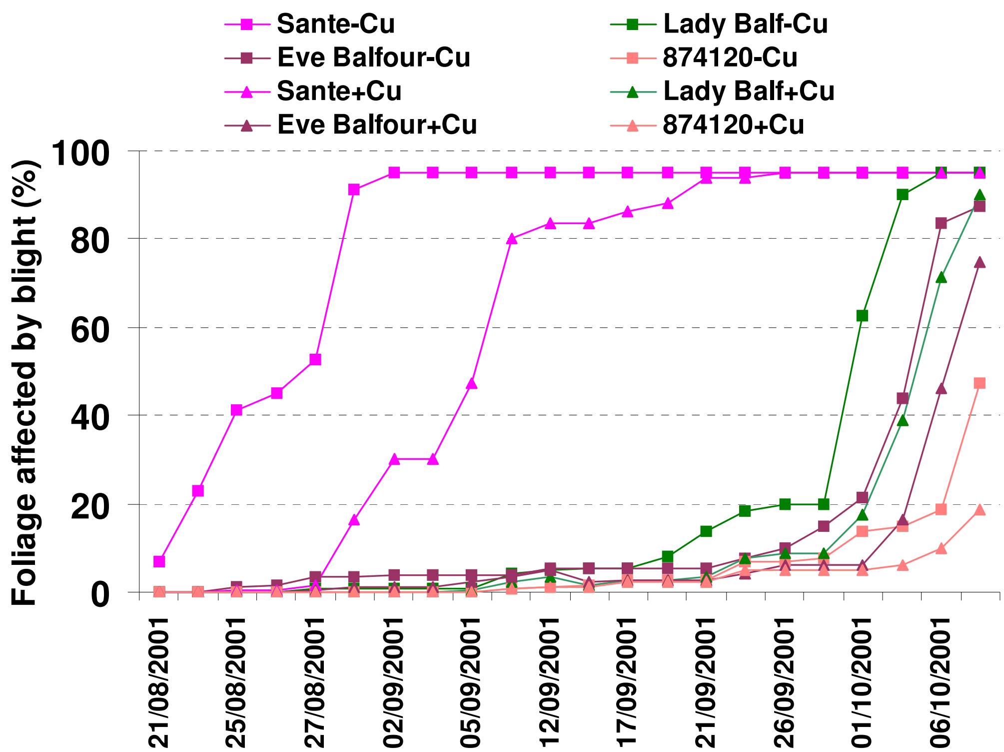
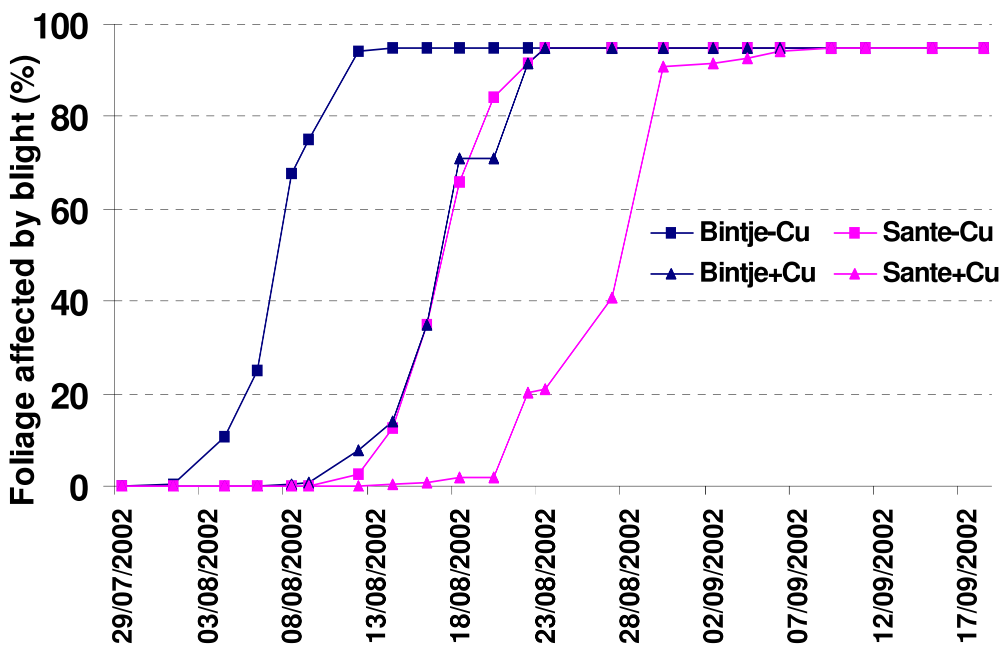
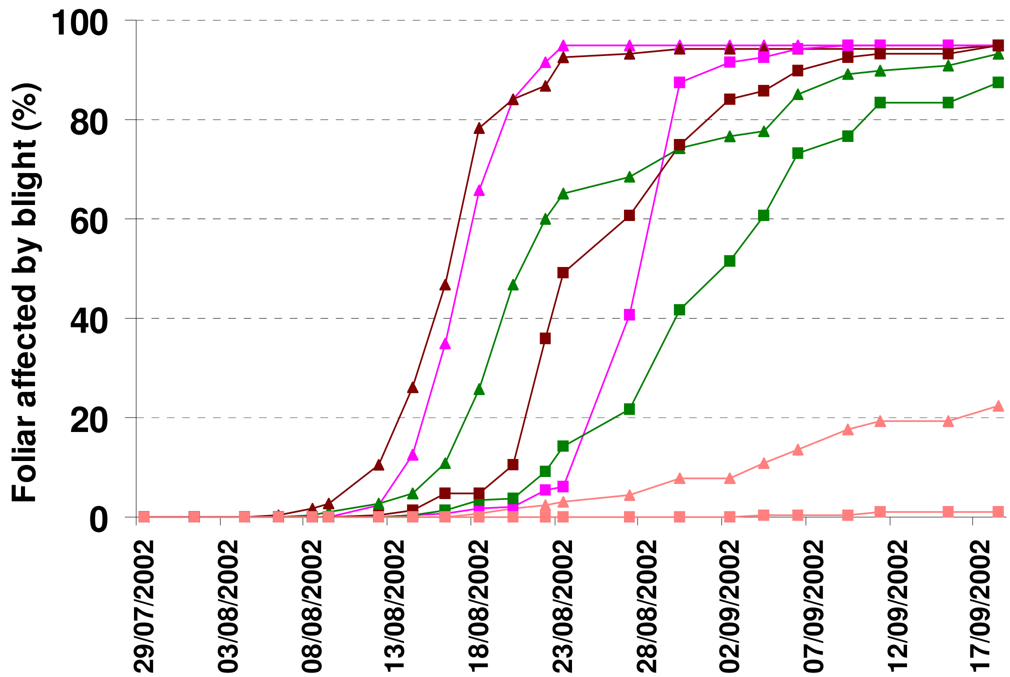


Figure 3.16 Effect of copper fungicide treatment on late blight development in selected potato varieties in 2002

(a)



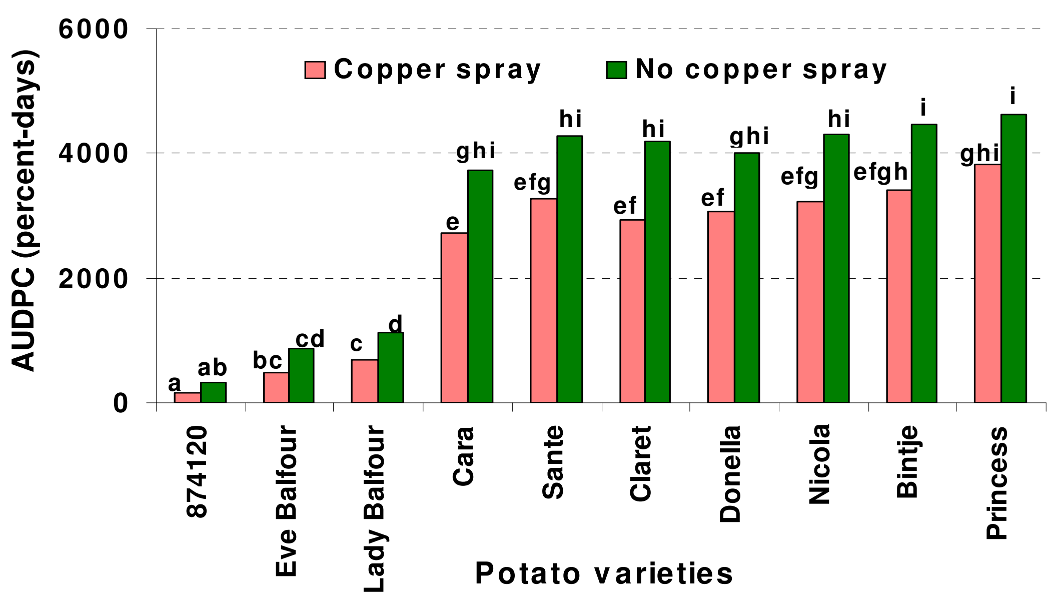
(b)



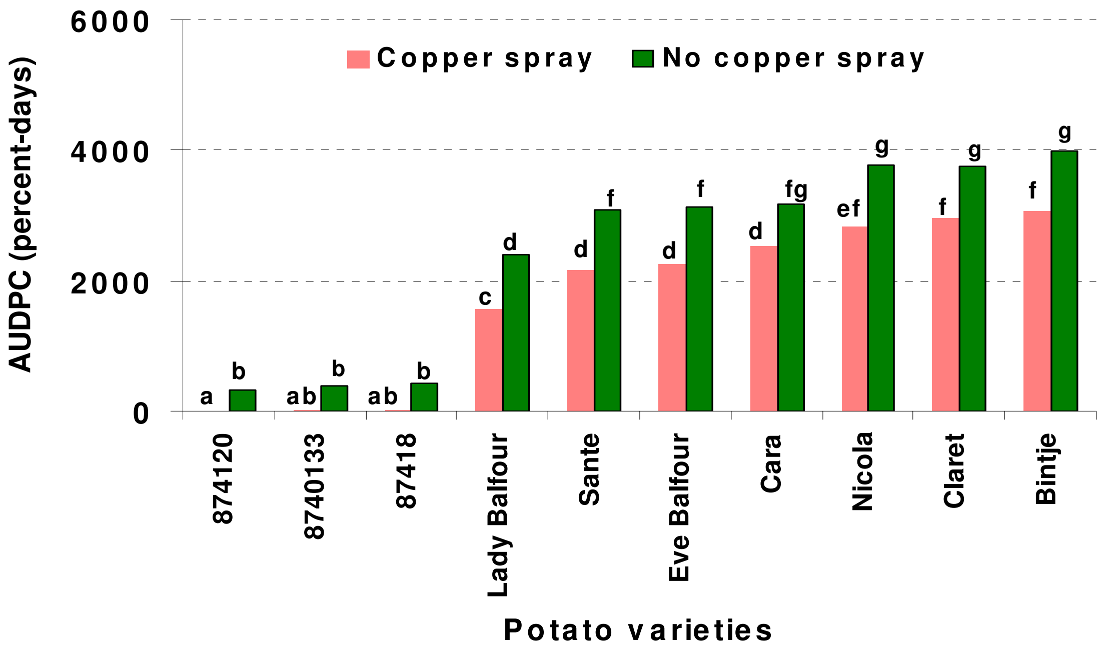
(For key for Figure 3.16b, see Figure 3.15b on the previous page)

Figure 3.17 Effect of copper fungicide treatment on late blight development in selected potato varieties in (a) 2001 and (b) 2002.

(a)



(b)


6000
4000
2000
0
AUDPC (percent-days)
Copper spray
No copper spray
a
b
ab
b
ab
b
c
d
d
f
d
f
d
fg
ef
g
f
g
f
g
874120
8740133
87418
Lady Balfour
Sante
Eve Balfour
Cara
Nicola
Claret
Bintje
Potato varieties

In 2002, the AUDPC showed the same trend as in 2001. Copper fungicide decreased the severity of foliar disease development in most varieties of potato, except for the highly resistant Sarpo varieties; 8740133 and 87418 (Figure 3.17). In both 2001 and 2002 Cu-fungicides resulted in a significant effect on blight levels in the resistant variety Lady Balfour and in 2002 also in Eve Balfour.

Effect of variety choice and copper fungicide use on tuber yields in organic potato production systems

There was a significant difference between variety and copper fungicide application with respect to potato yields. On average copper fungicide increased tuber yield by about 2.5t/ha (20.6%) in 2001 (Figure 3.18a) and 6.6t/ha (22%) in 2002 (Figure 3.18b). The tuber yield of all varieties in 2002 was higher than in 2001, reaching approximately 33t/ha and 17.5t/ha respectively.

In 2001, resistant varieties such as 874120, Sante, Lady Balfour and Eve Balfour gave significantly higher tuber yields than the more susceptible varieties Cara and Donella. However, there was no significant difference in tuber yield between the other susceptible varieties (Claret, Bintje and Nicola) and the more resistant varieties Lady Balfour, Eve Balfour, 874120 and Sante (Figure 3.18a).

In 2002, the tuber yield of the more susceptible varieties Nicola and Bintje was significantly lower than the yield of the more resistant varieties (Eve Balfour, Lady Balfour, Sante, 874133, 87418 and 874120). There was no significant difference in the tuber yield of resistant varieties (Figure 3.18b).

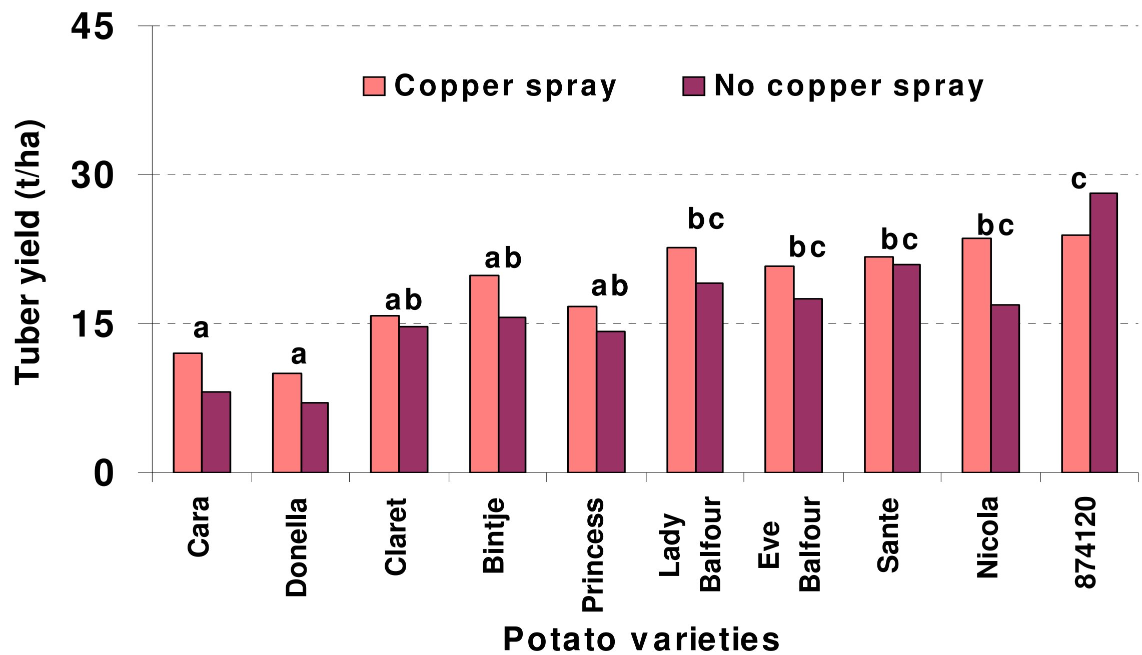
In 2001 and 2002, there were significant differences in yield fractions between varieties and between copper treated and untreated plots. In all treatments most tubers were in the 55-70 mm and 35-55 mm grades. Copper treated crops gave bigger tuber sizes than non-copper treated crops.

In 2001, the percentage of tuber yield in the >70mm 55-70 mm and < 35 mm grades were higher in copper treated crops. Also, there was a significant interaction between potato varieties and copper treatments with respect to the tuber size grade >70mm (Table 3.1 and 3.2).

In 2002, in some treatments there were no tubers < 35 mm and so this set of data could not be analysed. There were differences in the yield of tuber size >70 mm between copper treated and non-copper treated plots and between varieties of potato (Table 3.4 and 3.5). The percentage of tuber yield >70 mm from copper treated crops was higher than that of untreated crops (Table 3.5). The percentage of yield tubers in sizes 35-55 mm and 55-70 mm showed a significant interaction between the factors variety and copper treatment (Table 3.6).

Figure 3.18 Effects of copper fungicide treatment on yield of selected varieties in (a) 2001 and (b) 2002

(a)



(b)

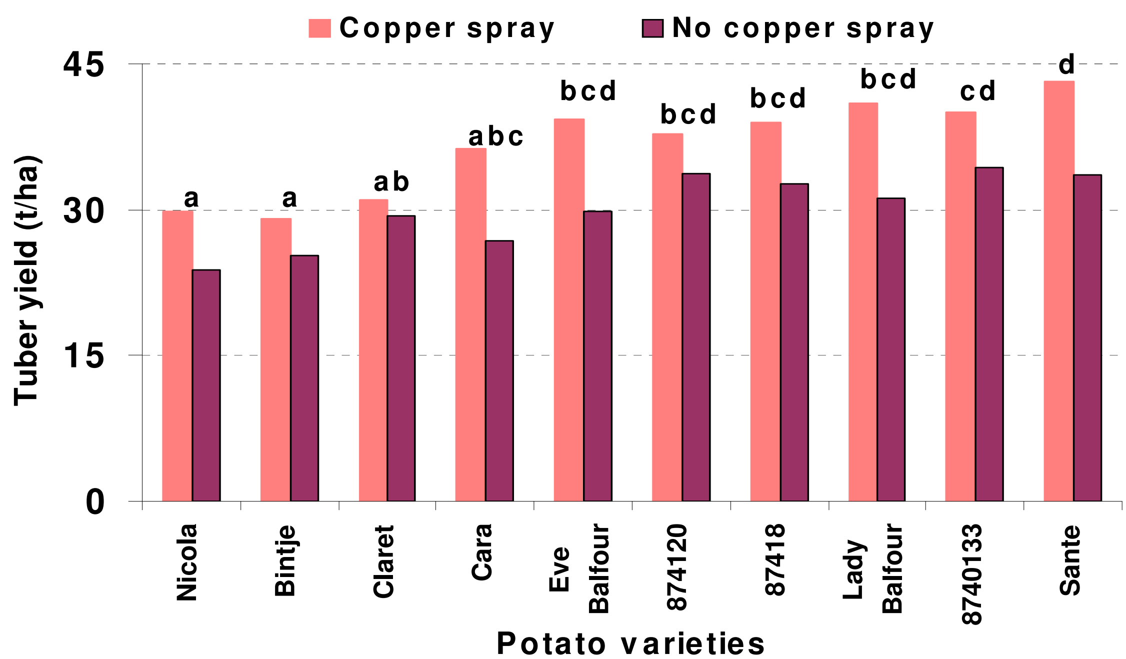


Table 3.1. Yield fraction > 70mm (as % of total yield) of copper sprayed and unsprayed varieties of potato in 2001. Different letters at different rows in the same column indicate significant differences (P<0.05) according to Tukey's Honestly Significant Difference (HSD) test.

Potato varieties	Tuber size >70mm (% of total yield)		SE
	Copper sprayed	No copper sprayed	
Lady Balfour	60.23a	45.25b	0.6
Eve Balfour	62.56a	48.02b	0.6
874120	38.56b	30.05bc	0.6
Cara	26.04bc	0.00c	0.6
Claret	55.64a	30.48bc	0.6
Nicola	8.48c	0.00c	0.6
Sante	75.02a	12.79c	0.6
Bintje	24.31bc	12.37c	0.6
Donella	27.99bc	22.61bc	0.6
Princess	23.81bc	12.15c	0.6

Table 3.2. Yield fractions; 55-70mm, 35- 55 mm and <35 mm (as % of total yield) of copper sprayed and unsprayed treatments in 2001. Different letters at different rows in the same column indicate significant differences (P<0.05) according to Tukey's Honestly Significant Difference (HSD) test.

Tuber size (mm)	Yield fractions (% of total yield)		SE
	Copper sprayed	No copper sprayed	
55-70	24.02a	20.84b	0.16
35-55	32.18b	55.38a	0.15
<35	3.54a	2.41b	0.09

Table 3.3. Yield fraction <35 mm (as % of total yield) of different varieties of potato in 2001. Different letters at different rows in the same column indicate significant differences (P<0.05) according to Tukey's Honestly Significant Difference (HSD) test.

Potato varieties	Yield fractions (% of total yield)			
	Tuber size 35-50 mm	SE	Tuber size <35 mm	SE
Lady Balfour	29.17b	0.15	0.99b	0.2
Eve Balfour	24.22b	0.15	1.44b	0.2
874120	39.55a	0.15	1.32b	0.2
Cara	62.48a	0.15	2.71ab	0.2
Claret	33.27b	0.15	0.57b	0.2
Nicola	60.68a	0.15	4.79ab	0.2
Sante	39.22b	0.15	0.06b	0.2
Bintje	49.37a	0.15	1.02b	0.2
Donella	46.75a	0.15	8.42a	0.2
Princess	53.11a	0.15	8.38a	0.2

Table 3.4. Yield fractions > 70mm (as % of total yield) of copper sprayed and unsprayed varieties of potato in 2002. Different letters indicate significant differences (P<0.05) according to Tukey's Honestly Significant Difference (HSD) test.

Potato varieties	Tuber size >70mm (% of total yield)	SE
Lady Balfour	11.57d	0.27
Eve Balfour	12.35d	0.27
874120	19.85c	0.27
Cara	22.75b	0.27
Claret	27.85a	0.27
Nicola	39.86a	0.27
Sante	18.41c	0.27
Bintje	33.31a	0.27
87418	32.00a	0.27
8740133	15.33cd	0.27

Table 3.5. Yield fraction >70mm (as % of total yield) of copper sprayed and unsprayed treatments in 2002. Different letters indicate significant differences (P<0.05) according to Tukey's Honestly Significant Difference (HSD) test.

Tuber size (mm)	Yield fractions (% of total yield)		SE
	Copper sprayed	No copper sprayed	
>70	14.58a	9.19b	0.12

Table 3.6. Yield fractions; 55-70 mm and 35-55 mm (as % of total yield) of copper sprayed and unsprayed treatments in 2002. Different letters at different rows in the same column indicate significant differences (P<0.05) according to Tukey's Honestly Significant Difference (HSD) test. SE = Standard Error

Potato varieties	Tuber size 55-70mm (% of total yield)			Tuber size 35-55mm (% of total yield)		
	Copper sprayed	No copper sprayed	SE	Copper sprayed	No copper sprayed	SE
Lady Balfour	58.14a	64.45a	3.1	12.01c	19.78c	2.8
Eve Balfour	57.79a	63.05a	3.1	12.48c	14.30c	2.8
874120	65.16a	64.27a	3.1	19.03c	23.14c	2.8
Cara	65.12a	67.73a	3.1	23.10c	27.24c	2.8
Claret	65.44a	56.43a	3.1	28.30c	41.98b	2.8
Nicola	57.79a	47.14b	3.1	39.69c	51.78b	2.8
Sante	59.82a	63.80a	3.1	18.22c	25.07c	2.8
Bintje	63.02a	57.47a	3.1	33.88c	42.25b	2.8
87418	62.19a	47.01b	3.1	62.19a	48.93b	2.8
8740133	62.04a	64.52a	3.1	15.45c	14.98c	2.8

Effect of variety choice and copper fungicide use on tuber blight infection in organic potato production systems

In both seasons, there were highly significant differences (P<0.01) in tuber blight infection between the ten varieties of potato in terms of both percentage of infected tubers and weight both at harvest and after storage. However, there was no difference in tuber blight development between copper fungicide treated and untreated crops of the same variety. In 2001, Bintje showed the highest percentage tuber blight at harvest (almost 40%) and after harvest (15 %). There were no significant differences in tuber blight incidence in the rest of the potato varieties in both first and second assessments. In 2002, the highest levels of tuber blight were recorded in Bintje, Nicola, 874120 and 8740133 at both assessment times.

Tuber blight affected tuber yield according to varietal susceptibility. The more susceptible the more yield loss. In some susceptible varieties such as Bintje, about 5t/ha of tubers were infected with tuber blight in 2001 and 2t/ha in 2002 (Table 3.7).

Table 3.7 Average marketable yield of copper sprayed and unsprayed varieties in 2001 and 2002

Potato varieties	Year					
	2001			2002		
	Total tuber yield (t/ha)	Infected tubers (t/ha)	Marketable yield (t/ha)	Total tuber yield (t/ha)	Infected tubers (t/ha)	Marketable yield (t/ha)
Lady Balfour	20.83	0.46	**20.37**	36.04	0.01	**36.03**
Eve Balfour	19.13	0.52	**18.61**	34.54	0.32	**34.22**
874120	25.97	0.38	**25.59**	35.72	1.01	**34.71**
Cara	10.10	0.23	**9.88**	31.55	0.17	**31.38**
Claret	15.20	0.30	**14.91**	30.18	0.50	**29.68**
Donella	8.54	0.43	**8.11**	-	-	**-**
Sante	21.32	1.06	**20.26**	38.40	0.41	**37.99**
Bintje	17.78	4.67	**13.11**	27.19	2.05	**25.14**
Nicola	20.24	0.69	**19.55**	26.82	1.87	**24.95**
Princess	15.45	0.43	**15.02**	**-**	**-**	**-**
87418	-	-	**-**	35.81	0.33	**35.48**
8740133	-	-	**-**	37.12	1.75	**35.37**

Discussion

Foliar blight severity differed considerably between varieties of potato and between copper treated and untreated crops. The differences between varieties reflected the level of foliar blight resistance which is determined by potato genetics. Tuber yields in 2001 were lower than in 2002. The most likely reasons for this were; (i) in 2001, planting was delayed by approximately 1 month, until 25 May and (ii) differences in blight development pattern in the 2 years. In 2001 a period of high blight pressure early in the growing season resulted in rapid destruction of the foliage for most varieties. On the other hand in 2002 defoliation by blight occurred over a longer time period which probably also accounted for the higher incidence of tuber blight in 2002. Novel varieties (Lady Balfour, Eve Balfour, 874120, 87418 and 874133) from breeding programmes at SCRI (Scotland, UK) and Sarpo (Hungary) were shown to have higher levels of foliar blight resistance than the most blight resistant varieties currently used in organic production in the UK (e.g. Sante, Cara). In particular, foliar blight development in these novel varieties (without Cu-treatment) was lower or similar to foliar blight development in Sante and Cara crops, which were treated with Cu-fungicides, in 2 growing seasons with relatively high late blight pressure (2001 and 2002). It would therefore be tempting to conclude that resistant varieties such as Balfour or Sarpo lines could provide the basis for commercially viable organic main crop potato production systems, which do not use Cu-fungicides. However, yields of the Balfour varieties were reduced by approximately 15-25%, when no Cu treatments were applied, which represented a similar yield loss to that observed in some foliar blight susceptible crops. Even the Sarpo varieties which showed very low blight severity (<20% leaf area affected), one month after Sante and Cara had been defoliated by blight showed approximately 10-15% higher yields when Cu-fungicides were applied in 2002 trials, but this difference was not statistically significant. This could have been due to the late development of tubers in the Sarpo varieties and/or a nutrient effect of the Cu applied to the foliage (Cu is known to be an important micronutrient for plants and may be present in insufficient levels in organically managed soils). Use of resistant varieties is therefore potentially the most efficient method to improve yields and yield stability.

Very little is known about the suitability of different blight resistant varieties for different organic potato markets (direct consumption, starch, chips, crisps etc.). Currently most markets and especially the multiple retailers still demand the well-known, but more susceptible cultivars. For example although the Sarpo varieties (874120, 874133 and 87418), which were bred in Hungary were highly resistant to late blight and gave high tuber yields, they have not been introduced on a wide scale in the UK market despite two of them being on the National List and therefore commercially available in the UK. Growers who wanted to plant these varieties have been discouraged by their customers (especially the multiple retailers) on the grounds of fears about market acceptability (William Weatherspoon; personal communication). There is also a concern about the risk of introducing seed potato tubers that could be contaminated with exotic strains of the fungus leading to increase virulence and genetic variation.

There was no correlation between foliar blight and tuber yields in some early bulking varieties of potato such as in Nicola, Claret and Bintje in 2001. Although the levels of foliar blight were high, these are early bulking varieties, so that the plants were likely to achieve higher yields earlier, before the disease infected the crop . For some late maturing varieties such as Eve Balfour, Lady Balfour and Sante, although the levels of foliar blight were quite high, the tuber yields were also high. This is thought to be due to their greater tolerance of the disease, because of their vigorous foliage.

The percentage of tuber infection in most varieties of potato in 2001 and 2002 at the first assessment (at harvest) was higher than at the second assessment (after storage). This because tubers became infected when spores were washed down to exposed tuber faces at the base of the infected potato plant. Also they were infected during digging and during harvest when fresh wounds and bruises were exposed to the fungus infected foliage. However, the second assessment was done after the tubers were kept in the cold room with moisture and temperature levels that suppressed infection. In the cold room the temperature was much lower than the optimum temperature for infection which is 18-22℃. Comparing the percentage of tuber infection between in 2001 and 2002, it was higher in 2001 because the strain of the blight fungus in that season was the local strain which was more virulent than in 2002, when plots were inoculated with a fungal suspension. Differences in tuber blight infection in different potato varieties can be described by the differences in periderm development, lenticel resistance and tuber maturity. The defence reaction of the tuber surface usually increases with maturity. This resistance component is marked by reduced hyphal growth and sporulation of *P. infestans*. The level of foliar blight was not found to be closely related to tuber infection resistance, which is in agreement with results of other studies. Tuber blight had an important effect on tuber yield especially in tuber blight of susceptible varieties such as in Bintje and Nicola.

SWITZERLAND (CH)

Materials and Methods

In both 2001 and 2002, the trial was installed at Gut Rheinau in Eastern Switzerland on a sandy soil on a biodynamic farm. In 2001, five new, blight resistant potato varieties (Appell, Derby, Innovator, Markies, Naturella) were compared with two varieties which are frequently grown in Swiss organic agriculture (Agria, Charlotte), and two reference varieties which were included at all sites (Bintje, Santé). All varieties were the same in 2002 with the exception that Markies was replaced with Eden.

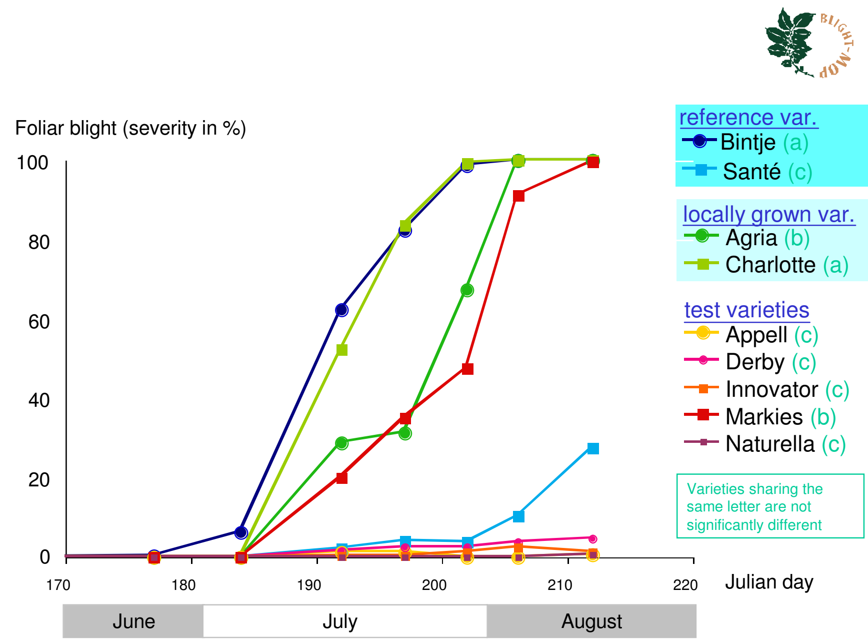
Potatoes were chitted on 22 February and planted on 30 April 2001 and 25 February and 18 March respectively in 2002. The haulms were mechanically destroyed on 7 August and the tubers harvested on 21 August 2001 (21 August and 4 September 2002).

Results and Discussion

2001 Growing Season

1) Foliar blight
Disease development is shown in Figure 3.19. Late blight (*Phytophthora infestans*) was first observed on 26 June.There were highly significant differences between varieties, but no significant differences between blocks. Tukey test discriminated between highly susceptible varieties (Bintje, Charlotte; stAUDPC = 32-34), intermediately susceptible varieties (Agria, Markies; stAUDPC = 20-23) and hardly susceptible varieties (Santé, Derby, Innovator, Appell, Naturella; stAUDPC = 0.07-3.0).

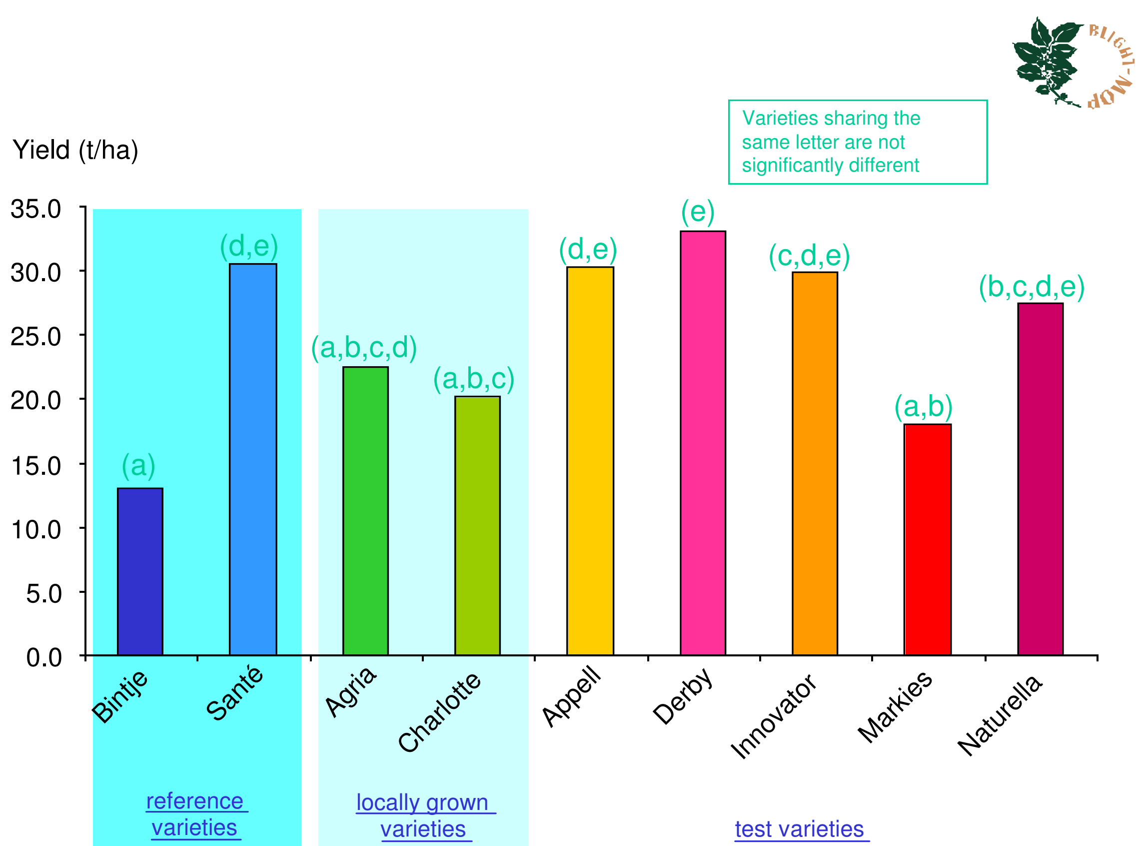
Figure 3.19 Development of foliar blight over the season



2) Yields

Yields are shown in Figure 3.20. There were significant differences between varieties and between blocks.

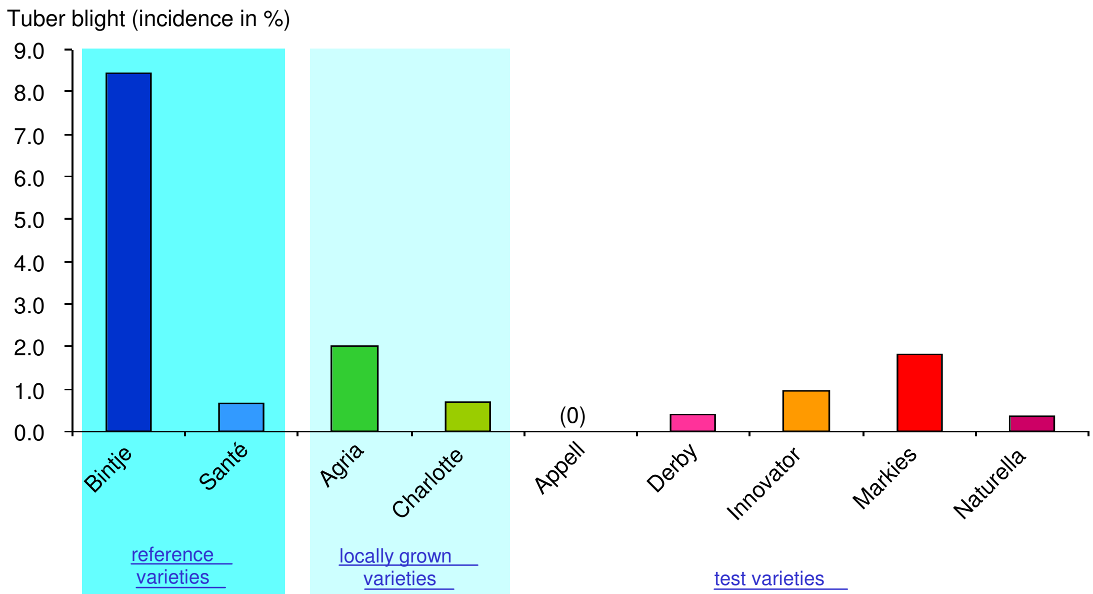
Figure 3.20 Yield



3) Tuber blight

Incidence of tuber blight is shown in Figure 3.21. There were no significant differences between varieties, nor between blocks.

Figure 3.21 Tuber blight (no significant differences)



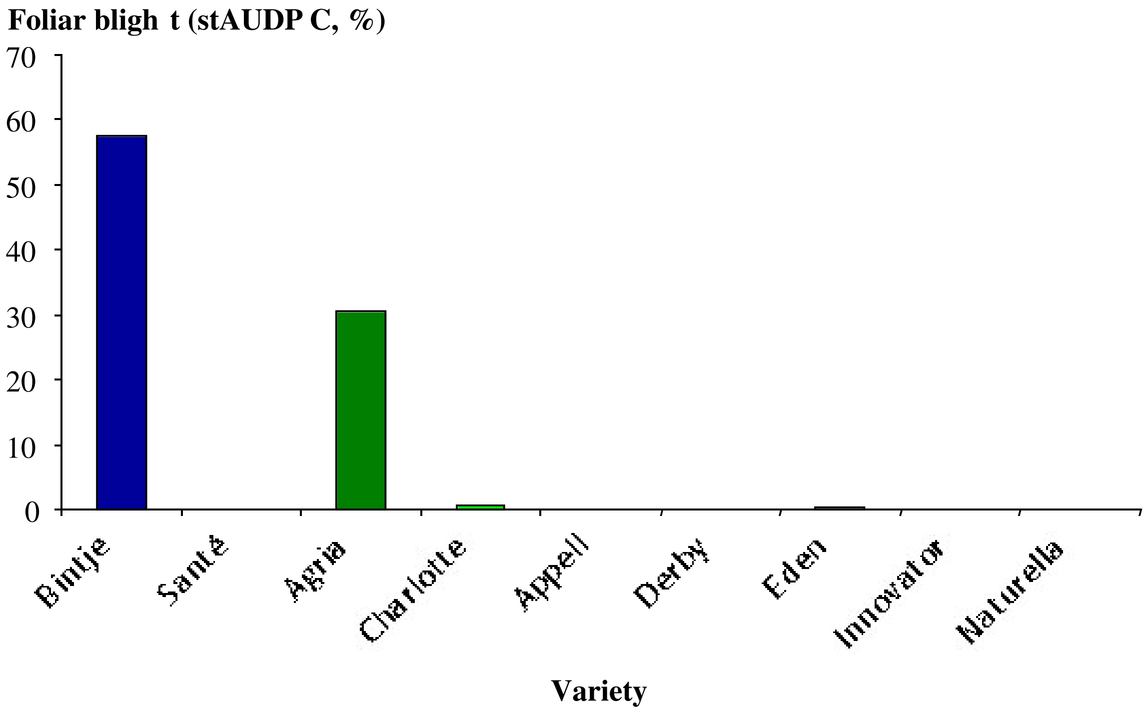
Results

2002 Growing Season

1) Foliar blight

Foliar blight (stAUDPC) is shown in Figure 3.22. Late blight (*Phytophthora infestans*) was first observed on 19 July.There were highly significant differences between varieties, but no significant differences between blocks. Because the epidemic arrived late, Charlotte and Sante were very little affected, as their foliage was already senescent at that time.

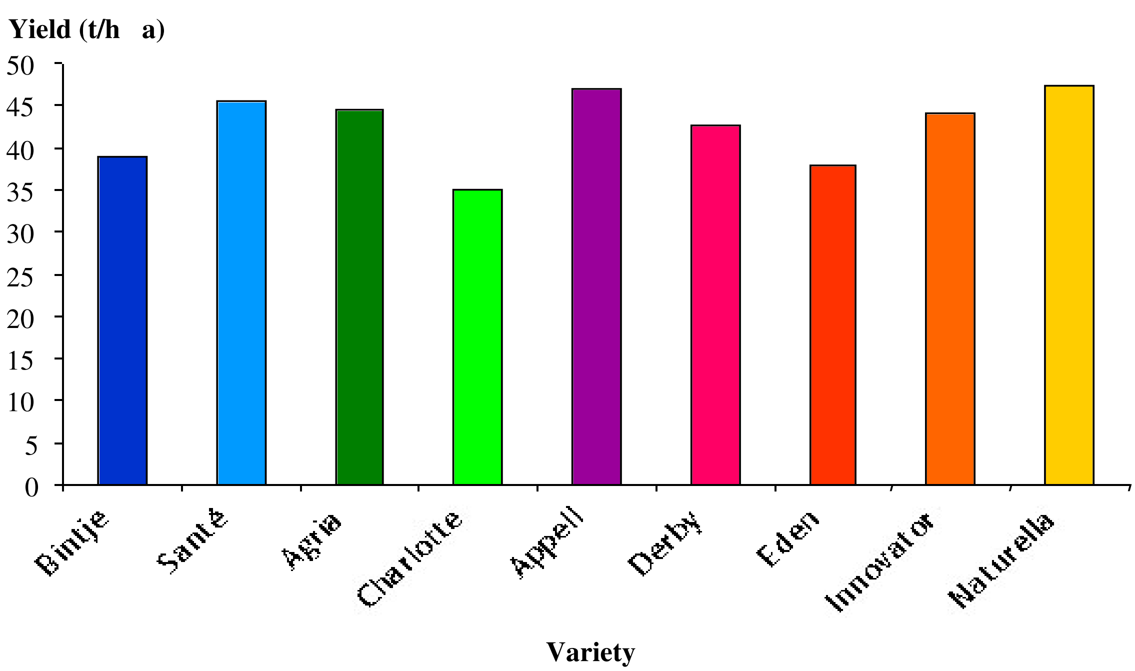
Figure 3.22 Foliar blight



2) Yields

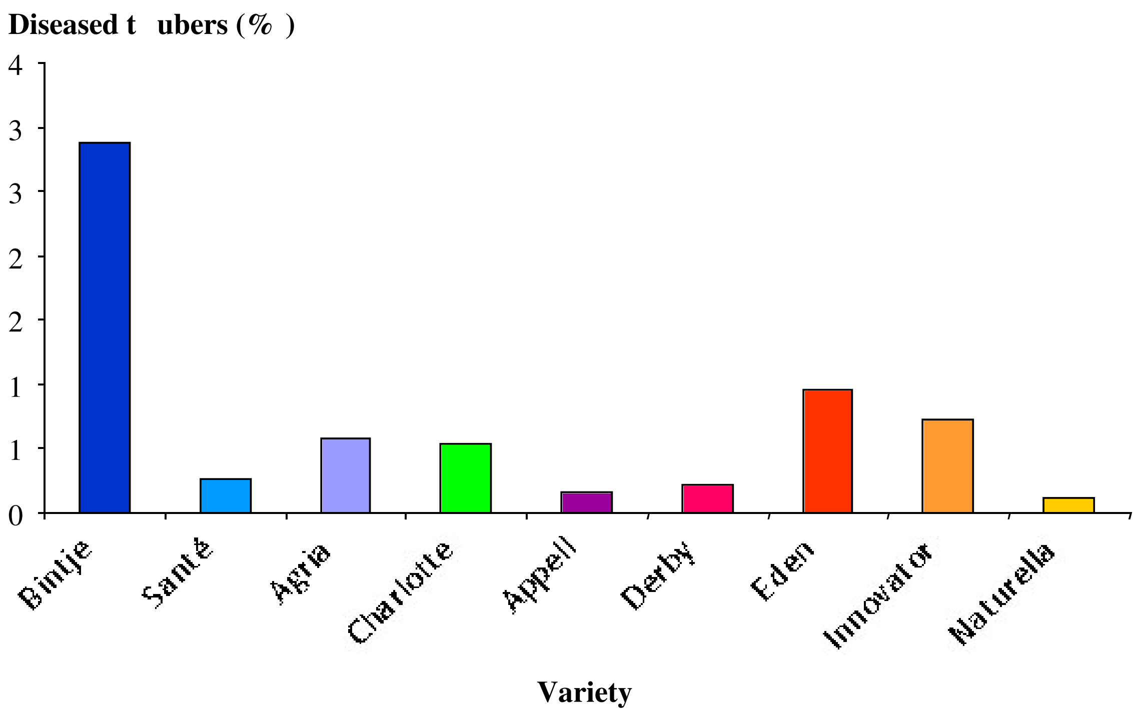
Yields are shown in Figure 3.23. None of the test varieties was significantly different from Agria, the most frequently grown variety.

Figure 3.23 Yields



3) Tuber blight
Incidence of tuber blight is shown in Figure 3. Tuber blight was significantly higher in Bintje than in all other varieties; there were no significant differences between any of the other varieties.

Figure 3.24 Tuber blight



Discussion and Conclusions

In 2001, four out of the five test varieties (Appell, Derby, Innovator, Naturella) suffered significantly less foliar blight than the two most frequently grown varieties. Probably, as a consequence of this, all of them also gave higher yields than the locally grown varieties. Tuber blight was not significantly different between varieties but Appell, Derby, Innovator and Naturella tended to have less tuber blight than the locally grown varieties. In 2002 all five test varieties (Appell, Derby, Innovator, Naturella, Eden) suffered significantly less foliar blight than the most frequently grown variety Agria, while yields and tuber blight were similar.

Taking into account all of the available agronomic information, it seems possible to substitute to a considerable extent the presently grown varieties with Appell, Derby, Innovator or Naturella. This agronomic strategy should reduce blight pressure in Swiss organic agriculture drastically.

However, before these varieties can be introduced into organic agriculture on a large scale, it must be made sure that they are accepted by market partners. Preliminary consumer investigations suggested that Appell, Derby and Naturella are promising candidates for the retail market. Innovator can be used for processing to flakes (frigemo, pers. comm.), and a large industrial partner expressed great interest in this variety.

OVERVIEW OF ASSESSMENT OF VARIETY PERFORMANCE IN DIFFERENT EU REGIONS AND ORGANIC PRODUCTION SYSTEMS

These experiments were designed to determine whether growing more resistant varieties is a promising strategy to eliminate or reduce the need for applications of copper-based fungicides in organic potato production. The potential reduction of foliar blight infection at local and regional level gained from substituting a currently grown variety with a new, more resistant one, was assessed and compared with the effect of a copper-based fungicide. Such an approach - growing varieties with better blight resistance than those grown currently which require little or no protection with copper fungicides - seems desirable.

It was clear that in each of the four countries, some of the new potato varieties are much less susceptible to foliar and tuber blight than most of the varieties currently grown. However, they are only commercially viable in organic farming if their agronomic characteristics e.g. adaptation to local climate; freedom from physiologically caused quality defects; resistance to major pests and diseases and tuber characteristics e.g. shape, size, skin finish, storage and culinary or processing properties) are comparable or superior to those of the varieties that are currently grown for the organic market sector. However, acceptability of any variety may vary from country to country and also from region to region within a country.

Some varieties have shown high degrees of foliar resistance against late blight, but for most varieties, the genetic basis of resistance is incompletely known and the risk of resistance breakdown in varieties with R-gene-based resistance is difficult to estimate. However, if such a variety is grown on a small area e.g. in organic production, the period of effectiveness of the R-gene may be extended. Nevertheless, to minimize the risk of resistance breakdown and its adverse consequences, growers should further diversify within a season wherever possible, by using varieties with genetically different resistance.

Blight control with copper fungicides

In the UK, copper treatments (the only effective fungicides currently permitted for blight control in organic agriculture) reduced foliar blight and yield on average by between about 20 and 30%. However there were no significant effects on tuber blight. In the UK 2001, the most resistant varieties were 87418 (Axona), 874120 (Sarpo Mira) and 8740133 (Tominia) and these showed very low levels of foliar blight infection even where copper oxychloride was not applied so that applying copper-based fungicides to them gave virtually complete control. In practice, in such highly resistant varieties, the limited benefits of using copper-based fungicides might be difficult to justify. However yield was improved by the fungicide even in the most resistant varieties. Furthermore, as copper-based fungicides are inexpensive and quick to apply, growers who have used them previously may be reluctant to dispense with them unless forced to do so because of the insurance they offer against the risk of disease in all varieties. Longer term, it is desirable to grow organic potatoes without copper-based fungicides to meet the demands for their elimination as previously outlined in this report. In the short term however, where severe blight pressure is the norm, limited use of copper fungicides may be necessary until other, effective blight management practices are available.

In this study, the effectiveness of two components of an integrated late blight management system in organic production - more resistant varieties and copper-based fungicide treatments - was investigated in four European countries and the implications would seem to be the same for all countries.
i.e. a significant reduction of late blight incidence and less need for fungicide treatments. However, this may be short lived if resistance is solely based on R-genes. In some countries and for some market outlets it seems that a blight susceptible variety can be replaced with a blight resistant one but not necessarily for other outlets. In the short term, the availability of organically produced seed potatoes may further limit the choice of varieties which can be grown in organic agriculture and this applies to all varieties whether they are resistant or susceptible, novel or long standing. Limited supplies, especially of newer, resistant varieties may result in higher seed and ware prices, but this is not necessarily the case. An analysis applied to the Swiss organic market showed that highly blight resistant varieties account for about 13% of the total area and the experimental results showed that another 35% could also be grown with the resistant varieties tested, resulting in almost one-half of the total

production area being grown with more resistant varieties. Moreover, assuming that resistant varieties require 0 to 33% of the copper-based fungicides used to protect currently grown susceptible varieties, a reduction of 16.5 to 50% of copper fungicides could be achieved by growing them and similar values could apply for other European countries.

In conclusion, growing blight resistant varieties in organic farming should be encouraged to the maximum extent possible. Currently, this approach used alone seems unlikely to bring about a complete end to the use of copper-based fungicides. Therefore, the integrated systems approach which is advocated by the Blight-MOP programme is needed, which combines as many control measures as possible, but including resistant varieties wherever feasible as a major foundation. Measures which have limited efficacy on their own may be useful, if they have a synergistic effect when used in combination with other components such as those tested in other parts of the programme and considered in subsequent sections of this report.

References

Campbell, C. L. and Madden, L. V. (1990*). Introduction to Plant Disease Epidemiology*. John Wiley & Sons; New York.

James, C. (1971). *A manual of assessment keys for plant disease*. Canada Department of Agriculture.

Note: Please also refer to the following publication describing the experiments reported in Section 1 of this Chapter.

Speiser, B., Tamm, L., Amsler, T., Lambion, J., Bertrand, C., Hermansen, A., Ruissen, M. A., Haaland, P., Zarb, J., Santos, J., Shotton, P., Wilcockson, S., Juntaharathep, P., Ghorbani, R., and Leifert, C. (2006). Field tests of blight control methods for organic farming: tolerant varieties and copper fungicides. *Biological Agriculture and Horticulture*, 23 (4), *393-412.*.

Section 2: Clonal diversity, race spectrum and aggressiveness of *P. infestans* populations found in organic production units in different areas on the EU

Summary

The suitability of potato varieties to be grown in different areas of the EU depends on the effect of *P. infestans* race spectrum on their resistance. Potential for breakdown of resistance was assessed by analysing virulence spectrum, race complexity and aggressiveness of *P. infestans*- isolate populations collected from infected leaves of the same reference cultivars grown in organic systems in France (F), Norway (N), Switzerland (CH) and United Kingdom (UK). (An objective of the Blight-MOP project was to evaluate the potential impacts of variety resistance on *Phytophthora infestans* (late blight) infection in organic potato cropping systems, as described in Section 1 of this Chapter). This assessed the risk of possible shifts in regional/national late blight populations towards increased pathogenicity that can be linked with a shift towards the growing of more late-blight resistant varieties in organic potato crops in different areas of the EU. Isolates of the late blight populations collected in the field from the two common reference varieties - a susceptible (cv. Bintje) and a moderately resistant (cv. Santé) cultivar - in the experiments made in 2001 in CH, F, N, UK were characterised at Plant-RI (NL) using neutral DNA markers, virulence testing and aggressiveness assays. Thus it was possible to compare their diversity and pathogenicity, and test whether isolates collected from cv. Santé were more pathogenic (virulent and/or aggressive) than those from cv. Bintje. Insight into the pathogenicity

traits of these populations could help to identify potato varieties that are best suited for use in organic farming in these countries.

All isolates collected from CH, F, UK and N were late blight strains of the new European *P. infestans* population introduced around 25 years ago. AFLP fingerprinting, mating type and mtDNA haplotype data showed that isolates from N and CH were more variable than those from F and UK. In both F and N, several groups of isolates had identical AFLP fingerprints and in isolates of both Bintje and Santé. On the other hand, in the UK and CH, all isolates had different fingerprints.
Three of the four haplotypes of *P.infestans* (Ia, Iaa and Ib) were found. In both F and UK, haplotypes Ia and IIa were present in equal amounts. Both these haplotypes are found in the new population introduced in Europe in the late 1970s. In CH, only Ia type isolates were found. Haplotypes Ia and Ib were found in equal numbers in N, which is remarkable since the Ib haplotype represents the old *P. infestans* population, which has been replaced by new population strains in most of Europe.
All isolates from CH, F, UK were of the A1 mating type. The A2 mating type was found only in N where more than half of the isolates were A2. This suggests that the sexual stage of the pathogen may be present in N. The rare combination of A2 type isolates with the Ib type mitochondria in N, indicates that sexual crosses have occurred between the "old" and "new" populations of *P. infestans* in this country and that the offspring of these crosses still prevail in the population.
There was no evidence of differences in frequency and distribution of virulence factors between Bintje and Santé isolates on a European scale but there were differences in virulence spectrum between individual countries. CH isolates contained most virulence factors on average (CH Bintje 5.6 / CH Santé 6.8 virulence factors/isolate), and N isolates the least (N Bintje 3.7 / N Santé 5.2).
There were no significant differences in infection efficiency (IE) between isolates from Bintje and Santé but N isolates from Bintje showed the highest IE values.
The new *Phytophthora infestans* population is present in all four countries, but remarkably, in Norway some traits of the old population introduced before the 1970s prevail. Isolates that share the same DNA fingerprint (clonal lineage) but can differ slightly in pathogenicity traits, such as virulence and aggressiveness is another remarkable feature.

Isolates from the 4 countries were fairly complex (but less so than the population found in the Netherlands) and populations differed in virulence spectrum and race complexity. However, no difference in race complexity could be related to the two cultivars, Bintje and Santé, from which the isolates were collected. This indicates that no population sub-structuring based on virulence is to be expected when more susceptible varieties are replaced with more resistant ones. The results suggests that no significant shifts towards increased pathogenicity are to be expected when organic potato production utilizes more resistant potato cultivars as a component of an integrated late blight management system. However, future shifts towards virulence to new R-genes bred into new cultivars (e.g. Biogold), cannot be predicted on the basis of these Blight-MOP project experiments.

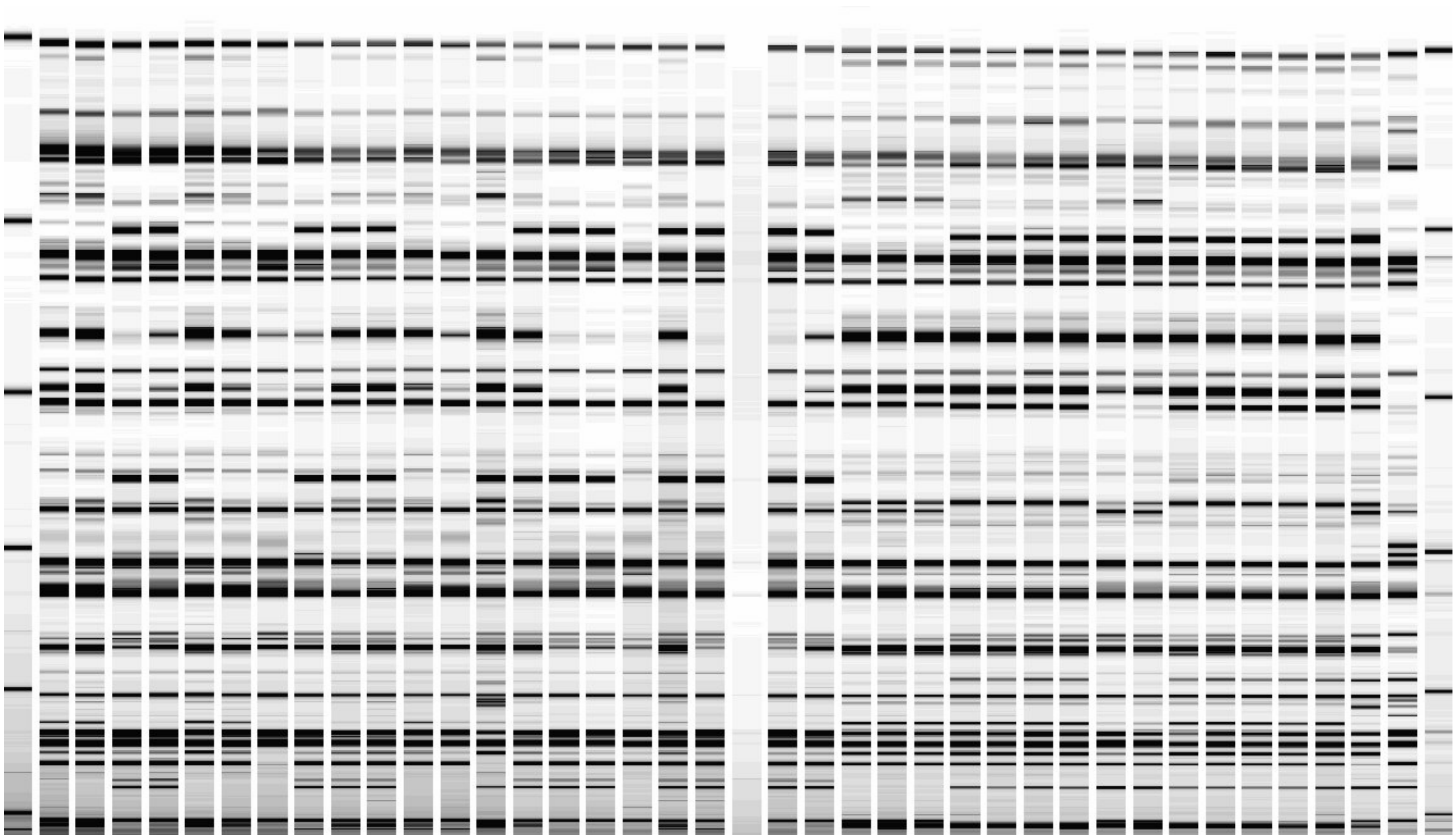
Introduction

With the predicted increase of organic potato production in Europe, and the preference for growing more late blight resistant potato varieties, it is of strategic importance to make a risk assessment of possible shifts in regional/national late blight populations towards increased pathogenicity that can be linked with a shift towards the growing of more resistant cultivars. The chance of infection and disease progress is affected both by varietal resistance and by the genetic make-up of the pathogen. An objective of the Blight-MOP project was to evaluate the potential impacts of variety resistance on *Phytophthora infestans* (late blight) infection in organic potato cropping systems. Plant-RI (NL) assessed the late blight populations collected in the field from a susceptible (cv. Bintje) and a moderately resistant (cv. Santé) cultivar in the experiments that were made in 2001 in France (F), Norway (N), The United Kingdom (UK) and Switzerland (CH) to assess potato variety performance in organic systems and reported in the previous section of this chapter. Isolates of the late blight populations from the four countries were characterized using neutral DNA markers, virulence testing and aggressiveness assays. This made it possible to compare their diversity and pathogenicity, and test whether isolates collected from cv. Santé were more pathogenic (virulent and/or aggressive) than those from cv. Bintje. Insight in the pathogenicity traits of these populations could help to identify potato varieties that are best suited for use in organic farming in these countries.

Materials and Methods

Isolate collection: In France, Norway, the United Kingdom and Switzerland, *Phytophthora* isolates were collected in the variety assessment field trials in 2001. At least 54 isolates were sampled in each country at various times after natural late blight infection. Twenty-seven isolates were sampled from Bintje (B, susceptible) and Santé (S, moderately resistant) plots, sent to Plant Research International and stored in liquid nitrogen. Because of problems with a large number of Swiss isolates, a new batch of these isolates was collected in 2002.

Figure 3.25 AFLP fingerprint of Norwegian isolates, collected in 2001, with primer combination E21/M16



M [Santé isolates][Bintje isolates] P V M

M= marker (50 bp ladder)
P = Pic99016 (Mexican reference strain)
V = Vk1.4oud (Dutch reference strain)

AFLP fingerprinting: This technique gives an indication of population diversity. Extraction of DNA from the isolates was followed by digestion of nDNA with restriction enzymes and amplification with labelled primers, according to the Amplified Fragment Length Polymorphism (AFLP)-protocol used for *P. infestans* (Flier, 2003). Two different primer combinations were used in the fingerprinting experiments, so for each isolate, two fingerprints were generated. Two reference isolates were included in each gel, one from the proposed centre of origin of *P. infestans* (the Toluca Valley of central Mexico) and one from the Netherlands. Fingerprints were generated on an ALF sequencer, and the fingerprint images were analysed with Imagemaster software. One AFLP-fingerprint for a subset of Norwegian isolates is shown in Figure 3.25 with 50 bp markers in lane 1 and 40, and the reference isolates in lane 38 and 39.

mtDNA: The mitochondrial DNA present in *P. infestans* isolates can be divided in 4 types. The presence of certain types of mtDNA indicates the origin or background of the isolates, and/or of the parental types from which they are derived. The mtDNA typing is based on restriction patterns of 2 regions of amplified mitochondrial DNA, according to Griffith and Shaw (1998).

Mating type: For *P. infestans*, two mating types are known (A1 and A2). The occurrence of two mating types indicates that the pathogen is bipolar and heterothallic, it needs a combination of isolates of opposite mating type to form sexual progeny. Sexual recombination between *P. infestans*' isolates could give rise to new combinations of pathogenic traits, resulting in a more aggressive population of the pathogen. These mating types do not correspond to dimorphic forms of the pathogen, but are distinguished by the production of specific hormones that induce the formation of sexual organs in the opposite mating type. Fusion of male and female gametangia (antheridia and oogonia) leads to formation of persistent diploid sexual spores, or oospores (Ko, 1988; Judelson, 1996). For each isolate, the mating type was determined by testing against 2 strains with known mating type. In this way, the potential for generating sexual offspring was tested for each region.

Virulence: Monogenic resistance genes (R-genes) from *Solanum demissum* were used in potato breeding programs, to infer resistance to *P. infestans*. Of the 11 R-genes from *demissum* identified so far, most are overcome by the corresponding virulence factors of *P. infestans*. The number of virulence factors in *P. infestans* isolates varies, from isolates with no virulence factors, which are only able to infect R-gene free potato varieties (i.e. Bintje), to isolates which carry (almost) all virulence factors. It is important to know which virulence factors are present in regional pathogen populations, because potato varieties that will be susceptible to the prevailing *P. infestans* population can be identified and hence the usefulness of certain potato varieties in a particular region can be determined. Isolates were tested in detached leaf assays. Each isolate was tested on two occasions, using 2 replicates (2 leaves of each R-gene plant/experiment). The leaf material originated from the international R-gene differential set. Each potato plant carries a single R-gene (R1-R11) and Bintje was included as an R-gene free control (R0). Leaves (lower side) were spray-inoculated with $3x10^4$ zoosporangia /ml and incubated at 15°C at a 16h light/8h darkness interval for 7 days (first 24h in total darkness). After one week, the leaves were assessed for percent leaf area affected by late blight, and sporulation.

Infection efficiency: For each isolate, the infection efficiency (IE) was measured. This parameter indicates the relative chance of successful infection for each zoosporangium. It is also an indicator of the aggressiveness of isolates. Fully- grown Bintje leaves were drop inoculated at 10 sites/leaflet with 10 µl of a suspension of $1x10^4$ spores/ml and incubated at 15°C at a 16h light/8h darkness interval for 7 days (first 24h in total darkness) and infection efficiency was assessed 7 days post inoculation. Fifteen leaves were tested in each experiment and the experiments were repeated twice. IE was expressed as the chance p that a single spore in the inoculum was successful, estimated from the fraction of inoculum droplets that resulted in growing lesions, as $p=1-H^{1/k}$ (Swallow, 1987); H is the fraction of unsuccessful inoculations and k is the number of spores in each inoculum droplet.

Lesion Growth Rate (LGR): The ability of an isolate to spread into healthy plant tissue after successful infection is an important parameter in disease progression and another indicator of the aggressiveness of isolates. LGR can be determined by assessing lesion spread after point inoculation of large Bintje leaves. A single 10 µl droplet of a suspension of $5x10^4$ spores/ml was applied on the lower side, at the centre of the leaves. After 7 days, lesion spread was measured in two dimensions and the LGR was calculated in mm per day.

Sporulation: Another important parameter in disease progression is sporulation and indicates the disease pressure that an isolate can give in a potato crop. An isolate that can generate more sporangia per area of infected leaf tissue has a higher chance of contributing its genetic material to the next generation, and so has an evolutionary advantage. The leaves from the LGR experiment were rinsed in Coulter Counter fluid, the spore concentration was measured and the amount of spores per square cm of infected leaf tissue was calculated.

Results and Discussion

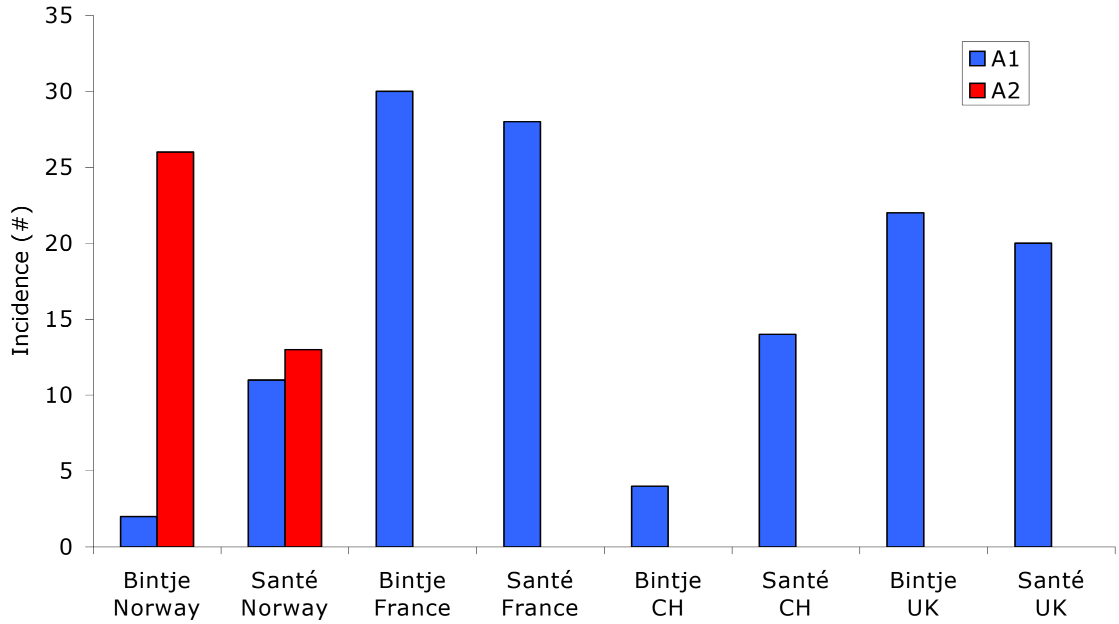
Isolates collected from CH, F, UK and N were all classified as late blight strains belonging to the new European *P. infestans* population, introduced around 25 years ago. The variation in Norwegian and Swiss isolates was much higher than those collected in France and the UK based on AFLP fingerprinting, mating type and mtDNA haplotype data.

AFLP fingerprinting: Almost all isolates collected in 2001 (and the Swiss isolates collected in 2002) were successfully fingerprinted for both primer combinations. Analysis of the fingerprint patterns commenced by setting up a basic gel for each primer combination and all subsequent Blight-MOP gels were linked to these basic gels, in order to facilitate comparison between countries.
There were several groups of isolates with identical fingerprints in both France and Norway and groups that comprised of isolates of both Bintje and Santé. On the other hand, in the UK and Switzerland, all isolates had different fingerprints.

mtDNA: Three of the four haplotypes of *P.infestans* (Ia,Iaa and Ib) were found in these experiments. In both France and the UK, haplotypes Ia and IIa were present in equal amounts. These are both haplotypes found in the new population, which was introduced in Europe in the late 1970s. In Switzerland, only Ia type isolates were found. In Norway, haplotypes Ia and Ib were found in equal numbers, which is remarkable since the Ib haplotype represents the old *P. infestans* population, which has been replaced by new population strains in most of Europe.

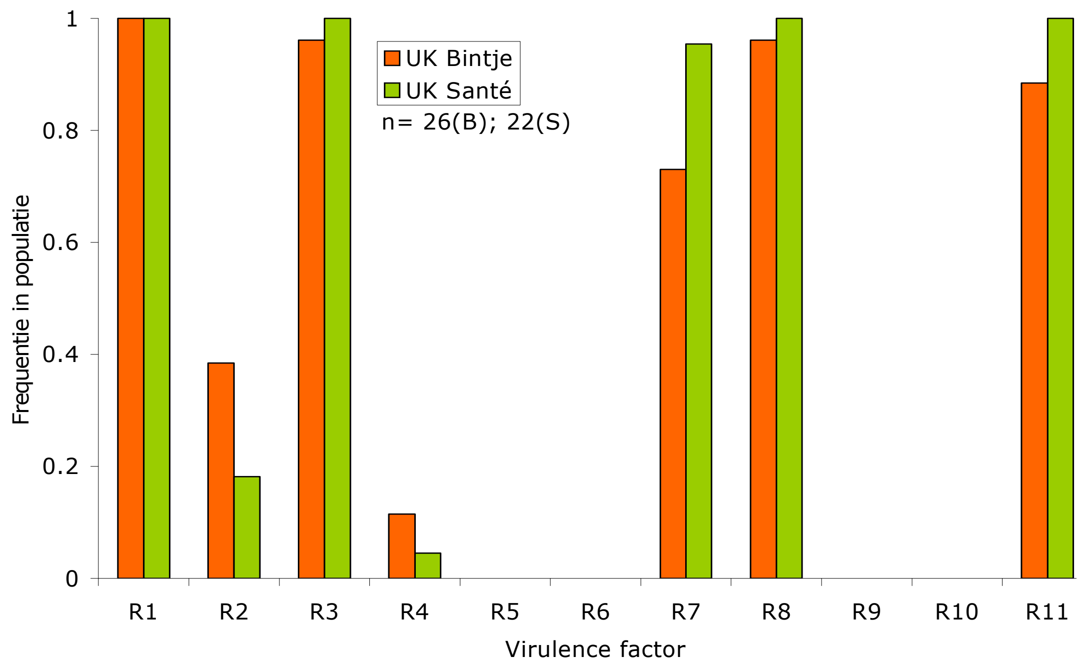
Mating type: The A2 mating type was found only in Norway: more than half of the Norwegian isolates were of the A2 mating type. All other isolates were of the A1 mating type (Figure 3.26). This suggests that at least in Norway, the sexual stage of the pathogen may be present. The rare combination found in Norway, of A2 type isolates with the Ib type mitochondria, indicates that sexual crosses have occurred between the “old” and “new” populations of *P. infestans*, and that the offspring of these crosses still prevail in the population.

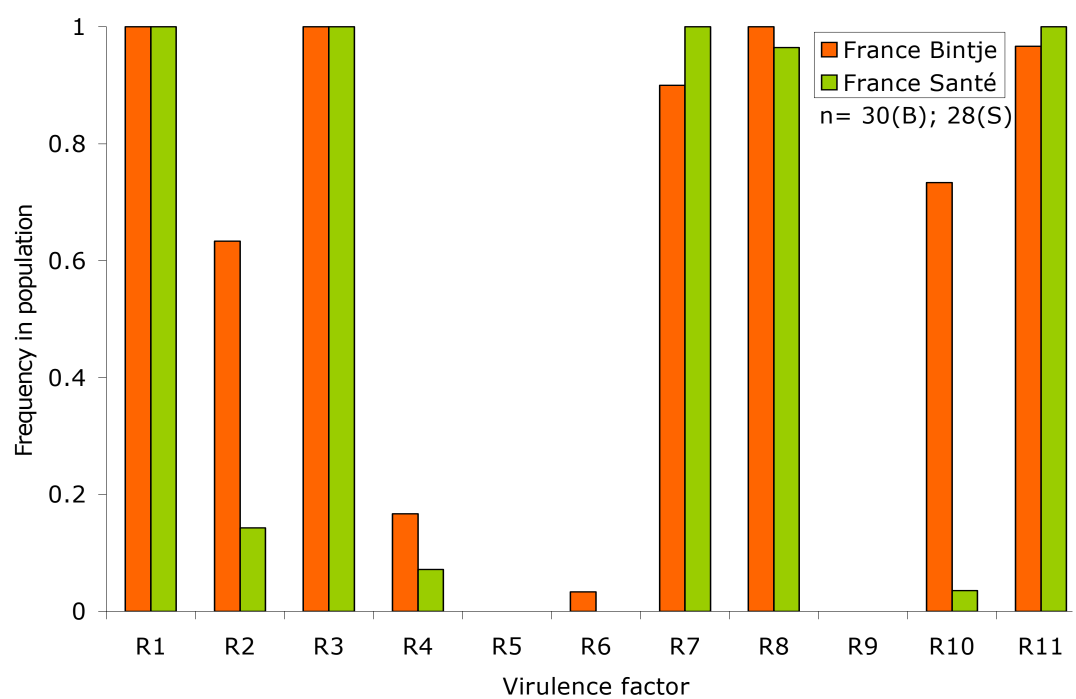
Figure 3.26 Distribution of mating types in isolates cvs. Bintje and Sante in Norway, France, Switzerland and UK collected in 2001.

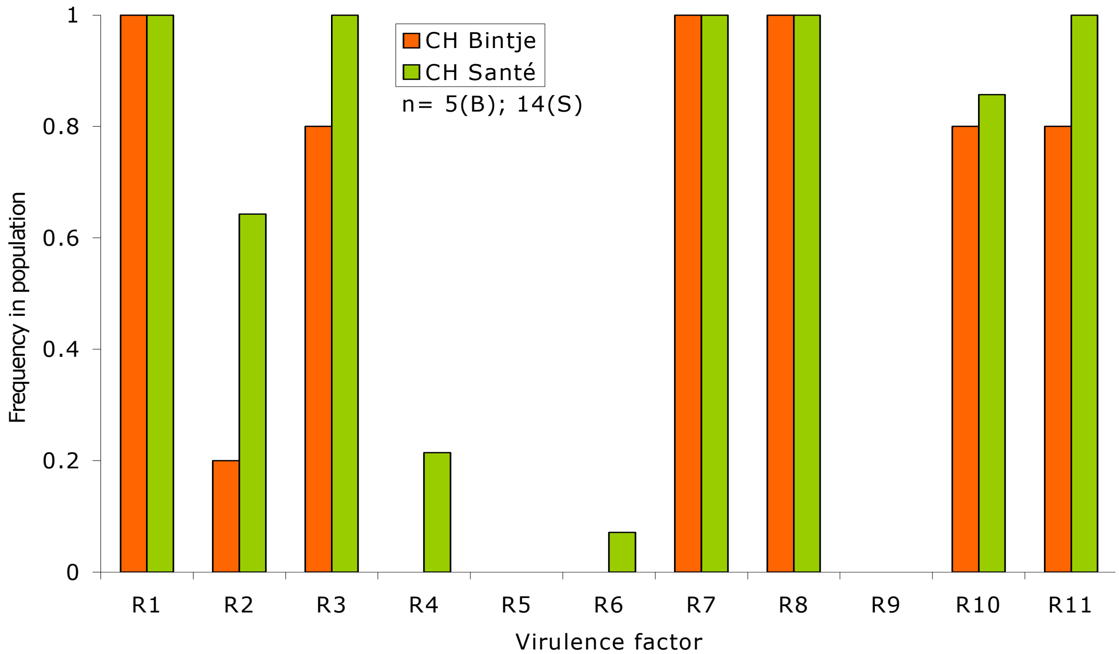


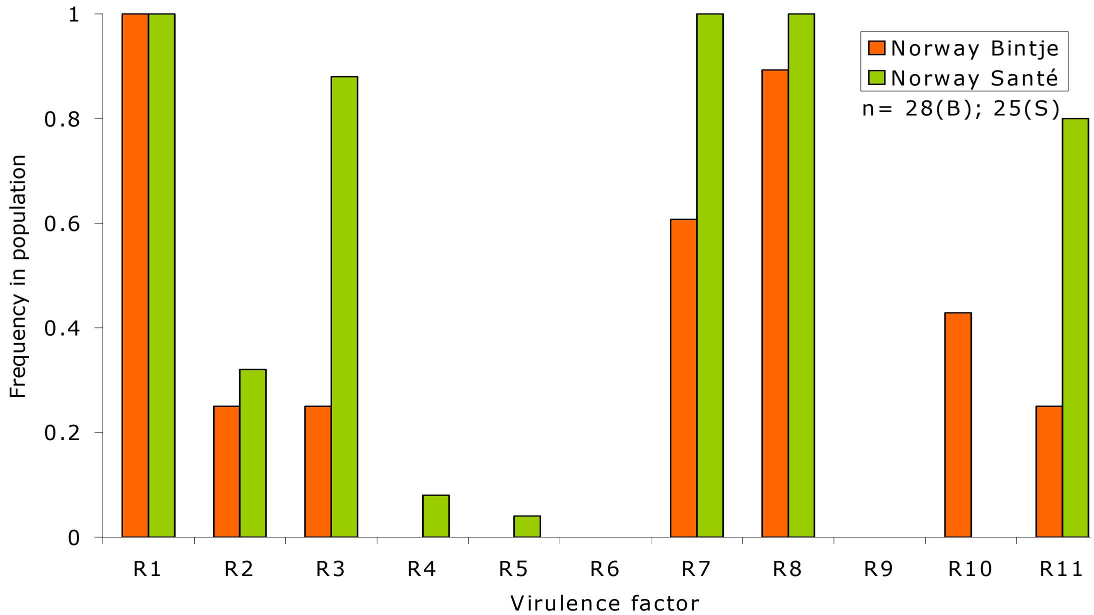
Virulence: Figure 3.27 show a graphical representation of the virulence factors present in Bintje and Santé isolates in Norway, France, Switzerland and the United Kingdom. Statistical testing using the Chi Square goodness of fit test indicated no differences in frequency and distribution of virulence factors between Bintje and Santé isolates on a European scale. There were differences in virulence spectrum between individual countries. Isolates from Switzerland contained on average most virulence factors (CH Bintje 5.6 / CH Santé 6.8 virulence factors/isolate), and Norwegian isolates contained the least virulence factors (N Bintje 3.7 / N Santé 5.2). For some R-genes i.e. R2 and R4, the number of isolates that can overcome these specific resistance genes seems high compared with previous studies (Lebreton, 1999) but this can be partly explained by the method of data analysis. The expression of foliar late blight resistance by the R2 and R4 R-genes is not absolute and phenotypically, these genes react more like a partial resistance gene. Instead of the definitive, clear-cut situation that is normal for R-gene controlled resistance (100% infection vs. zero infection visible), for these genes there is an intermediate class (40 to 60% infected, with some sporulation). In these experiments this intermediate class was included in the fully compatible class on the basis that the isolates can infect the R2 and R4 plants, and form progeny on the leaf surface, although less efficiently than fully compatible isolates. Whilst the inclusion of the intermediate class gave a slight over-estimation of virulence this was preferable to under-estimation.

Figure 3.27 Distribution of virulence factors in United Kingdom (UK), France, Switzerland (CH) and Norway isolates collected in 2001






CH Bintje
CH Santé
n= 5(B); 14(S)
Frequency in population
1
0.8
0.6
0.4
0.2
0
R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
Virulence factor



Norway Bintje
Norway Santé
n= 28(B); 25(S)
Frequency in population
0
0.2
0.4
0.6
0.8
1
R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
Virulence factor

Infection efficiency/ LGR/ Sporulation: No significant differences were found in infection efficiency between isolates sampled from Bintje and Santé, although the isolates collected in N from Bintje showed the highest IE values. No differences were found between the late blight populations from CH, F, UK and N. Average lesion-area was slightly larger in isolates collected from Bintje, compared with Santé. Significant countries by cultivar interactions were observed, with highest values present in UK isolates (Santé) and F (Bintje), respectively. Significant effects of cultivar and cultivar by country interaction was detected for sporulation density (Table 3.7).

Table 3.7: Average sporulation densities (sporangia/mm^2) of Blight-MOP isolates

	CH	F	N	UK
Bintje	466.2	275.6	280.7	425.2
Santé	245.8	292.2	241.5	264.4

Figures 3.28 and 3.29 show the associations between IE and sporulating lesion area and IE and sporulation density respectively.

Figure 3.28 The association between IE and sporulating lesion area

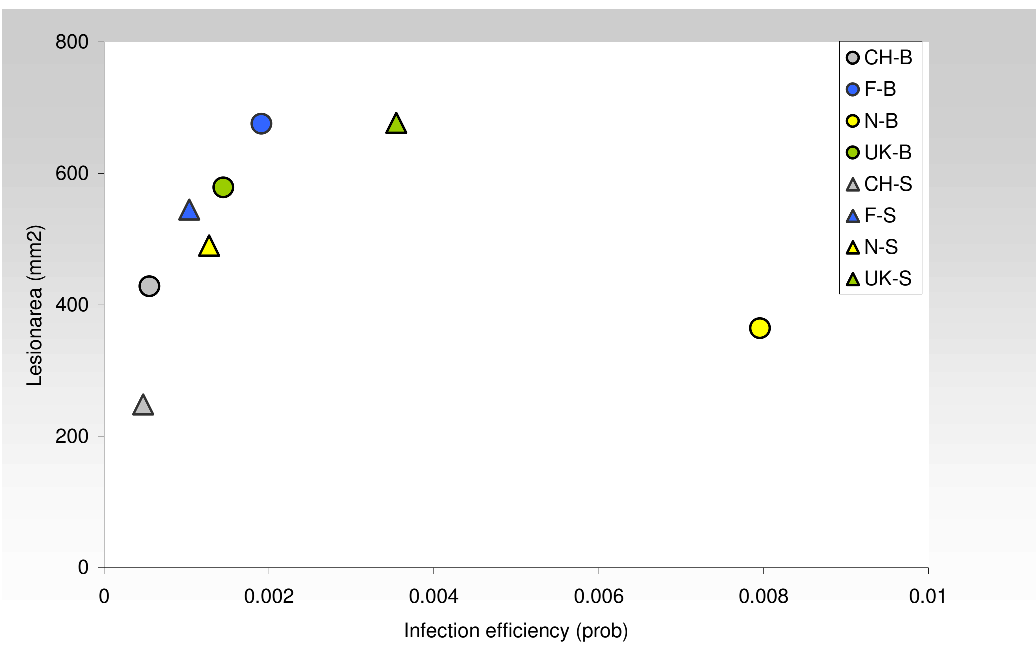
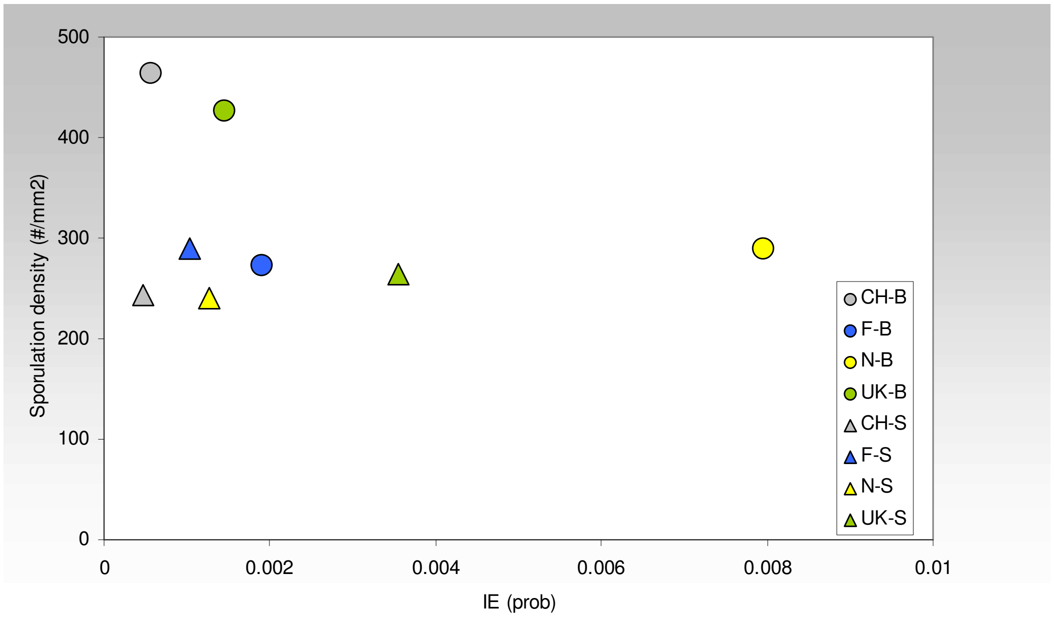


Figure 3.29 The association between IE and sporulation density



Conclusions:

In all countries, the new *Phytophthora infestans* population is present, which can be inferred from mtDNA and virulence data. In Norway, some traits of the old population (the population introduced before the 1970s) prevail, which is quite remarkable. Another remarkable feature is that isolates that share the same DNA fingerprint (clonal lineage) can still differ slightly in pathogenicity traits, such as virulence and aggressiveness.

Isolates from the 4 countries were fairly complex, but less complex than the population found in the Netherlands. Clearly, populations of Phytophthora collected from the four countries differed in virulence spectrum and race complexity. However, no difference in race complexity could be related to the two cultivars (Bintje and Santé from which the isolates were collected) indicating that no population sub-structuring based on virulence is to be expected when more susceptible varieties are replaced with more resistant ones. It appears that, based on these results no significant shifts towards increased pathogenicity are to be expected when organic potato production utilizes more resistant potato cultivars as a component of an integrated late blight management system. This may however not exclude future shifts towards virulence to new R-genes bred into new cultivars (e.g. Biogold), which cannot be predicted based on the experiments performed in the Blight-MOP project.

References:

Flier, W. G., Grünwald, N. J., Kroon, L. P. N. M., Sturbaum, A. K., Bosch, G. B. M. van den, Garay-Serrano, E., Lozoya-Saldaña, H., Fry, W. E., Turkensteen, L. J. 2003. The population structure of *Phytophthora infestans* from the Toluca Valley of central Mexico suggest differentiation between populations from cultivated potato and wild *Solanum* species. *Phytopathology* (accepted) Vol. **93**.

Griffith, G. W., Shaw, D. S., 1998. Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected following PCR amplification of DNA from pure cultures or from host lesions. *Appl. Env. Microbiol*. **64**: 4007-4014.

Judelson, H. S., 1996. Recent advances in the genetics of oomycete plant pathogens. *MPMI* **9**-6: 443-449

Ko, W. H., 1988. Hormonal heterothallism and homothallism in Phytophthora. Ann.Rev.Phytopathology 26: 57-73.

Lebreton, L., Lucas, J.-M., and Andrivon, D. 1999. Aggressiveness and competitive fitness of *Phytophthora infestans* isolates collected from potato and tomato in France. *Phytopathology* **89**:679-686.

Swallow, W. H., 1987. Relative mean squared error and cost considerations in choosing group size for group testing to estimate infection rates and probabilities of disease transmission. *Phytopathology* **77**: 1376-1381.