Chapter 4:

Diversification Strategies

Section 1: Alternate rows of resistant & susceptible varieties to control the development of late blight and identification of mechanisms for disease reduction and yield increase

Section 2: Variety mixtures

Section 3: Intercropping

Section 4: Consolidated Report on Diversification Strategies

Chapter 4: Diversification Strategies

General Introduction

In response to consumer demand (market pressure), organic farmers may need to produce some potato varieties that are blight susceptible. Indeed results from the survey reported in Chapter 2 confirm this. Previous experience has shown that late blight development in susceptible varieties can be reduced by adopting diversification strategies. Such strategies include growing varieties with different types and levels of resistance in alternating rows or in random mixtures or intercropped with different species that are unaffected by late blight. Whilst the survey of 'state of the art' techniques in Chapter 2 did not identify these to be used at present, it is essential to assess their potential for commercial application.

Alternating rows: Late blight infection in susceptible varieties can be reduced and their yields increased significantly by growing these varieties in rows alternating with other varieties displaying a partial (non-race specific) resistance to the pathogen. However, the performance of such varietal associations has not been assessed with different densities of partially resistant and susceptible varieties or with mixtures of partial and complete resistance (mediated by undefeated, race specific R-genes). Greater knowledge and understanding of the underlying mechanisms for the improved performance of such "associations" and influence of environmental conditions is needed to improve their reliability. Potential mechanisms include: physical barrier to inoculum dispersal caused by rows of resistant plants; progressive adaptation (during the course of the epidemic) of the pathogen genotypes to one or the other variety used in the association, thus restricting pathogen movement between varieties; compensatory growth effects between varieties. Field experiments that test the efficacy of alternating rows of different varieties with different types and levels of resistance can be used to investigate these 3 potential mechanisms by i) modelling the spread of late blight epidemics in pure and associated plots. ii) measuring variety-specific adaptation of *P. infestans* genotypes to determine to what extent any observed temporal adaptation of the pathogen population structure contributes to the performance of associations in controlling late blight. iii) quantifying competition between varieties in associated crops which may account for yield benefits that are independent of the effect of associations on disease control.

Variety mixtures: There is now substantial evidence that the development of air-borne pathogen epidemics can be significantly restricted by the use of variety mixtures, even if the mixtures contain only three or four different forms of resistance. The most important mechanisms which are thought to restrict epidemic development in mixtures are spatial separation of plants carrying the same resistance genes, barrier effects and induced resistance (i.e. plant A induces resistance in its neighbour B by plant A allowing the persistence of a pathogen race which is virulent to A, and avirulent to, but resistance inducing in B). Barrier effects and induced resistance should play a greater role when plants are mixed within rows than when planted in alternating rows. However, improvements in disease control in varietal mixtures must be weighed against additional costs for separating the varieties prior to sale and only certain types of mixtures may be of practical value depending on the target market (fresh, processing, industrial).

Intercropping: Once primary infection has taken place, the relatively heavy spores of *P. infestans* are mainly distributed within the field by rain splash and through direct contact of leaves. Separation of single or multiple rows/beds of potato by suitable barrier crops of a different species should restrict the development of blight epidemics by restricting row-to-row dispersal. However, the microclimatic conditions within the crop will be affected by the type of intercrop and the width of the potato beds, which may influence the efficacy of intercropping.

Effectiveness of restriction of dispersal is expected to be greater in potato varieties with some resistance than in fully susceptible crops. Intercropping with relatively tall crops such as wheat should significantly delay the spread of blight and increase yields compared with shorter crops such as clover. Furthermore, the protective, barrier effect should increase as the strips of wheat get wider.

Section 1: Alternate rows of resistant & susceptible varieties to control the development of late blight and identification of mechanisms for disease reduction and yield increase

Summary

In Denmark, experiments in 2001 and 2002 showed no significant reduction of potato late blight in susceptible varieties grown in alternate rows with varieties possessing either a partial (non-race specific) or race specific resistance. However, in 2001, late blight infection was significantly delayed in the resistant variety Kuras (with high race-specific resistance) when alternated with the susceptible variety Oleva. The delay of approximately one week occurred after the adjacent rows of Oleva were defoliated by leaf blight. Such an effect was absent in 2002 however.

Trials carried out during two consecutive years in France in Brittany showed no significant disease reduction in mixed (alternating rows of contrasting varieties) vs pure (single variety) plots irrespective of the planting pattern (with single or twin rows of varieties alternating with other varieties) and of the type of resistance used. However, the spatial distribution of disease was clearly different in pure and in mixed plots. These results were consistent with those from similar experiments made in Denmark with the same protocol, but different varieties. The three cultivars chosen displayed no competitive interactions when mixed. Populations of the pathogen differed according to host genotypes. Isolates virulent to Naturella (possessing the R2 resistance gene) were recovered almost exclusively from this cultivar, and proved weakly aggressive on Bintje (susceptible) or Désirée (partially resistant). Isolates collected on Bintje were most aggressive on both Bintje and Désirée. There was no clear indication of a strong correlation between pathogenicity characteristics and AFLP fingerprints. These patterns were similar in collections made over the two successive years. It was concluded that alternating rows of susceptible and resistant potato cultivars have a limited potential to control late blight in environments highly conducive to the disease, but may help manage the evolution of pathogen populations for virulence and aggressiveness.

Introduction

Market demands require that organic producers grow some blight susceptible potato varieties. Previous experience has shown that late blight development on susceptible varieties can be reduced significantly by growing these varieties in rows alternating with other varieties displaying a partial (non-race specific) resistance to the pathogen (Andrivon et al, 2003). Previous experiments had also shown potential for blight control in mixtures between susceptible cultivars and cultivars with partially defeated R genes (Garrett et al., 1999). However, the performance of such associations has not been assessed with different densities of partially resistant and susceptible varieties or with mixtures of partial and complete resistance (mediated by undefeated, race specific R-genes).

The underlying mechanisms for the improved performance of such "associations" are unknown and performance was found to vary depending on environmental conditions (Pilet, 2003). To improve the reliability of associations it is therefore essential to gain a better understanding of the underlying mechanisms. Three potential mechanisms were investigated in this project:

(i) Physical barrier to inoculum dispersal caused by rows of resistant plants;
(ii) Progressive adaptation (during the course of the epidemic) of the pathogen genotypes to one or the other variety used in the association, thus restricting pathogen movement between varieties;
(iii) Compensatory growth effects between varieties.

Under hypothesis (i), it would be expected that disease will progress more rapidly along the rows than across the rows in associated crops, but not in pure plots where resistance to disease spread will be identical in all directions**.** Under hypothesis (ii), pathogen populations would be expected to be more pathogenic on the cultivar they were collected from than on other cultivars. Finally, under hypothesis (iii), part of the yield benefits observed previously in associations would be expected to be due to a better use of resources required for growth by the different cultivars when associated than when pure (for instance due to their differences in growth rate), independently of the effect of associations on disease control.
Experiments were made in Denmark and France in both 2001 and 2002 to test the efficacy of alternating rows of different varieties with different types and levels of resistance on infection with late blight and crop yield. In France, the field experiments were also used to analyse epidemiological, ecophysiological and evolutionary mechanisms implicated and identify and understand underlying mechanisms for the improved performance of such “associations” outlined above. Approaches included modelling the spread of late blight epidemics in pure and associated plots; measuring variety-specific adaptation of *P. infestans* genotypes to determine the extent to which any observed temporal adaptation of the pathogen population structure contributes to the performance of associations in controlling late blight; quantifying yield effects which may be due to competition and better resource use rather than to better disease control.

DENMARK (F)

Materials and methods

In 2001 and 2002, the effect of a diversification strategy on the severity of late blight using alternating rows of tolerant and susceptible varieties was tested in the field. Seven identical treatments were included in both years, but an extra treatment was included in 2002 (Table 4.1). In 2001 infection was delayed in the resistant variety mixed with a susceptible one. This could have been due to a change in the crop’s microclimate and/or greater availability of soil nutrients to the resistant variety following early defoliation of the susceptible variety by late blight. To test this hypothesis, the additional treatment in 2002 (treatment 8) assessed the effects of mechanically defoliating rows of the susceptible variety (Oleva) when foliage blight reached 50% severity on the neighbouring row of the resistant variety Kuras. Treatments were arranged in a completely randomised block design with four replicates and individual plots were 8 rows wide and 10m long. Plots were assessed for disease incidence and severity on each plant of each harvest area (1 m) at weekly intervals from when late blight first appeared. Yield, tuber blight and size classes were assessed in all plots.

Table 4.1 Details of varieties and row configurations used in 2001 and 2002 in Denmark

Treatment	Varieties
1	Oleva – Oleva – Kuras – Kuras
2	Oleva – Kuras
3	Kuras (high race specific resistance)
4	Danva (high partial resistance)
5	Oleva – Danva
6	Oleva – Oleva - Danva – Danva
7	Oleva (susceptible)
8*	Kuras: canopy of every second row was removed at 50% defoliation of Oleva

* Only in 2002

Results and Discussion

In 2001, blight infection in the susceptible variety (Oleva) was not decreased by growing it in either single or double rows alternating with either single or double rows of a variety with partial (non-race specific) resistance i.e. Danva or race specific resistance i.e. Kuras. However, the resistant variety Kuras showed a significant delay in blight of approximately one week when mixed with the susceptible variety Oleva. This delay occurred almost a month after neighbouring rows of Oleva had been destroyed by leaf blight (Figure 4.1).

Mixing the susceptible variety Oleva with partially or race-specific resistant varieties had no effect on the tuber yield of the susceptible variety. However, the yield increase in the resistant variety Kuras was consistent with the delay in foliar blight (Figure 4.2). The incidence of tuber blight was very low in 2001 (0-2% in all varieties) and there were no differences in tuber blight within the individual varieties.

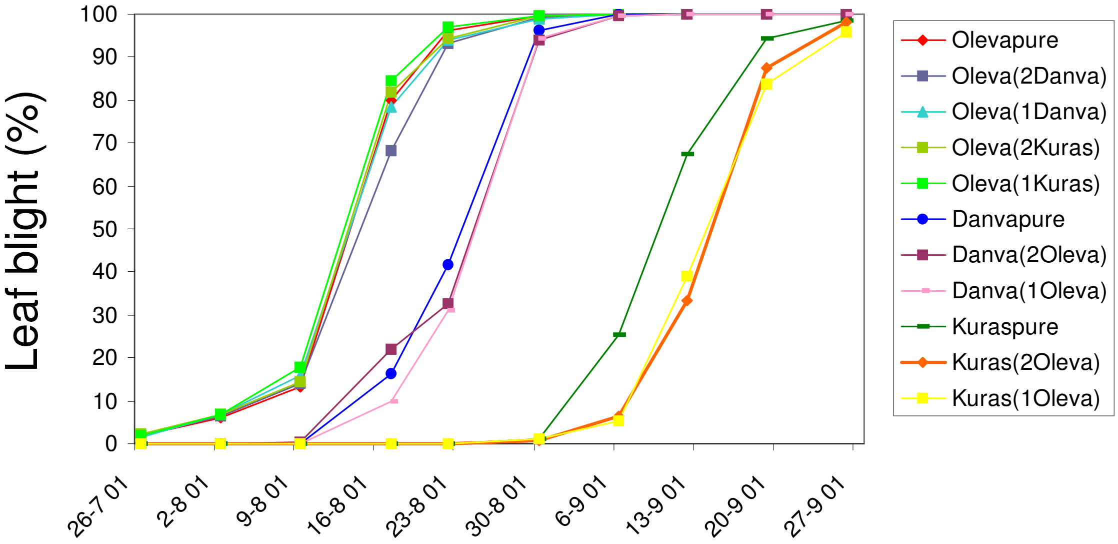

Figure 4.1. The effect of alternating single and double rows of susceptible (Oleva), partially resistant (Danva) and race-specific resistant (Kuras) varieties on foliage blight infection in individual varieties within the treatments in 2001.

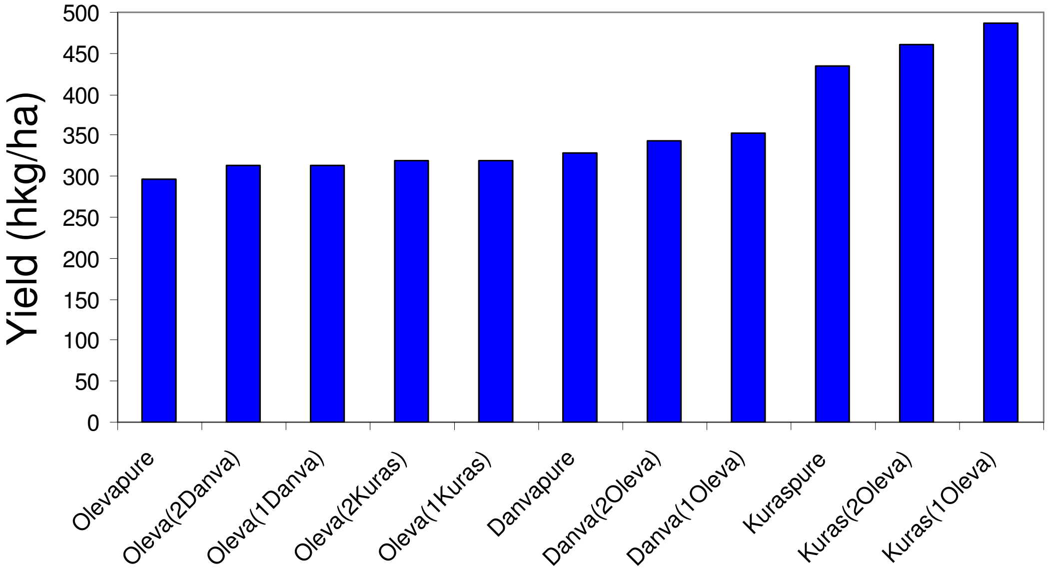

Figure 4.2. The effect of alternating single and double rows of susceptible (Oleva), partially resistant (Danva) and race-specific resistant (Kuras) varieties on yields of the individual varieties within the treatments in 2001.

In 2002, infection of the susceptible variety Oleva was not delayed by alternating rows with either Danva (partial resistance) or Kuras (race specific resistance). Furthermore, there was no delay in blight development in either Kuras or Danva when rows were alternated with either one or two rows of the susceptible variety Oleva. However, there was a yield increase of about 20% in the susceptible variety Oleva when grown in either single or twin rows alternated with single or twin rows of the resistant variety Kuras (Figure 4.3) possibly because of better resource use. There was no delay in the late blight epidemic in Kuras when the neighbouring row of Oleva was defoliated when it reached 50% blight infection. Yields of the resistant variety Kuras were unaffected by alternating it with either one or two rows of Oleva.

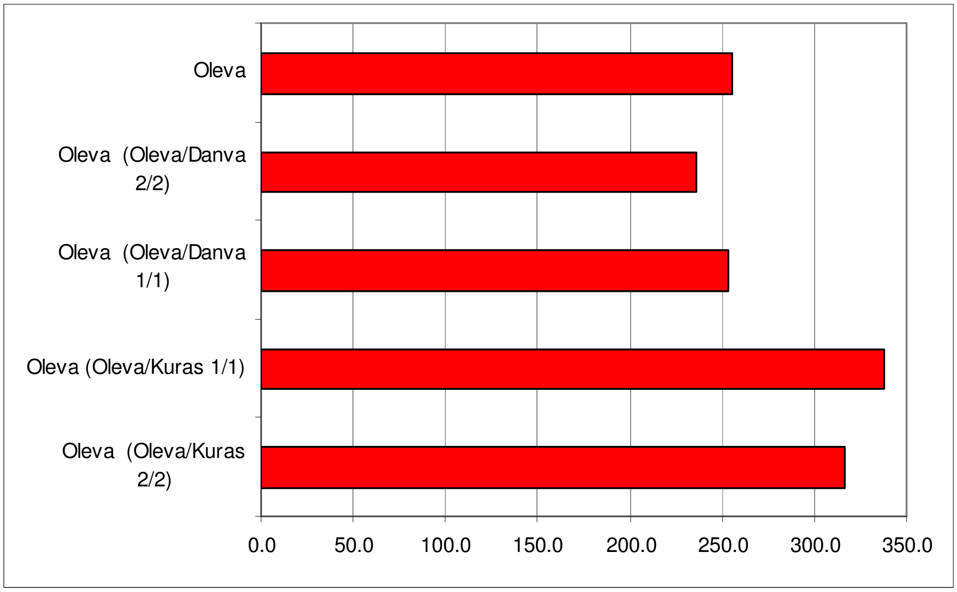

Figure 4.3. Potato yield (hkg/ha) in variety Oleva and different mixtures with Oleva, in 2002.

Conclusions

In 2001, alternating rows of resistant and susceptible varieties delayed foliar blight in the variety Kuras with race specific, late blight resistance. However, as this effect was not observed in 2002, the evidence - based on the two years trial - regarding the possibility of reducing blight in susceptible varieties by growing them in rows alternating with other varieties displaying either a partial (non-race specific) or a race specific resistance to the pathogen was inconclusive.

FRANCE (F)

Materials and methods

Field trials design

Two field experiments were performed in 2001 in Ploudaniel and Le Rheu (western France) and one experiment in 2002 in Ploudaniel. Blight pressure is consistently high in Ploudaniel (where the site is close to the sea with high humidity/rainfall and mild temperatures), but more erratic in Le Rheu (with a drier climate). The basic design was a randomised block design (2 blocks), with 8-10 row-plots of 10 plants of the following combinations of the 3 cultivars Bintje (B: susceptible), Désirée (D: partially resistant) and Naturella (N: race-specific resistant, gene R2): pure B, pure D, pure N, BD, BN, BBDD and BBNN. All trials were under natural late blight infection, with the first symptoms appearing in early June (Ploudaniel 2002), end of June (Ploudaniel 2001) or early July (Le Rheu 2001). All plots were left unsprayed throughout the epidemic. A second set of plots, identical in design, was established in Le Rheu (2001) and in Ploudaniel (2001 and 2002), and sprayed as needed to avoid blight contaminations. This second set of plots was used to measure competition effects between cultivars in the absence of disease.

Late blight monitoring

Late blight severity was scored visually, as the percentage of foliage area diseased, according to a modified pictorial scale (Andrivon et al., 2003). Disease scorings were performed weekly on every plant of each plot, providing a complete dynamic map of disease progress over time. The scorings began when the first symptoms were seen, and continued until the susceptible plants were destroyed.

Plant growth monitoring

For the competition experiments, plant growth was assessed by measuring ground coverage using a square 1 x 1 m grid, subdivided into 10 x 10 cm quadrats, placed on stands over the top of the canopy. The ground surface covered was estimated by counting the proportion of 'empty' quadrats on three different spots in each plot. The measurements were made at 4-6 dates at 1-2 wks intervals, depending on growing conditions.

Isolate adaptation

Isolates were collected from the 2001 and 2002 trials in Ploudaniel, at a time when all three cultivars showed infection. Blighted leaf tissue (ca 25-30 leaflets per cv) were collected separately in the field and their spatial position recorded (row and plant within the row), only in the pure plots. Isolation of *P. infestans* from these leaves was carried out in the laboratory on potato slices, and isolates were maintained on pea agar. 20-25 isolates per cv were thus obtained. The aggressiveness of the 2001 and 2002 collections of isolates to each of the three cvs was tested by inoculating detached 6-10 leaflets from greenhouse-grown plants, using the methodology described by Lebreton et al (1999). Lesion size, infection efficacy (i.e. the proportion of inoculated leaflets producing a sporulating and expanding lesion) and spore production per lesion were measured. The virulence of the isolates in the collection was also determined by inoculating detached leaflets of potato clones with known R genes.

Isolate fingerprinting

Isolates were fingerprinted using AFLP profiles generated with two different primer pairs (Mse-CC/Eco-GA and Mse CG/Eco AC). The banding patterns were detected on an ABI Prism capillary sequencer, and converted to a genetic distance matrix. A phenetic tree was computed from that distance matrix with the UPGMA clustering algorithm of the Bionumerics software.

Results

Late blight progress in pure and alternated plots

No significant effects of associations on blight development were detected in either 2001 or 2002 in France. No significant yield differences were detected either (Figure 4.4). These results are consistent with those obtained in Denmark during the same period.

Epidemiological mechanisms and population evolution

Barrier effects

The observation of disease patterns in pure and alternated plots showed a clear dependence of spatio-temporal disease spread according to the type of plot and to the efficacy of resistance. In pure plots of susceptible and partially resistant cultivars, the disease pattern was usually focal. In the race-specific resistant Naturella, disease was patchy and severity remained low. In alternated plots, the architecture of the canopy was reflected in the disease patterns, with the most severely diseased plants being those from the susceptible cultivars (Figure 4.5).

The statistical analysis of these patterns was attempted, but faced several difficulties. The most important of these was a consequence of the relatively small size of plots and rapid spread of the pathogen. This made the use of methods based on incidence data (where plants are assessed as either diseased or healthy), such as ordinary runs (Madden et al., 1987) or 2D-class analysis (Nelson et al., 1992) impossible, as the proportion of diseased plants went from 0 to 100 % in a very short time. Furthermore, the reduction of the unit area occupied by each genotype in alternated plots generated high border effects. It is therefore impossible to find the range (i.e. the distance between plants at which spatial autocorrelation stabilizes) in analyses based on disease severity, such as spatio-temporal autocorrelation or geostatistics. Therefore appropriate statistical methods to analyse the disease patterns in such plots were sought. To this end, discussions on this topic were initiated with Prof. Larry Madden (Ohio State University, Wooster OH) during the International Workshop on Plant Disease Epidemiology (Landerneau, April 2005).

Competition

Despite their different growth habits (Désirée and Bintje being ramose (heavily-branched) cultivars while Naturella is an erect one), there was no evidence of competitive interactions between cultivars in mixed plots, neither for growth of the canopy (Figure 4.6) nor for final yield in the presence or absence of late blight (Figure 4.7).

Isolate adaptation

All isolates from the 2001 collection, and all 71 isolates but four from the 2002 collection were of the A1 mating type. The 4 A2 isolates from the 2002 collection were isolated from Naturella, alongside A1 isolates, in the two pure plots.

AFLP fingerprints revealed the presence of six closely related AFLP groups among the 70 isolates of the 2002 collection. Two groups (AFLP 3 and 4) largely dominated the collection, while the other four groups were restricted to 1-4 isolates each. The four isolates with A2 mating type were identical, and clustered alone in AFLP group 5. The same AFLP groups - except AFLP 5, found in 2002 only, and a seventh group represented by only one isolate from the 2001 collection - were found in the 2001 and 2002 collection. This indicates a general stability of the genetic composition of the population present in Ploudaniel in these two consecutive years.

In both years, isolates virulent to R2 were found almost exclusively among those collected from Naturella. The race diversity was greater among isolates from Naturella than among isolates from either Bintje or Désirée.

In both years, the isolates from Naturella were on average less aggressive to cvs Bintje and Désirée than those collected on these cvs. The isolates collected from Bintje were on average more aggressive to both Bintje and Désirée than those collected from Désirée. These data confirm the findings on the 2001 collection of isolates. There was no difference in mean aggressiveness between AFLP groups 3 and 4, whereas AFLP group 5 (A2 isolates) were significantly less aggressive.

(These results are the subject of the paper 'Does selection by resistant hosts trigger local adaptation in plant-pathogen systems?' (J. Montarry, R. Corbière, S. Lesueur, I. Glais and D. Andrivon; Journal of Evolutionary Biology 2005 – in press; doi 10.1111/j.1420-9101.2005.01005.x), and available on-line).

Discussion

The lack of performance of alternated rows of susceptible and resistant cultivars to control late blight was disappointing, since earlier experiments had shown significant reduction of disease progress in mixed plantings in France (Andrivon et al., 2003). However, later experiments in conditions highly conducive to late blight had shown that mixture performance was low under high disease pressure (Pilet, 2003; Garrett et al., 2001), but can be increased when disease pressure is lowered (Garrett et al., 2001; Pilet et al., 2006). Alternating rows therefore appears as a potentially useful component of an integrated late blight control strategy, particularly in environments marginally favorable to late blight (continental climates for instance). It was indeed used as one of the strategies tested in model and link farms with reasonable success, described in Chapter 8: Integrated Systems Approach.

From an epidemiological standpoint, the main mechanism acting in alternated plots is likely to be barrier effects to spore transmission. As shown by Maria Finckh and her group at Kassel University (D) on their work on Intercropping described in Section 3 of this Chapter, this mechanism will act best if the geometry of the plot is orientated relative to the prevailing wind direction. Although this is not always possible for practical reasons (particularly in small fields), it is an important factor to bear in mind when attempting to optimize the benefits from cultivar diversification schemes at the plot scale. While no competition effects between cultivars were observed in these trials in France, the results obtained by Scott Phillips in the UK described in Section 2: Variety mixtures in this Chapter using other cultivars showed that this absence of competition in mixed plantings is not general, but specific to particular cultivar combinations.

The population characterisation of the 2001 and 2002 collections of isolates from one location (Ploudaniel) raised two interesting results:

- **the distribution of aggressiveness was heterogeneous between cultivars**, with the most aggressive isolates predominating on the most susceptible host. This might support the idea of making mixtures of partially resistant cvs only, rather than introducing a susceptible cultivar in the combination.
- **the same pathogen genotypes persisted in the population over consecutive growing seasons**, including the less frequent ones. This finding differs from observations in other climates (e.g. the Netherlands), where the turnover of genotypes is rapid (Drenth *et al*., 1994). This difference is possibly due to the reproductive status of the two populations (asexual in France, largely sexual in the Netherlands), but also possibly to climatic differences between the two locations (milder winters in France possibly allowing a large fraction of the population to survive, as compared to the more rigorous winter conditions in the Netherlands which would eliminate a sizeable fraction of the isolates surviving on infected tubers). This is now being investigated in more detail, by monitoring the survival ability of isolates with different levels of aggressiveness in three different climatic environments.

The data collected provide a strong experimental basis to undertake a modeling approach of population changes in response to selection by resistant cultivars. Such a tool would enable prediction of which isolates will predominate in the pathogen population, depending on their respective fitnesses on susceptible and resistant cultivars and on the proportion of these cultivars in the area considered. A prototype version of this model, based on the simple selective model of Leonard (1977), was developed recently (Montarry et al, 2005), but it accounts only for the selective effects during the epidemic phase. It needs now to be expanded to include other important features, such as local survival and gene flow through short and long-distance transportation of spores and/or infected tubers, for which we still lack appropriate data.

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Figure 4.4- Late blight progress and tuber yield in pure and alternated plots of potatoes under natural epidemics. Blue boxes : cv Bintje, susceptible; orange boxes: cv Désirée, partially resistant; green boxes: cv Naturella, race-specific resistant (R2 resistance gene). Solid parts of the yield bars = marketable yield; striped parts of the yield bars = unmarketable yield (tubers less than 35 mm, diseased tubers, etc…). Data shown are from the Ploudaniel experiment in 2002.

Late blight progress

Tuber yield

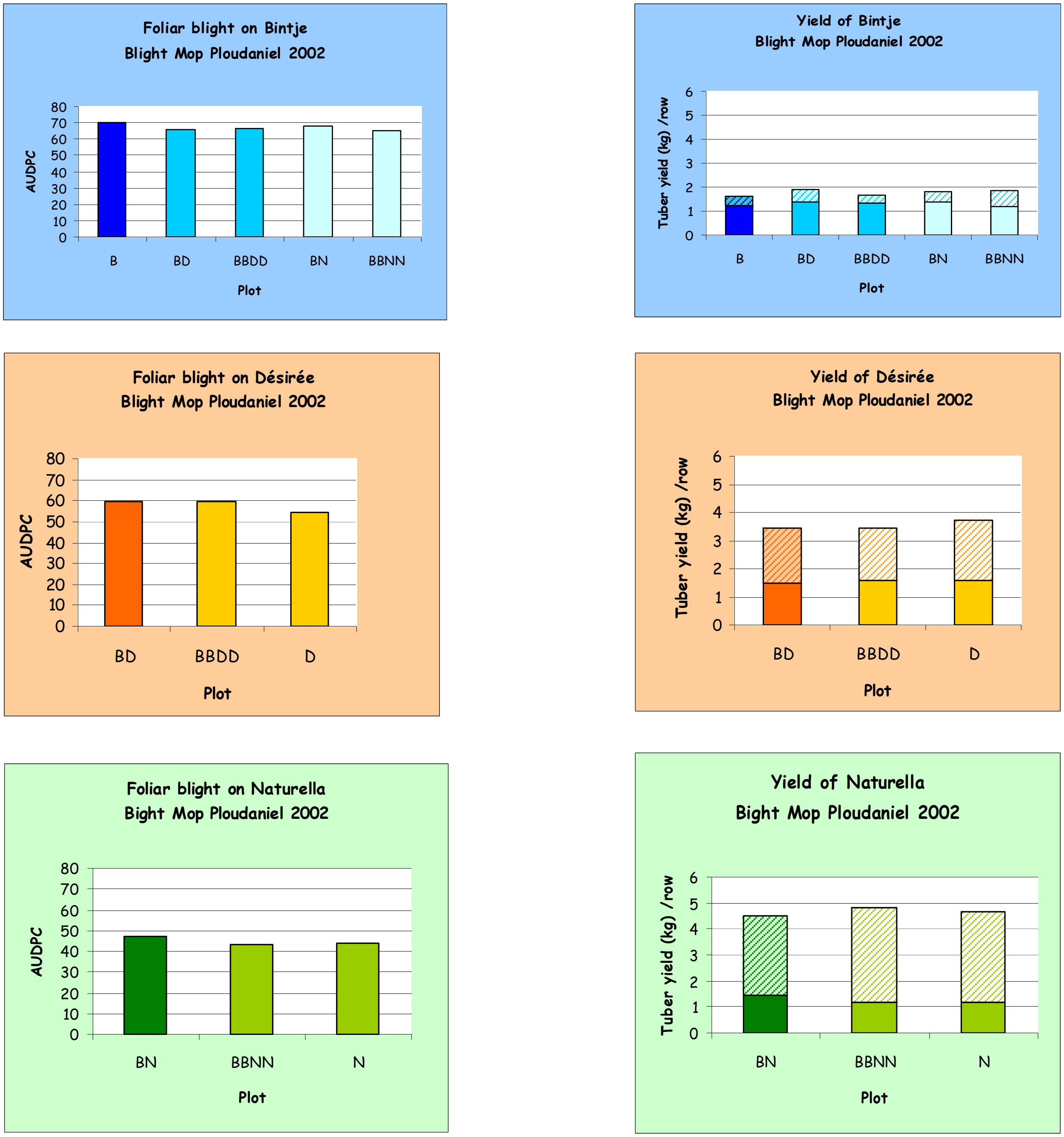


Figure 4.5. Mapping of late blight progress in pure and alternated plots of susceptible (Bintje , B) and resistant (Désrée, D; Naturella, N) potato cultivars at three different stages of the epidemic: beginning '86 days after planting), middle part (96 says after planting) and end (103 days after planting). Disease severity was assessed visually and transformed to a color code (see legend). The small colored squares in each plot represent individual plants. Data are from Le Rheu (Brittany, western France) in 2001. The patterns observed in the other experiments in Le Rheu (2002) and Ploudaniel were similar to those illustrated here.

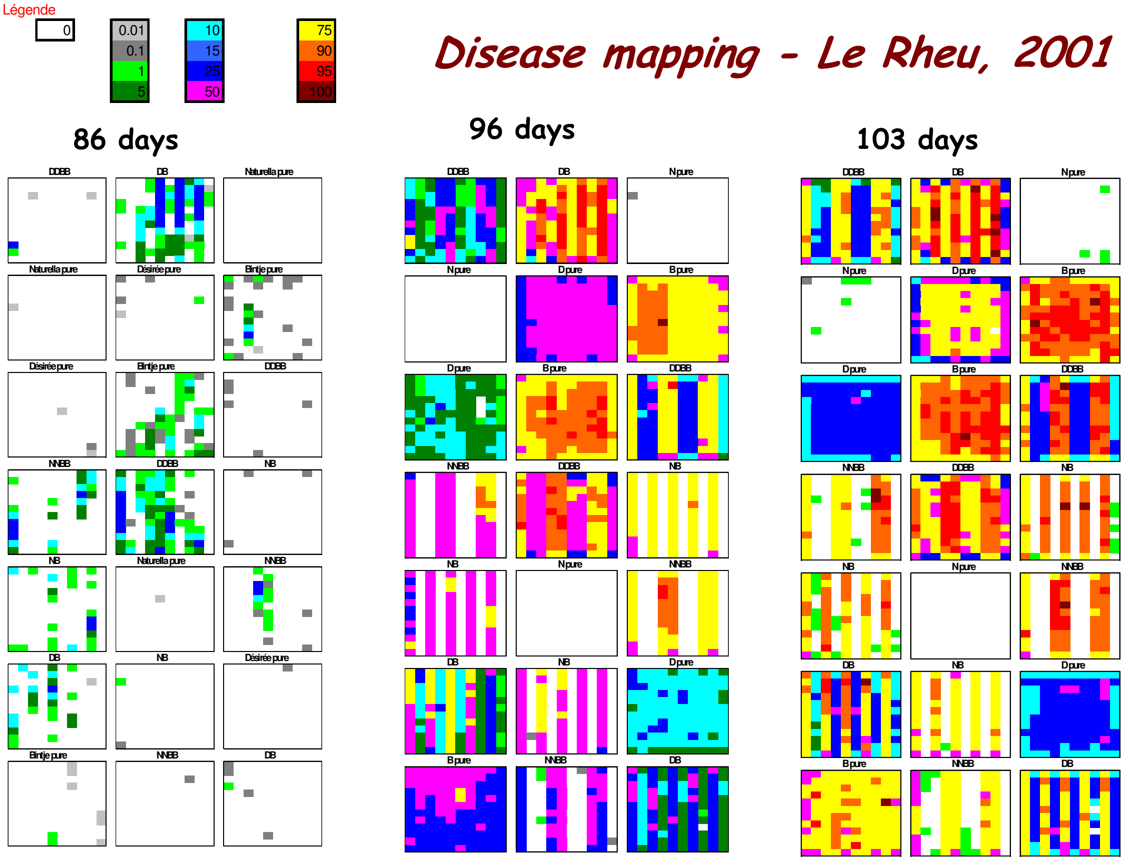


Figure 4.6. Growth of potato cultivars Bintje (B), Désirée (D) and Naturella (N) in pure and alternated plantings in the absence of late blight. Ground coverage was estimated using a 1 m² grid layed on top of the canopy. Growth was recorded from the date when 50 % of the plants had emerged until the first late blight symptoms appeared in the trial. Data are from the 2001 experiment in Le Rheu.

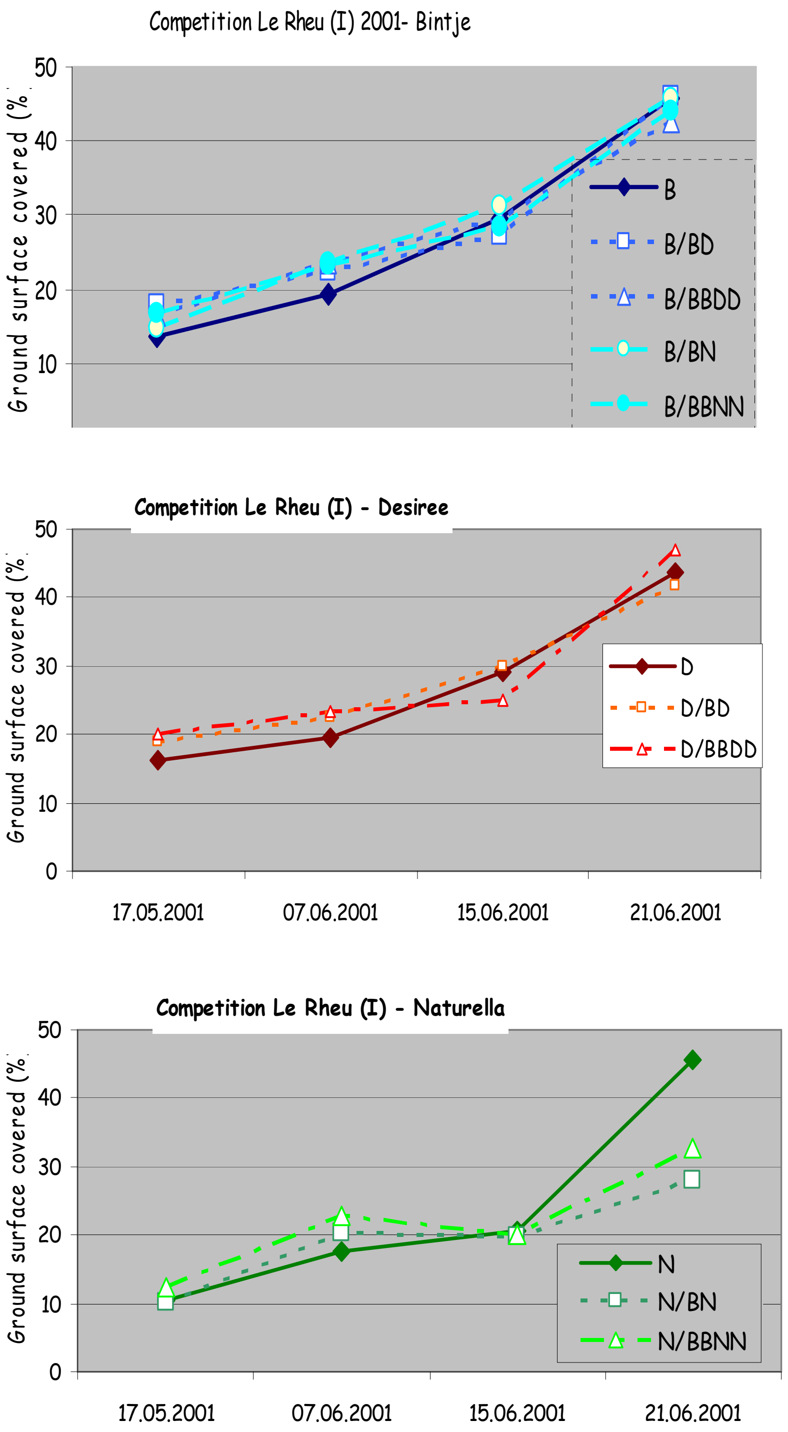
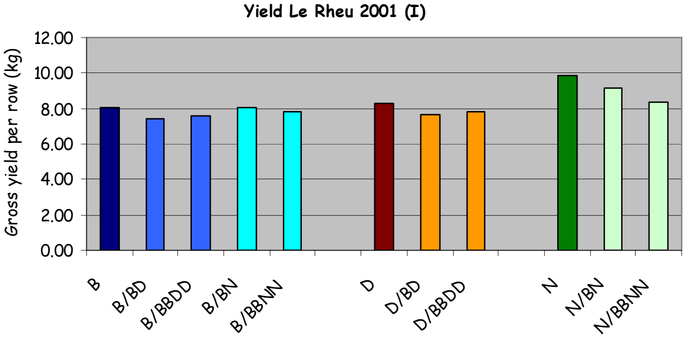
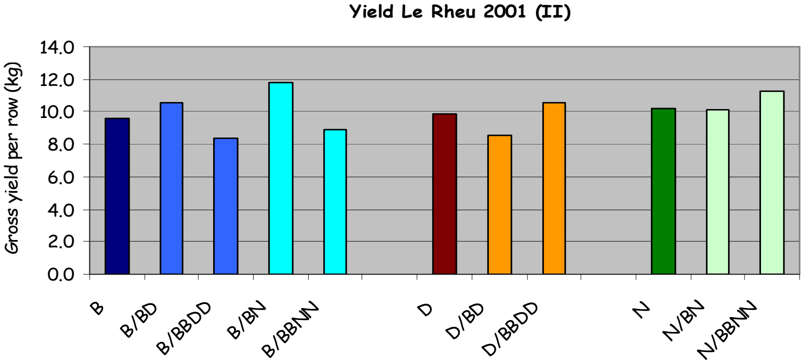


Figure 4.7. Yield of potato cultivars Bintje (B), Désirée (D) and Naturella (N) in pure and alternated plantings exposed to late blight or protected from it by regular fungicide applications. None of the differences between yields for a given cultivar and protection status are statistically significant.



Unprotected plots

Fungicide-protected plots



Section 2: Variety mixtures

Summary

Experiments were made in the United Kingdom (UK) and Germany (D) between 2001 and 2003 to investigate growing mixtures of varieties of potatoes (with varieties mixed within the row, rather than different varieties being gown in alternate rows as described in Section 1 of this Chapter) as a diversification strategy for the management of late blight. In the UK, there were 4 experiments testing effects of variety mixtures and pure stands on blight development and severity. Susceptible or moderately resistant varieties suitable for commercial cropping were infected to similar extents in mixtures and pure stands. In two of three years, infection in variety Sante (moderately susceptible variety) was slightly less when mixed with Cara (moderately resistant), but Cara was unaffected. Mixing a late blight immune/near immune partner (Tominia) with either Cara or Sante reduced disease in these latter two varieties.

However, it seemed that mixtures of varieties did not suppress the disease to an extent which exceeded that provided by the average of the resistance of individual components/varieties within the mixture. Larger plots were more infected than small plots in terms of AUDPC, but the disease progressed more rapidly in small plots. Planting density had no effect on rate or extent of infection.

Variety Cara consistently yielded better in mixtures with different varieties than expected, demonstrating positive combining ability: Appell performed worse than expected, showing negative combining ability. The combining ability of other varieties depended on their companion varieties. Overall, mixtures improved yields by about 5% compared with the yields of individual components (indicating better resource use), but whilst certain varieties showed an improvement, other varieties' yields were depressed. The advantages shown by Cara improved as the number of varieties in the mixture increased whereas Sante yielded progressively less (other varieties showed no response) and for Cara the relative benefits of mixtures over varieties was affected by plant density. It is possible that plant-pathogen interactions in some varieties in some conditions may further improve the performance of variety mixtures above that provided by favourable plant-plant interactions.

Different varieties were used in Germany (D) in 2002 and 2003 – Agria and Simone (white skinned) and Laura and Rosella (red skinned). They were grown in pure stands and four mixtures of a red and white skinned variety either following grass/clover or wheat to study effects of nutrient availability on late blight and plant-plant interactions. Yields were about 18.5t/ha in 2002 and 37.8t/ha in 2003 and in both years, tuber bulking was slower and yields less after wheat, for both pure stands and mixtures, although not significantly so. Blight was severe in 2002 but not in 2003 because of very dry conditions but severity and rate of infection was less (but not significantly) following wheat than grass/clover. Effects of mixtures on disease were small compared with pure stands. The Laura-Simone mixture was most effective (these two varieties represented the upper and lower ends of the susceptibility spectrum), especially under low disease pressure after wheat than grass clover in both years. Whilst mixing varieties did not affect overall yield, the least competitive variety in the mixture produced fewer oversized and more undersized tubers than in pure stand. Competitive interactions between varieties were similar in both years and were unaffected by nutrient availability.

Introduction

UNITED KINGDOM (UK)

1) The effect of potato variety mixtures on epidemics of late blight, in relation to plot size and level of resistance

Summary

Potatoes of a number of varieties of contrasting levels of resistance were planted in pure or mixed stands in four experiments over three years. Three experiments compared the late blight severity and progress in mixtures with that in pure stands. Disease on susceptible or moderately resistant varieties typical of those in commercial use was similar in mixtures and pure stands. In two of three years, there were slight reductions on cv. Sante, which is moderately susceptible, in mixture with cv. Cara, which is moderately resistant. Cara was unaffected by this mixture. Mixtures of an immune or near-immune partner with Cara or Sante substantially reduced disease on the latter. The effect on blight severity of the size of plots of individual varieties or mixtures was compared in two experiments. Larger plots had a greater area under the disease progress curve, but the average rate of disease progress was greater in smaller plots; this may be because most disease progress took place later, under more favourable conditions, in the smaller plots. In one experiment, two planting densities were used. Density had no effect on disease and did not interact with mixture effects. The overall conclusion is that, while mixtures of potato varieties may be desirable for other reasons, they do not offer any improvement on the average of the disease resistance of the components.

Introduction

Large plantings of single genotypes dominate modern cropping systems. Such production systems are convenient, but can favour plant disease epiphytotics. The vulnerability of single genotypes is well illustrated by the late blight epidemics that devastated Irish potato production in the 1840s (Bourke, 1964). These epidemics resulted from the introduction of *Phytophthora infestans* into crops of a susceptible variety (*cv.* Lumper) grown across large areas. Such uniformity contrasts with natural ecosystems in which host genotypes are usually both locally diverse and distributed in small patches (Jarosz and Burdon, 1991); this means that natural ecosystems are usually less prone to rapid and severe epidemics.

The intimate mixing of varieties is a proven method in certain cropping systems of introducing diversity to reduce plant disease (Akem *et al.* 2000, Wolfe, 1985; Zhu *et al.* 2000). Variety mixtures may reduce disease in three basic ways (see Finckh & Wolfe, 1998 and Mundt, 2002): (1) resistance induction, by avirulent spores preventing or delaying infection by adjacent virulent spores; (2) barrier effects, with resistant plants acting as barriers to pathogen spread and (3) dilution of susceptibles, where there is an increased distance between plants of the most susceptible component for any particular pathogen genotype. Garrett & Mundt (1999) suggest five key characteristics of host-pathogen systems that predict host-diversity effects leading to reduced disease: (1) a small genotype unit area (GUA),by which is meant the area of a patch in the crop in which plant tissue has identical genetic composition (2) a shallow dispersal gradient of pathogen spores, (3) small lesion size, (4) short pathogen generation time and (5) strong host specialisation. Hence, there are inherent characteristics of both the host and pathogen that optimise mixture responses. These characteristics are common in small-grained cereals affected by rusts and mildews (Wolfe, 1985). However, potatoes are large broad-leaved plants, *P. infestans* causes large spreading lesions, and most functional potato resistance to *P. infestans* is considered race non-specific. (R-genes are commonly present, but virulence against all known R-genes is common, so they do not determine the observed resistance level of a cultivar).

These are all factors that should limit the usefulness of induced resistance, barrier effects and the dilution of susceptibles in variety mixtures.

Nonetheless, there has been some recent interest in the use of potato variety diversification to reduce late blight. Andrivon *et al.* (2003) found that late blight severity on a susceptible cultivar was significantly lower when the cultivar was grown in rows alternating with partially resistant cultivars than when it was grown alone. They showed that this was the result of cumulative effects over the course of the epidemic. In the USA, Garrett & Mundt (2000) found that disease severity on the susceptibles was reduced by around 37% in intimately mixed plots of 10 susceptible and 26 resistant potato plants. However, in Ecuador, Garrett *et al.* (2001) reported less convincing evidence of the effects of mixing potato varieties against late blight, with a large host-diversity effect for reduced disease only at the site most distant from commercial potato production.

Mundt (2002) points out the importance of spatial scale, in terms of plot size, in determining how mixtures affect disease. He explains that mixture efficacy is greater in production-scale situations than in small-scale experimental plots. One reason for this is that interplot interference is particularly important in variety mixture trials as the effect of reducing the pathogen's infection efficiency through dilution of inoculum can be negated by large amounts of external inoculum. Another reason is that focus expansion is faster in a pure stand than in a mixture, but this only causes a difference if it takes a focus a substantial time to cover a field. The autoinfection/alloinfection ratio is central to mixture performance, as mixtures are unlikely to have significant effects on disease unless there is substantial exchange of inoculum among different host genotypes in the population (Mundt, 2002). Autoinfections are those in which the donor is the same as the recipient and alloinfections are those in which the donor is different from the recipient. The GUA affects this ratio, as alloinfection increases with decreasing GUA so that mixture effects are favoured by smaller GUA (Mundt & Leonard, 1985). This can be further explored by examining the effects of planting density. Host diversity effects would be expected to be greater at higher planting densities because single genotypes, make up smaller areas as density increases and autoinfection should be reduced (Garrett & Mundt, 1999). Therefore, study of potato variety mixture performance should address aspects of spatial scale and the effects of different auto:alloinfection ratios at different planting densities, in different variety combinations. An improved understanding of the effect of potato variety mixtures on late blight is important because such mixtures may have a role to play in a durable 'systems approach' to disease management in low input agricultural systems (Mundt *et al.* 2002). Variety diversification may also help to reduce pesticide input in conventional systems (Andrivon *et al.*, 2003).

Materials and Methods

The site used for the trials is a certified (UK5) organic farm (Grid Ref: TM280790). The soil has a medium texture with approximately 20% clay and a typical pH of 7.5. Composted horse manure at 25 tha^{-1} was incorporated around two weeks before planting, 35 kgha^{-1} of potassium sulphate was incorporated prior to trials in 2002 and all trials followed the leguminous phase of a rotation. The trials were not irrigated and both pre- and post- emergence weeding was achieved with inter-row cultivation and re-ridging. Unless otherwise stated, the planting depth was around 10 cm with a 35 cm plant spacing within rows and 75 cm between rows, similar to common grower practice. Several varieties were used (Table 1), chosen to provide diverse foliage late blight resistance; as the work developed, different subsets of varieties were used in successive years. In particular, the most resistant varieties were not initially available. Most of the varieties are known to possess some R genes, although in all varieties except Appell and Tominia these are routinely overcome by UK populations of *Phytophthora infestans*. Resistance in Appell sometimes resembles the hypersensitive response associated with major gene resistance, so it may contain an R-gene that is functional in some seasons in the UK.

Tominia demonstrates characteristics of both race specific and race non-specific resistance responses (D Shaw, University of Wales, personal communication).
Late blight severity was assessed visually using the key of W. E. Fry http://ppathw3.cals.cornell.edu/Fry/protocols.htm, based on James, 1971). Disease severity was summarised as area under the disease progress curve (AUDPC; Campbell & Madden, 1990) and rate of disease progress, calculated as the slope coefficient of a regression of logit transformed of disease severity against time.

Effect of plot size on disease in variety mixtures

A trial was planted during the last week of April 2001 to fulfil various purposes. That relevant here was to examine the effect of plot size on potato late blight epidemics in varietally pure or mixed crops of potato; other data from the same field are reported in Phillips *et al.* .(2005a). The trial was a split-plot design. Main plots had large (9 m x 7.5 m) or small subplots (4.5 m x 4.5 m). Each subplot contained either a pure variety or an equiproportionate mixture of two to five varieties; a complete set of variety treatments were randomised within each main plot. For logistical reasons only two replicates were possible. Details of the variety mixtures and agronomy used are given in Phillips *et al.* (2005a). Varieties were compatible agronomically and had a range of foliage resistance to late blight. Late blight arrived as a natural infection around 9 August 2001. Severity was assessed visually on 9, 15, 20, 24, 28 August and 1 September.
In 2002, four varieties of potato with diverse foliage resistance levels against late blight (Table 1) were planted in plots of three sizes. Large plots were 5.25 m x 11.5 m, medium plots were 11.5 m x 3 m and small plots were 5.75 m x 1.5 m. The total area of each variety in each replication in small plots (28 plots per replicate), medium plots (8 plots per replicate) and large plots (4 plots per replicate) was approximately 60 m^2. The plots were arranged within and between blocks so that, as far as possible, no variety appeared next to itself. There were three replicates. It was planted on 29 April 2002. Late blight arrived as a natural infection around the 17 July 2002. Late blight was scored on the 17, 23 and 31 July, and 6 and 13 August 2002. The data were analysed using ANOVA in Genstat, one variety at a time.

Effect of planting density on disease in variety mixtures

This split-plot trial was designed to examine the effects on potato late blight of two-way variety mixtures at three planting densities. Planting began on 29 April 2002. Main plots were 10 m long x 7.5 m wide, with at least 1 m gaps, (occupied by oats) between plots. The trial contained six replications; willow trees separated replications across the trial. The main plot factor was planting density, with three levels, achieved by planting at 25 cm, 35 cm and 45 cm spacings within rows. The split-plot treatment factor had three levels: pure stands and a two-way random mixture of the varieties Cara and Sante. Late blight arrived as a natural infection around 13 July 2002. Late blight was scored on the 13th July, 17th July, 23rd July, 30th July and 5th August 2002. AUDPC and rate were analysed in each variety separately using a split-plot ANOVA procedure in Genstat.

The effects of two-way variety mixtures

This trial examined the performance of four maincrop varieties (Cara, Appell, Sante and Tominia) in pure stands and in all two-way mixture permutations (Table 1). Additional treatments were provided by mixing Cara and Sante with Tominia in the ratio 1:3. The trial had five blocks with plots 18.5 m long and 7.5 m wide, with a 1.5 m gap between plots. The varieties were chosen based on results from previous trials, to diversify for late blight resistance, to diversify for growth characteristics and to provide distinct tubers for separation at harvest (Table 4). Late blight arrived as a natural infection and was first scored on 1 August 2003. Plots were scored on 8, 12, 18, 28 August and 3 September 2003.

Results

Effect of plot size on disease in variety mixtures

Preliminary results from the 2001 experiment were summarised in Phillips (2002). There was no significant interaction in AUDPC between plot size and mixture composition. In cv. Verity, the most resistant, the AUDPC increased with the number of components in the mixture ($P < 0.01$), but in other varieties any trend was not significant. Larger plots had about 100 %-days more AUDPC on all varieties, regardless of their resistance level, in all mixtures (Table 2). This is not formally significant, but the power of the test is very low because of the shortage of degrees of freedom for error at the main plot level. There were no significant differences in rates of disease progress related either to plot size or to composition.

In 2002, Figure 1 shows that the GUA again influenced the severity of late blight. In cv Milva, Robinta and Sante late blight was least severe in the small plots and most severe in the large plots. In all varieties there were significant differences ($P< 0.01$) in AUDPC between plot sizes (Table 3). In Milva, Robinta and Sante small plots reached a diseased leaf area of 30% more than six days later than large plots. The more resistant variety, Appell, had little late blight; small plots had non-significantly more disease than medium and large plots. The rate of disease progress in Robinta and Sante differed significantly different between plot sizes, with disease progress faster in smaller plots (Table 4). Averaged over varieties, the rate of disease progress was greater in the small plots than in the large plots.

Effect of planting density on disease in variety mixtures

There were no interactions between the effects of mixing and planting density on either rate or AUDPC in either variety. Planting density did not affect disease in either variety (Fig 2). AUDPC in Cara was similar in pure stands and mixtures, but in Sante there was significantly less disease in mixtures (Fig 2B).

The effects of two-way variety mixtures

In 2001, the AUDPC in pure stands was essentially the same as in two-way mixtures, for all varieties (Table 2). In 2002 Sante had less disease in mixtures than in pure stands (Figure 2), but for Cara there was no difference in disease severity between pure stands and mixtures.

In 2003, there was negligible disease on Appell and Tominia, but late blight reached 100% in some plots containing Sante and 30% on some plots containing Cara. There were significant differences in disease severity on both Cara and Sante according to whether or not they were grown in pure stands, in mixtures with Appell, in mixtures with Tominia or in 1:3 mixtures with Tominia (Table 5). Both Cara and Sante plants had more disease in pure stands than in mixtures with the resistant varieties Appell and Tominia. Compared to pure stands, Sante had 49% higher disease severity when mixed with Cara and 53% lower disease severity when mixed with Appell. The best performing Cara mixture was with Appell; this mixture provided an 82% reduction in disease severity on Cara.

Discussion

In the present study, in both 2001 and 2002, the severity of late blight on potatoes was less in small plots than in larger plots. In modelling the effects of plot size on the apparent infection rate of late blight, Paysour & Fry (1983) found that late blight severity increased with plot size towards an asymptote. Lipps & Madden (1992) found that small plots of winter wheat had lower levels of powdery mildew *Blumeria graminis* than large plots, and that the dispersal gradient of *B. graminis* is similar to *P. infestans*. Similar effects of plot size have also been described for *Mycosphaerella graminicola* on wheat and *Pyrenophora teres* on barley (Burleigh & Loubane, 1984).

These effects occur because the proportion of inoculum lost from plots decreases with increasing plot size and because the probability of an initial focus forming from external inoculum is less in any given smaller plots. In the 2002 trial the small plots occupied the same overall area as the medium and large plots, so the total probability of infection was the same regardless of plot size (assuming the inoculum arrived at random). However, in a group of small plots, resistant plots may have slowed the spread of spores into less infected susceptible plots. The effectiveness of this depends on the level of the resistance in that genotype; on resistant genotypes more spores will be received than otherwise, and in fact, there was a small but significant increase in disease severity on Appell in the small plots, presumably because of this (Table 3).

Although AUDPC was lower in the smaller plots in 2002, the rate of disease progress was actually higher. Interplot interference and the delayed epidemic on the smaller plots explains this. As the epidemic was up to a week more advanced in the large plots, the spores distributed from these plots would have increased disease progress on the smaller plots during the middle of the general epidemic. This effect would increase the apparent rate of disease progress on the smaller plots, as observed.

This leads to the conclusion that if diversity is available for plant resistance against late blight, then disease severity will be reduced if any given mixture or variety is grown in more, smaller, plots rather than in fewer, larger, plots. This would especially be the case for simple mixtures of an immune and a susceptible variety or when the non-potato area is occupied by immune material. Fleming *et al.* (1982) also suggested that the perimeter to area ratio of a field is roughly proportional to the rate of losses of pathogen spores from that field. Since the perimeter to area ratio increases as area decreases for fields of a given shape, small fields may sustain less disease than large fields.

In the UK setting, it is sensible to assume that race-specific variation in the pathogen is unimportant because matching virulences to all combinations of deployed R-genes are common. Then reducing the GUA will have little effect if external inoculum is common because the advantage to the susceptible variety will be offset by disadvantages to the more resistant variety (also see Connolly *et al*., 1995). It is not clear whether there is a proportional response to reducing GUA in mixtures incorporating rate-reducing resistance. However, the results discussed above indicate that the benefits of improving the autoinfection:alloinfection ratio by reducing the GUA are heavily dependent on the resistance level and resistance type of the more resistant genotypes. It is possible that the performance of some mixtures will be better at larger GUAs, as the negative impact of spores on the more resistant variety will be less. This implies that there is an optimal GUA, dependent on the level and type of resistance being diversified and the dispersal characteristics of the pathogen. If race-specific variation is important in the mixture effect, and the pathogen is a mixture of simple races, then the usual arguments imply that the most intimate mixture will give the greatest effect.

Table 5 illustrates the importance of resistance levels in the performance of variety diversification to prevent late blight. Appell and Tominia sustained negligible late blight in 2003. In the case of Appell this is likely to be due to a useful major gene, and may therefore be unstable. In the case of Tominia, one of the Hungarian Sarpo family of potatoes, there is a high level of race non-specific resistance (D. Shaw, University of Wales, personal communication). The importance of resistance levels is illustrated in the effect of mixing Cara with Sante. Figure 2 and Table 5 show that Cara can restrict disease progress on Sante in variety mixtures but the 2001 experiment shows that the effect is not reliable. This is probably because Cara itself is not immune and does not ordinarily have useful major genes in the UK. Also, Sante is a small, determinate variety whereas Cara is a tall, spreading plant; this can create a damper microclimate in a mixed canopy than in a pure Sante crop, and encourage disease instead of restricting it. This probably happened in 2003, when the late blight epidemic was slowed by dry conditions during August. This is also likely to be the reason that Sante/Appell mixtures restricted late blight more than Sante/Tominia mixtures: Tominia foliage resembles Cara foliage and Appell foliage resembles Sante foliage. In 2003 mixtures of Cara and Sante with Appell had substantially less disease than mixtures with Tominia, although both varieties were nearly immune and had similar epidemiological effects in spacing the Cara and Sante plants and acting as 'immune' barriers to spore dispersal.

As discussed above, Table 5 shows that the relative resistance ratios of potato varieties are crucial to the performance of variety mixtures. Non-race-specific late blight resistance is genetically linked to late maturity (Thurston, 1971). This is an agronomic problem as the varieties to be mixed should be agronomically compatible, especially in maturity class. However, the largest epidemiological effects of diversification are in simple mixtures of an immune and susceptible, or where one or other of the varieties has functioning major gene resistance. Race specific resistance is not linked to maturity class. Therefore, potato variety mixtures to prevent late blight epidemics should diversify race non-specific resistance as far as possible and also diversify for major gene resistances for which matching virulence is rare. Garrett & Mundt (2000) also arrive at this conclusion, stating that, even if a sizeable proportion of the pathogen population has genes for overcoming the particular forms of qualitative resistance, deployment of genes for qualitative resistance in mixtures may still partition the pathogen population so that overall disease levels are reduced.

As Garrett & Mundt (2000) point out, host-diversity effect might be expected to be greater at higher planting densities because single plants, and thus genotypes, occupy smaller areas as density increases, which will reduce autoinfection. If an interaction between planting density and mixture effects could be identified, this would further highlight the function of the dilution of susceptibles and barrier effects in the prevention of disease. In Figure 2 there was no evidence for an effect of planting density on late blight severity nor any interaction between planting density and mixing. This confirms the results of Garrett *et al.* (2001) who report that there was no evidence for an influence of planting density on host-diversity effects for potato late blight. It seems that the mechanisms operating in the prevention of late blight by variety mixtures cannot be augmented by adjusting planting density within agriculturally relevant limits.

Garrett & Mundt (2000) reported consistent reductions in late blight severity under both focal and general patterns of primary inoculum, in mixtures of a highly resistant variety with a susceptible variety. Field trials of potato mixtures near Quito, Ecuador, did not reflect these results (Garrett *et al.*, 2001), possibly because external inoculum swamped mixture effects within plots. However, Garrett *et al.* (2001) used small plots and none of the varieties used had a level of resistance equivalent to the more resistant variety reported in Garrett & Mundt (2000) or of Appell and Tominia in the present study. All these results suggest that the epidemiological effects of potato variety mixtures are based mainly on replacing susceptible plants with resistant ones, so such mixtures will work best in large plots and with the best resistance available.

The use of variety mixtures to prevent plant diseases is an ecological approach to the problem of crop diseases. Potato mixtures exhibit important plant-plant interactions as well as plant-pathogen interactions, which may be thought of as resulting in a typical “ecological combining ability” for each variety (Phillips *et al.*, 2005a). Furthermore, Phillips *et al.* (2005b) showed that cultivars with positive general ecological combining ability are characterised by very strong intracultivar competition in pure stands. Yield improvements of between 5 and 10% have been routinely recorded in potato variety mixtures (Phillips *et al.*, 2005b and M. Finckh, personal communication). Therefore potato variety mixtures should be designed to improve yield of more resistant varieties by reducing the negative effects of intracultivar competition and to improve the yield of more susceptible varieties by reducing disease through mixture with a resistant variety. Diversification in this way could reduce the inputs needed to grow potatoes below those typical of large-scale monocultures.

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Table 1 The characteristics of varieties used

Variety	Growth Habit	Foliage Late Blight Resistance	Tuber Characteristics	Used in		
Verity	Very erect	High	White with pink spots	2001		
Nicola	Semi-erect	Low	White, kidney-shaped	2001		
Robinta	Semi-erect	Medium	Red, round	2001	2002	
Cara	Very erect	High	White with pink spots	2001	2002	2003
Sante	Spreading to semi-erect	Low to medium	White, round	2001	2002	2003
Appell	Spreading to semi-erect	High	White, round		2002	2003
Milva	Spreading to semi-erect	Low to medium	White, round		2002	
Tomina	Very erect	Very high	Purple, oval			2003

Table 2. Averages of Area Under Disease Progress Curves (%-day) of late blight on each variety growing in pure stands and two-component mixtures in plots of two different sizes in 2001.

	Plot type			Plot size[a]	
Variety	Pure[b]	Mix	Partners in mixture plots	68 m^2[c]	20 m^2
Cara	659	648	All	707	594
Sante	869	878	Not Robinta	892	860
Verity	767	693	All	785	650
Robinta	1300	1172	Cara, Verity	1233	1176
Nicola	450	480	Not Robinta	501	446

[a] Main effects of mixture and size only are shown; interactions were not significant

[b] For comparisons between pure varieties or mixtures, SED 65, 64 df

[c] For comparisons between plot sizes, SED 99, 10 df

Table 3. Area Under Disease Progress Curves (%-day) of late blight on four varieties growing in pure stands of three different plot sizes in 2002.

Variety	**63 m^2 plots**	**34 m^2 Plots**	**9 m^2 Plots**	**SED**[a]
Robinta	1448	1109	868	112, P<0.001
Sante	1753	1462	1226	168, P<0.01
Milva	1712	1316	1042	121, P<0.001
Appell	20	14	48	15, P<0.01
Mean	869	841	713	

[a] All single variety SED have 25 df

Table 4. Analysis of the logistic disease progress rates for potato late blight on four varieties at three different plot sizes in 2002. There were significant differences between plot sizes for Robinta, Sante and for the mean of all the varieties.

Variety	**63 m^2 plots**	**34 m^2 Plots**	**9 m^2 Plots**	**SED**[a]
Robinta	0.24	0.36	0.43	0.039, P<0.01
Sante	0.28	0.38	0.39	0.034, P<0.05
Milva	0.43	0.47	0.50	N.S
Appell	0.28	0.54	0.56	N.S.
Mean	0.31	0.44	0.47	0.044, P<0.01 (115 df)

[a] SED for single varieties have 25 df, for the mean 115 df

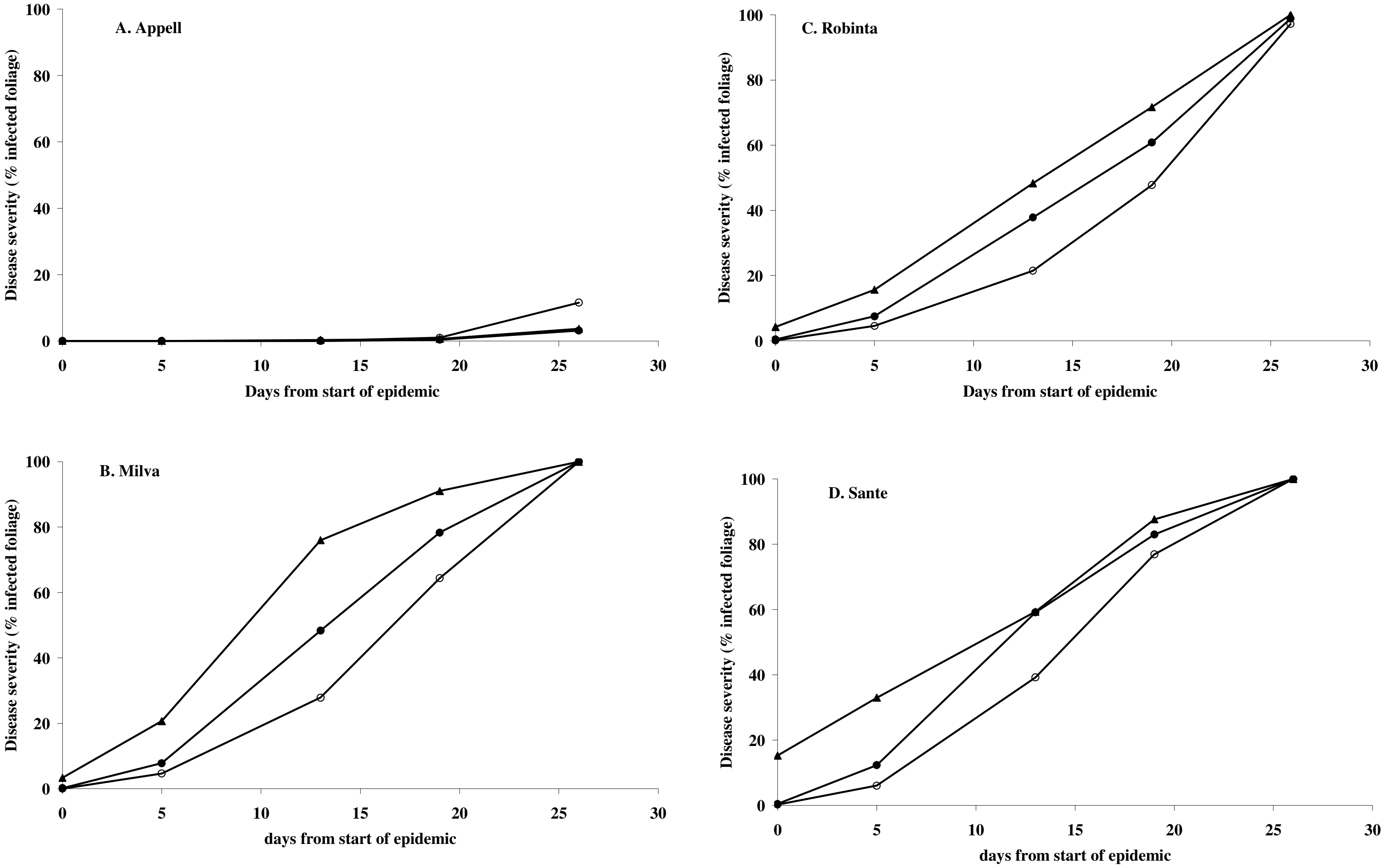
Table 5. Mean Area Under the Disease Progress Curve (AUDPC; %-day) of cv Cara and Sante in mixtures with other varieties.

		Variety A	
Variety B	**Proportion**	**Cara**[a]	**Sante**[b]
Cara	0.5	325	1683
Sante	0.5	333	1129
Appell	0.5	59	531
Tominia	0.5	142	1046
75% Tominia	0.75	66	862
SED, 16 df		76.9	282

[a] Differences between mixture partners, 4,16 df, P <0.01

[b] Differences between mixture partners, 4,16 df P <0.05

Figure 1 Disease epidemic curves for potato late blight on cvs Robinta, Sante, Milva and Appell grown in single variety plots of varying size in 2002. ○ 9 m^2 plots; ● 34 m^2 plots; ▲ 63 m^2 plots.



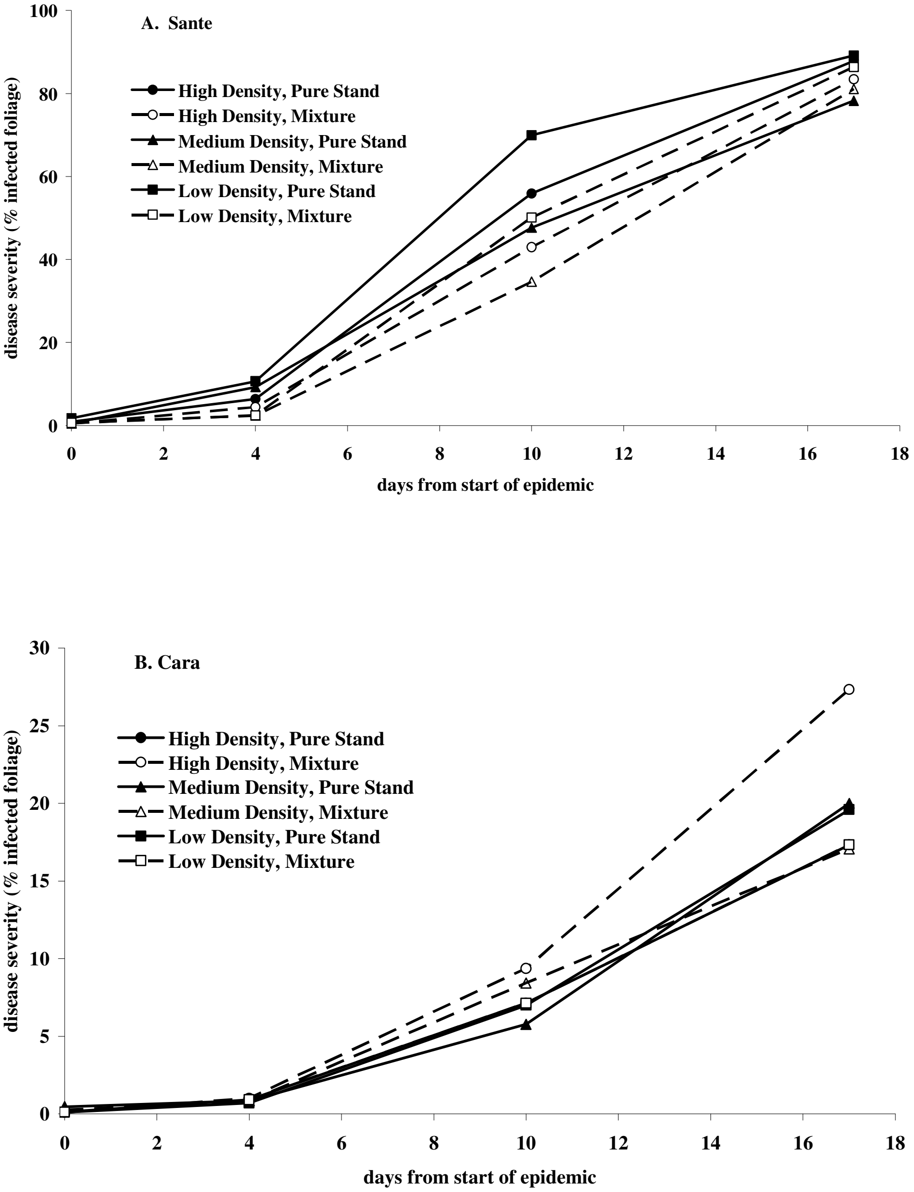


Figure 2 Potato late blight progress curves in 2002 on A. Cara and B. Sante in pure stands and mixtures with Sante or Cara at three planting densities.

2) The ecological combining abilities of potato varieties in variety mixures

Summary

In trials conducted over three years, potato cv. Cara consistently yielded better in mixtures with any of five other cultivars than expected from its representation in the mixture. This quality is referred to as positive combining ability. Cv. Appell consistently yielded worse than expected, so it had negative combining ability. Yields of other varieties depended on the partners in the mixture. Overall, variety mixtures consistently improved yields by around 5% above the mean of the components grown in pure stands. Improved yields were due to the advantages of the mixture for some varieties outweighing the disadvantages for others. There was no evidence for synergistic interactions between varieties in which all varieties in a mixture yielded more. However, it is possible that plant-pathogen interactions may improve the yield responses in potato variety mixtures beyond those expected from plant-plant interactions alone.

Introduction

A heterogeneous crop of a single species, achieved by intimately mixing varieties, has been suggested as a way to introduce functional biodiversity into cropping systems (Finckh & Wolfe, 1998). The creation of a heterogeneous crop can offer advantages over monocultures by restricting disease, (see Mundt 2002), improving pest control (see Altieri, 1999), increasing competition with weeds and stabilising yields (Finckh *et al.* 2000). Therefore, variety mixtures are well suited to low input production systems that do not rely on synthetic inputs to control weeds, pests and diseases and to provide high levels of fertility.

Potato variety mixtures have been studied for the prevention of late blight, caused by *Phytophthora infestans*. Garrett & Mundt (2000) and Andrivon, *et al.* (2003) showed that a heterogeneous crop of potatoes could delay and slow down late blight epidemics and significantly increase yields in comparison to the components in pure stands (increases up to 40% were recorded). The focus of these studies was the prevention of disease, or plant-pathogen interactions but yield responses in potato variety mixtures are also likely to involve plant-plant interactions (see Phillips, 2005a). It is therefore important to understand plant-plant interactions in potato variety mixtures when making decisions as to which varieties to grow together to maximise yield responses.

Positive interactions between varieties can be due to many biotic and abiotic factors; with the competition for resources and the prevention of diseases being two of the more important. Furthermore, Finckh & Mundt (1992) showed that competition for resources and the prevention of disease themselves interact to affect the performance of variety mixtures. So interactions between varieties in mixtures are complex and there is a need to discover variety combinations that ‘nick’ together well and to have some understanding of the mechanistic nature of positive interactions (see Phillips, 2005a). These understandings should inform decisions about which varieties to grow together and the expectations from mixing particular varieties under different conditions.

Knott & Mundt (1990) used mixing ability analyses, adapted from combining ability analyses used in plant breeding, to investigate general and specific combining abilities of wheat varieties. To calculate combining ability, each variety is grown in combination with every other variety in a diallel arrangement. The general combining ability is therefore calculated as the average performance of a cultivar in a mixture. Specific competing ability is an indication of how well certain combinations perform. The determination of the performance of potato varieties in variety mixtures is easier than in cereals in that the components can be easily separated if diversified for tuber characteristics.

The ecological combining ability of potatoes in mixtures is a combination of the yield of the varieties grown in monoculture, their relative competitive abilities, and the effects of diversification on weeds, pests and diseases. Mixing ability analyses across a number of environments should illuminate these complex interactions and their stability. Furthermore, mixing ability analyses help to highlight other aspects of ecological combining ability which extend to general agronomic compatibility, ease of separation at harvest (if required) and the effects of diversification on many variety specific maladies.

Materials and methods

The site used for the trials was a certified (UK5) organic farm (Grid Ref: TM280790). The soil has a medium texture with approximately 20% clay and a typical pH of 7.5. Composted horse manure at 25tha^{-1} was incorporated around two weeks before planting, 35kgha^{-1} of potassium sulphate was incorporated prior to trials in 2002 and all trials followed the leguminous phase of a rotation. The trials were not irrigated and both pre- and post- emergence weeding was achieved with inter-row cultivation and re-ridging.

Combining abilities in relation to number of components in the mixture

A trial was planted during the last two weeks of April 2001 to examine the effects of increasing the number of components in potato variety mixtures on the performance of individual varieties. The planting depth was 100mm with a 320mm plant spacing within rows; spacing between rows (ridges) was 500mm. Five cultivars were planted: Cara, Verity, Sante, Robinta and Nicola. These varieties were chosen as they are compatible agronomically and have diverse resistance to late blight and tuber characteristics which allow separation at harvest (see Table 1). Late blight arrived as a natural infection, first recorded on 9 August 2001.

The trial consisted of four blocks, two with large plots (9mx7.5m) and two with small plots (4.5mx4.5m). All treatments were randomized within blocks. The treatments were a subset of the permutations of the five varieties (see Table 2). Mixtures had equal numbers of tubers of each component and the seed tubers were planted randomly. All tubers from the centre two ridges of each plot were harvested and sorted into varieties; gross yield of each variety was measured within each plot.

The tuber yield per plant of each variety was analysed by ANOVA. Linear contrasts were used to ask: what is the performance of Variety A when in mixtures that contain Variety B? Regression was used to see if, for each variety, the number of components in variety mixtures altered tuber yield. Analysis used Genstat v4.2 (VSN, Oxford, UK)

Combining abilities of varieties in two-way mixture combinations

A trial was planted on 1 April 2002, designed to answer the questions: (1) are there particular varieties that perform well in mixtures? and (2) are there particular two-way variety combinations that perform well as mixtures? Varieties were chosen based on results from 2001, to diversify resistance levels, to diversify growth characteristics and to provide distinct tubers for separation at harvest (Table 1). The potato varieties Cara, Cosmos, Nicola, Robinta, Romano and Sante were divided into two maturity classes. Within each class, plots were planted with pure stands or each of the possible combinations of varieties (Table 3). The trial contained four blocks. Plots were 4.5m long and 7.5m wide with a 0.7m gap between them.

The planting depth was around 100mm with a 320mm plant spacing within ridges and 500mm between.. Late blight arrived as a natural infection and was first recorded on 13 July 2002. All tubers were harvested; the tubers from mixtures were separated into their two constituents and the gross yield for each variety recorded.

In 2003 a trial was designed to examine the performance of four maincrop varieties in pure stands and in all two-way mixture permutations. The four varieties were Cara, Appell, Sante and Tominia. These varieties were chosen based on results from previous trials, to diversify for late blight resistance, to diversify for growth characteristics and to provide distinct tubers for separation at harvest (Table 1). The trial had five blocks with plots that were 18.5m long and 7.5m wide with a 1.5m gap between plots. The planting depth was around 100mm with a 320m plant spacing within ridges and 500mm between. Late blight arrived as a natural infection and was first recorded on 1 August 2003. Tubers from mixture plots were separated into their two constituents and gross yield measured.

Tuber yields of each variety were analysed separately In order to understand the general combining ability of varieties ANOVA was used to examine the difference between variety yields in pure stands and in all two-way mixtures.

Results

Combining abilities in relation to number of components in the mixture

An indication of specific combining abilities of varieties when mixed together was obtained from the data from 2001 by examining the performance of a variety in mixtures that contained one of the other varieties. That is, asking the question, "what is the performance of variety *A* when in mixtures that contain variety *B*?". Table 4 shows that the responses of Cara and Sante to mixing with other varieties were general and not due to specific interactions between varieties. Cara generally responded positively and statistically significantly to all other varieties with around a 20% increase in yield. Sante responded negatively and statistically significantly to most of the other varieties with a decrease in yield between 15% and 30%. Neither Verity nor Robinta responded significantly to mixing with other varieties . The response of Nicola was unclear but there seemed to be a tendency towards negative general combining ability with other varieties, significantly so with Cara and Sante.

Combining abilities of varieties in two-way mixture combinations in 2002

The experiments in 2002 confirmed the results from 2001 (Table 5), with Cara plants yielding 18% more in mixed stands than in pure stands and none of the other varieties responding significantly to general mixing. There was weak evidence that Romano responded negatively and Cosmos positively to mixing with the other early varieties. Cara yielded more in combination with Robinta (20%) and Nicola (28%) than with Sante (7%)(Table 6), so the positive general combining response of Cara was nonetheless characterised by significantly better performance with some varieties than with others. There was limited evidence for specific mixture interactions in the other varieties (Tables 8 and 9).

In 2003 the general combining ability of Cara in two-way mixtures was again positive, with Cara plants yielding over 25% more in mixtures than in pure stands (Table 8). The only other variety with a significant overall response to mixing was Appell, yielding 10% less in mixtures than in pure stands. Other varieties showed a diversity of response depending on the mixture partner. .For instance, the increased yield of Sante plants in mixtures with Appell plants was significantly different from their decreased yield in mixtures with Cara and Tominia. Tominia also yielded significantly differently when mixed with different varieties; the relative response of Tominia plants was negative with Cara but positive with Sante and Appell.

Discussion

Wolfe (1987) summarised yield data for a large number of field trials in England over 11 years comparing the yields of different mixtures of three varieties of barley with their components grown as pure stands. He found a 7.9% increase in yield in mixtures. Yield increases of around 5% for variety mixtures have been reported in many different species (see Simmonds, 1962). In the data reported here the mean performance of two-way mixtures of Cara and Sante over 3 trials and 3 years was an increase in yield of 4% above the mean performance of the components in pure stands. As such, the performance of the Cara/Sante potato variety mixture is comparable with the performance of variety mixtures in other species. Increases in yield in potato variety mixtures have also been reported elsewhere (Garrett & Mundt, 2000 and Doney *et al.*, 1965).

Certain potato varieties, in common with other species (e.g. Knott & Mundt, 1990, Rao & Prasad, 1984, Early & Qualset, 1971 and Brim & Schutz, 1968) demonstrate statistically significant general combining abilities. Gizlice *et al.* (1989) working with soybean (in which they were able to separate the components) found that some genotypes consistently enhance blend performance regardless of the partner involved. Amongst the potato varieties under study here, the variety Cara consistently demonstrated positive general combining ability with other varieties, whereas the variety Sante consistently demonstrated negative combining abilities with other varieties. However, the concept of combining ability is misleading because it suggests that mixture performance is entirely the product of cultivar interactions in the mixtures. In actuality, mixture responses are only ever relative to intra-cultivar competitive interactions in pure stands, so the concept of ecological combining ability actually refers to the relative importance of intraspecific and interspecific combining ability (Phillips *et al.* 2005a).

As with soybean (Gizlice, *et al.* 1989), the majority of changes in variety yields in potato mixtures seem to be attributable to general combining ability. We hypothesise that positive general combining ability in potatoes is due in large part to reducing strong intracultivar competition in pure stands (Phillips *et al.* 2005a), rather than a particular ability to complement other varieties. On the other hand, potato varieties with negative combining ability are generally weaker competitors. It seems from our sample of varieties that most potato varieties do not demonstrate either positive or negative combining abilities in most mixtures. Therefore, the yields of most potato varieties are not likely to be consistently increased in mixtures. The 4% mean increase in yield of Cara and Sante mixtures occurred because the advantage to Cara of mixing with Sante was of greater overall value than the loss to Sante. Brim & Schutz (1968) also reported this effect in soybean. Phillips *et al.* (2005a) suggest that when resources are limiting productivity, Cara plants generally respond well to reductions in density and hence to intraspecific competition.

Part of the competitiveness of Cara, Verity and Tominia can be attributed to their greater height and foliar vigour (Table 1); similarly, the spreading, low-growing varieties Sante, Appell and Romano all tended to do poorly in mixtures, especially with the tallest varieties. However, this is only a partial explanation: Nicola performed badly in mixtures, but has a semi-erect habit. Thus, in looking for partners likely to give synergistic interactions, it may be best to choose among varieties of similar growth habit.

The majority of work on variety mixtures has focused on disease prevention. Garrett & Mundt (2000), Garrett *et al.* (2001) and Andrivon *et al.* (2003) investigated the influence of potato variety diversification on potato late blight, concluding that under certain conditions diversification can help slow down late blight epidemics (also see Phillips *et al.* 2005b). Finckh & Mundt (1992) point out that disease interacts with competitive ability in variety mixtures. This interaction may be important in potato variety mixtures, as, in mixtures of Cara and Sante, disease can be restricted on Sante (Phillips *et al.,* 2005b). This may compensate for the poorer inherent competitiveness of Sante. Heterogeneity for disease resistance can influence the outcome of competition in mixtures in many ways dependent upon the many combinations of intercultivar competition and disease resistance characteristics, both of which also interact with environmental conditions. Environmental interactions complicate assessment of the use of mixtures because the type, timing and severity of disease (for example) in low input systems are impossible to predict.

Phillips *et al.* (2005b) show that potato variety mixtures can reduce late blight on the more susceptible component in a two-way mixture. They also show that this effect is dependent on "epidemiological" combining ability, particularly in terms of diversity in resistance levels. In the 2003 trial reported here, late blight was restricted on Cara and Sante, the more susceptible varieties, when mixed with Appell and Tominia, both of which remained disease free (Phillips *et al.* 2005b). The reduction in late blight in these mixtures may have contributed to the positive yield responses of both varieties when mixed with Appell (Sante had a 20% increase, with an overall mixture effect of 4% Cara had a 30% increase, with an overall mixture effect of 3%).

In the trials reported here no one potato variety mixture significantly improved yields in both varieties above their mean performance in pure stands. Given this restriction, the theoretical ideal for a potato variety mixture is that the mixture would contain one variety in which intraspecific competition restricts yield in pure stands at normal planting density, and should offer heterogeneity in disease resistance (in the sense described by Garrett & Mundt 1999). There may also need to be diversification for tuber skin types to allow separation at harvest.

Low input systems, for example organic systems, heavily restrict the use of synthetic inputs to prevent disease and to provide fertility (Lampkin, 1998). Therefore, a variety mixture that reduces competition for scarce and valuable resources that limit the yield of a variety in pure stands, coupled to the prevention of an airborne fungal disease that cannot be controlled by inputs, is a sensible strategy for such systems to adopt. Hence, heterogeneity, in particular variety mixtures, may be a more appropriate approach in low input systems than in conventional industrialised production systems, where resources are less scarce and diseases are more easily controlled. This is further underlined if we recognise that the concept of ecological combining ability also extends to the insurance offered by heterogeneity against the many hundreds of potato maladies (fungal, bacterial or physiological disorders) that are affected by variety characteristics. The concept of ecological combining ability can also be extended beyond harvest to the marketing of the mixture, because varieties that are diverse for tuber characteristics are important for successful varietal separation and tubers that have similar quality characteristics are important in variety mixtures that do not need to be separated. It is interesting to note that the emphasis on local marketing in low input systems makes potato variety mixtures more appropriate in both economic and biological terms in these systems than in industrialised conventional systems.

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Table 1 The characteristics of potato varieties used in the 2001 trial to examine the effects of increasing the number of components in variety mixtures (Scottish Agricultural Science Agency, 2003)

Variety	**Growth Habit**	**Foliage Late Blight Resistance**	**Tuber Characteristics**	**Maturity class**	**Used in trials in**
Cara	Very erect	High	White with pink spots	Maincrop	All years
Nicola	Semi-erect	Low	White, kidney-shaped	Second early	2001,2002
Robinta	Semi-erect	Medium	Red, round	Maincrop	2001,2002
Sante	Spreading to semi-erect	Low to medium	White, round	Maincrop	All years
Verity	Very erect	High	White with pink spots	Maincrop	2001
Appell	Spreading to semi-erect	Very high	White, round	Maincrop	2003
Tominia	Very erect	Very high	Purple, oval	Maincrop	2003
Romano	Spreading to semi-erect	Medium	Red	Second early	2002
Cosmos	Semi-erect	Medium	White, oval	Second early	2002

Table 2 Varieties and mixtures planted in 2001, with the abbreviations used

Pure Stands	Two component mixtures	Three component mixtures	Four component mixtures
Cara (Ca)	CaNi	CaVeNi	CaVeNiRo
Nicola (Ni)	CaRo	CaVeRo	CaVeSaRo
Robinta (Ro)	CaSa	CaVeSa	CaVeNiSa
Sante (Sa)	CaVe	CaNiSa	
Verity (Ve)	NiSa	VeNiSa	
	VeNi		
	VeSa		
	VeRo		

Table 3 Varieties, maturity classes and mixtures planted in 2002.

Maturity Class	Varieties (pure stands)	Mixture Treatments
Second Early	Cosmos (Co[a]), Romano (Ro), Nicola (Ni)	CoRo, CoNi, RoNi
Maincrop	Cara (Ca), Sante (Sa), Nicola (Ni), Robinta (Rob)	CaSa, CaNi, CaRob, SaNi, SaRob, NiRob

[a] Variety abbreviations used to refer to mixture components

Table 4. Mean yields per plant in pure stands of each variety in 2001 and percentage differences from these in mixtures containing each other variety.

	yield per plant (g)				
Grown:	Cara	Sante	Verity	Nicola	Robinta
In monoculture	**927** (16.8)	**798** (21.1)	**786** (18.8)	**657** (21.3)	**775** (22.3)
In mixture with:					
Cara		-18**	1	-19*	-9
Sante	22**		14	-17*	0
Verity	23**	-16*		-15	-3
Nicola	21**	-11	11		-5
Robinta	21**	-30***	9	-11	
Mean Mixture Effect	21.5	-18.7	8.7	-15.4	-4.1

*P<0.05

**P<0.01

***P<0.001

Table 5. Tuber yield (gplant^{-1}) of varieties in pure stands and two-way mixtures in 2002

	Cara	Sante	Robinta	Nicola	Cosmos	Romano
Pure stands	973	980	794	787	720	710
Two-way mixtures	1151	1031	798	796	821	627
P value	<0.05	0.40	0.95	0.90	0.10	0.06
Test statistic, t	2.35,	0.93,	0.06,	0.19,	1.69,	-2.11
df	14	14	14	22	10	10

Table 6. The percentage difference between tuber yields in pure stands of each maincrop variety and in mixtures with each other variety in 2002.

		yield per plant (g)			
	Grown	Cara	Sante	Robinta	Nicola
Yield per plant (g)	in monoculture	1106	1018	797.5	758
SEM		37	24	22	18
Percentage difference from monoculture	In mixture with				
	Cara	0	-1.0	-8.4	-12.3
	Sante	6.8	0	-2.1	-5.0
	Robinta	20.0	6.5	0	2.9
	Nicola	28.0	10.0	11.7	0
Significance of differences		P<0.01	P=0.22	P=0.08	P=0.07
L.S.D. (9 d.f.)		13.2	12.1	15.1	12.2

Table 7. Mean yields per plant in pure stands of each second early variety in 2002 and percentage differences from these in mixtures containing each other variety

		Variety		
	Grown in	Nicola	Cosmos	Romano
Mean gplant^{-1} (SEM)	In monoculture	840.2	787.0	654.5
(SEM)		23.3	30.5	21.5
Percentage difference from monoculture	In mixture with			
	Nicola	0	18.4	-15.7
	Cosmos	5.5	0	-8.0
	Romano	15.0	9.8	0
Significance of differences		P=0.14	P=0.31	P=0.12
L.S.D. (6 d.f.)		14.87	24.24	16.67

Table 8. The mean tuber yield (gplant^{-1}) of varieties in pure stands and two-way mixtures in 2003

	Cara	Sante	Appell	Tominia
Pure stands	767	832	1078	749
Two-way mixtures	964	794	974	830
Significance	P<0.001	P=0.5	P=0.02	P=0.42
Test statistic, t	-4.67	-0.65	-2.69	-0.83
df	18	17	18	18

Table 9. Mean yields per plant in pure stands of each variety in 2002 and percentage differences from these in mixtures containing the other varieties

		Variety			
	Grown	Cara	Sante	Appell	Tominia
Mean gplant^{-1}	In monoculture	915	804	1000	810
SEM		26.4	39.8	19.4	42.1
Percentage difference from monoculture	In mixture with				
	Cara		-16.0	-13.0	-8.5
	Sante	28.7		-7.6	28.7
	Appell	30.4	19.7		12.4
	Tominia	18.0	-17.3	-8.5	
		P<0.01	P<0.01	P=0.06	P<0.01
L.S.D. (12 d.f.)		14.0	18.1	9.2	19.2

3) The effect of competition within and between varieties on the yield of potato variety mixtures

Summary

In mixture with several other cultivars, individual potato plants of cv. Cara yielded more, the more components were in the mixture, whereas plants of cv. Sante yielded less. A range of other cultivars showed no response. Equiproportionate mixtures of cv Cara and cv Sante increased yields on average by 5% over the mean of the two pure stands, independent of planting density. This was due to increases in Cara plant yield, while Sante plant yields were unchanged. At low density, Cara plants in pure stands outyielded those in mixtures, but at medium or high densities, plants in mixtures outyielded those in pure stands.

Introduction

Monocultural plant communities dominate modern agriculture. Monocultures are crops composed of a single species and a single variety; hence the degree of heterogeneity within such communities is severely limited. The development of monocultural crop communities in agriculture has been underpinned by the use of synthetic inputs to control ameliorable factors (especially weeds, pests and diseases) and provide high fertility (see Donald, 1968). However, in production systems where synthetic inputs are restricted, the diversification of cropping systems can buffer against weeds, pests and diseases and it can compensate for variable and lower fertility (Finckh, *et al.*, 2000). Analogous buffering effects are seen in natural communities, where productivity and ecosystem functioning have been linked to functional biodiversity in plant communities (Tilman, *et al.,* 2001).

One form of within-crop diversification that has received considerable attention is the use of variety mixtures, providing a heterogeneous crop of a single species (see Wolfe, 1985). There are many crop species for which positive mixture responses have been reported (e.g. Rao & Prasad, 1984; Frey & Maldonado, 1967; Zhu *et al.,* 2000; Gizlice *et al.,* 1989) but there are also some mixtures where responses were either neutral or negative (e.g. Baker & Briggs, 1984). It is important to recognise that mixtures with increased yields are more likely to be reported in scientific papers than mixtures with reduced yields (see Mundt, 2002). Nevertheless, the evidence seems to be cumulatively powerful in favour of positive mixture performances, which are frequently a few percent higher than the mean of the components in pure stands. However, it is not clear what the basic mechanisms are that determine these relative yield responses. This is hardly surprising if we consider that the yield of any mixture is the result of complex interactions. Examples of the complexity of interactions in variety mixtures are those caused by abiotic factors such as nutrient and moisture levels (see Hartmann & Allard, 1964) or biotic factors such as plant-pathogen interactions (see Garrett & Mundt, 1999). Also, abiotic and biotic interactions are not mutually exclusive when mediating interactions amongst varieties (see Finckh & Mundt, 1992; Knott & Mundt, 1990).

Garrett & Mundt (2000) and Andrivon *et al.* (2003) report significant reductions in late blight (caused by *Phytophthora infestans*) by mixing a resistant potato variety with a susceptible variety. Garrett & Mundt (2000) also report that tuber yield from both varieties in mixtures was increased in mixtures compared with pure stands. This work prompted Mundt *et al.,* (2002) to conclude that diversification of host resistance in potatoes, by growing variety mixtures, could have an important role in an integrated approach to disease management and resistance durability. However, the focus of these conclusions was plant-pathogen interactions, neglecting competitive interactions between potato varieties. Competitive interactions amongst varieties are likely to be important in potatoes as potato cultivars demonstrate unique inter- and intra-competitive responses (Love *et al.,* 1995) and such competitive responses are important in variety mixtures (e.g. Khalifa & Qualset, 1974). Moreover, Doney, *et al.* (1965) report strong non-additive competition responses in a two-way variety mixture of potatoes, in which the mixed plots outyielded the mean of the pure plots by 6.5%.

The trials reported here address, in potatoes, the effects on plant-plant interactions of: (1) the proportions of each variety in a mixture, (2) the density of planting of a mixture and (3) interactions between these. The study of these factors is more informative than replacement series experiments as they reveal whether any mixture response is due to an inhibition of resource acquisition in pure stands or synergism in variety mixtures (see Snaydon, 1991).

Materials and Methods

The site used for the trials is a certified (UK5) organic farm (Grid Ref: TM280790). The soil has a medium texture with approximately 20% clay and a typical pH of 7.5. Composted horse manure at 25tha^{-1} was incorporated around two weeks before planting, 35kgha^{-1} of potassium sulphate was incorporated prior to trials in 2002 and all trials followed the leguminous phase of a rotation. The trials were not irrigated and both pre- and post- emergence weeding was achieved with inter-row cultivation and re-ridging.

Effect of the number of components in mixtures

A trial was designed to examine the effects of increasing the number of components in potato variety mixtures. The trial was planted during the last week of April 2001. It consisted of four blocks, two with large plots (9m x 7.5m) and two with small plots (4.5m x 4.5m); all treatments were randomised within blocks. The different plot sizes were chosen to examine epidemiological effects; these are reported elsewhere. The treatments were permutated from five varieties (see Table 1). These varieties were chosen as they are compatible agronomically and they offer diversity for foliage resistance to late blight. These varieties also have different tuber characteristics to allow for separation of varieties at harvest. The mixtures had equal numbers of tubers of each component and the seed tubers were planted randomly. The planting depth was around 100mm with a 280mm plant spacing within the row and 500mm between rows.. Late blight arrived as a natural infection around 9 August 2001. All tubers from the central two rows of each plot were harvested and sorted into varieties. Gross yield was measured for each variety within each plot.

The present paper reports only the tuber yield (g plant^{-1}) of cvs. Cara and Sante . The outlying Cara plants that yielded less than 600 g plant^{-1}and the outlying Sante plants that yielded more than 1200 gplant^{-1} were excluded from analysis. The tuber yield of Cara and Sante plants in variety mixtures was related to the number of components in the mixture using simple linear regression in Genstat.

Interactions between mixture composition and planting density

A trial was planted on 29 April 2002 to examine the effects on tuber yield of planting density, a mixture of the two cultivars and any interactions between these two factors using a split-plot design. The main plot factor was planting density with 250mm, 350mm and 450mm spacings within the row; spacing between rows was constant at 500mm. The split plot treatment factor was proportion of Cara (or equivalently, of Sante) with 0, 25, 50, 75 and 100% as the levels. The plots were 10m long x 7.5m wide with at least 1m gaps (occupied by oats) between plots. The trial contained six replications and lines of willow trees separated replications across the trial. Late blight arrived through natural infection around 13 July 2002. Tubers from mixtures were separated into their two constituents and gross yield measured.
The effects of treatments on the yield of each variety were analysed separately, with g plant^{-1} as the response variate, using Genstat v4.2 (VSN, Oxford).

Interactions between frequency in mixture and planting density

A trial was planted on 29 April 2003 to examine the effects on tuber yields of both density and proportions of varieties Cara and Sante, and interactions between these two factors. Table 2 lists the treatment factors and their levels which were arranged in a split plot design, with planting density (3 levels) as the whole plot factor and proportions of Cara (0, 25,50,75 and 100%) as the sub-plot factor. There were four replications. Plots were 7.75m long x 2.25m wide; there were at least 0.5m gaps between plots. Late blight arrived through natural infection around 1 August 2003. Tubers from mixtures were separated into their two constituents and gross yield measured.

Yield (t ha^{-1}) of each planting density was examined separately. A linear contrast was used to test whether the yield of a variety changed linearly in proportion to their frequency in the mixture; contrast coefficients were -0.625, -0.375, 0.375, 0.625 for 25, 50, 75 and 100% of the variety. In addition, the performance of each of the mixtures was compared with the mean of the components in pure stands. Finally, the effects of proportions in mixtures, planting density and any interactions between these factors on yield per plant was analysed separately for each variety.

Results

Effect of the number of components in mixtures

Cara and Sante were the only two of the five varieties used that showed general responses to mixing. Cara plants yielded more, the more components there were in a mixture (Fig. 1) whereas Sante plants yielded less (Fig. 2). Yields of the other varieties did not vary with the number of components in variety mixtures.

Interactions between mixture composition and planting density

Considering all planting densities together there was no overall effect of mixing Cara with Sante on the tuber yield of Cara plants (Table 3). However, there was a significant effect of planting density, with Cara plants at the lowest density producing 64% more tubers (by weight) than those at the highest density ($P < 0.001$ for the main effect in ANOVA). The interaction between density and mixture was significant ($P<0.001$). When planted in mixtures, Cara plants yielded 2.4% more at high planting densities than when planted in pure stands, and 6.0 % more at medium planting density. However, at low planting density Cara plants in pure stands outyielded those in mixed stands by 6.4%.

Table 4 shows that there was no effect of mixing Sante with Cara on the tuber yield of Sante plants at any planting density. The only significant treatment effect on Sante plants was the 27% increase in tuber yield at a low planting density, compared to high planting density.

To summarise: plants of both varieties individually yielded more at lower planting densities, but there was a significant interaction between the effects of mixing and planting density on Cara, so that Cara plants yielded more in mixed stands than in pure stands at high and medium planting densities but less at a low planting density.

Interactions between frequency in mixture and planting density

High Planting Density

On average, mixtures yielded 8.9% above the mean of the component pure stands (Fig. 3). This improvement applied to all the mixture combinations. It was due mainly to a significantly better than expected performance of Cara ($P<0.001$) while Sante plants performed as expected.

Medium Planting Density

As at high density, Cara plants yielded more than expected from their frequency in the mixture ($P<0.001$), while Sante plants performed as expected (Fig. 4). Despite this, there were no significant differences between actual yields of mixtures and those expected from the proportions of each component.

Low Planting Density

The only statistically significant response found was the 11.4% lower than expected yield from 25Ca:75Sa mixtures ($P<0.05$), due to a reduction in the yield of Sante plants (Fig 5). However, across all mixture combinations Sante plants yielded the same as expected from their performance in pure stands.

Yield per plant

Cara plants yield more when surrounded by more plants of Sante (Table 5). In other words, for Cara, intra-genotypic competition is greater than inter-genotypic competition. The strength of this competition is illustrated by the 32.6 % increase in the yield of individual Cara plants grown at the low rather than the high planting density. This was even stronger with Sante, which yielded 46% more at low than at high density (Table 6).However, the improved yield of plants in pure stands indicates that for Sante plants, inter-genotypic competition with Cara plants is greater than intra-genotypic competition. The balance of effects led to Sante plants yielding more when contributing 25% of the mixture rather than 50% or 75%.

Discussion

The results reported above confirm that yields in potato variety mixtures can be increased above the mean of the components in pure stands, as observed by Garrett & Mundt (2000) and Doney *et al.* (1965). The medium planting density used in these trials is the spacing commonly used by growers. In both 2002 and 2003 at the medium planting density a 1:1 mix of Cara and Sante increased yields by 5% above the mean of the components in pure stands. Some individual results were spectacular. A mixture of Cara and Sante in 2003 at a high planting density with 25% Cara yielded 23% more than the expected yield of the components in pure stands (Fig. 3).

In all the trials and mixtures described here Cara was the more aggressive competitor. In other words, Cara plants acquired disproportionate amounts of resources (Fig. 1). This has two possible explanations: (1)

individual Cara plants interact negatively in pure stands (the resource acquisition of individual Cara plants is inhibited in a monospecific plant community) and/or (2) that there is a synergistic interaction between Cara and other varieties in variety mixtures thereby increasing tuber yields. Because the relationship is asymmetric, it is presumably not simply niche differentiation.

In 2002 and 2003 both Cara and Sante plants responded positively to reductions in planting density, as expected from the law of constant yield. The experiments were not designed to give information on the resources limiting individual plants, which may anyway have varied within and between seasons, especially since the trials were not irrigated.

There have been few studies where the interaction between different resource levels and mixture performance has been examined. Frey & Maldonado (1967) found that the advantage of oat mixtures increased as stress increased, the stress being high temperatures late in the season. Hartmann & Allard (1964) found that moderate moisture stress saw large competitive effects in favour of mixtures. Hartmann & Allard also found that moderate or high nutrient levels increased this favourable mixture performance. They concluded that the competition between the two wheat varieties studied was primarily for moisture but that the intensity of the competition depended on the nutrient level. It is interesting to note that at low planting densities the performance of Cara was better in pure stands than in mixtures. Thus, the competitive relations between varieties may actually reverse at altered densities. This indicates that niche differentiation offers advantages for the more competitive variety when competition is strong but no advantage or even disadvantages when the degree of competition is limited. This conclusion is compatible with the findings of Frey & Maldonado (1967); Hartmann and Allard (1964) and Allard (1961) that yield increases in mixtures are greatest when resources are few and when competition between plants for those resources are greatest. There is an advantage to variety mixtures for a variety where intracultivar>intercultivar competition, but only when competition is strong. This should have the desirable effect of stabilising yields between places and seasons.

The results from 2003 confirm the conclusion from 2002; However, at a low planting density, Cara performed significantly worse than expected when it constituted 25% of the mixture (Fig. 4); this is not likely to be the influence of Sante plants, but more likely to be due to an improved performance of Cara in pure stands at this planting density. In other words, Cara plants at low planting density, or when competition for resources is low, interact more positively than Cara plants in mixtures with other varieties.

If interactions among plants in accessing resources are limited then there is evidence that pure stands offer advantages over mixtures. Therefore, the type, timing and level of limitation to resources is likely to influence competitive interactions. In organic systems it is impossible to predict the type, timing and intensity of limitations of resources. However, it is likely in low input systems that one or more resources will be limiting at one or more times during a season and plant-plant interactions are likely to influence the amount of the resources available for yield production. Therefore, it would seem that mixtures of varieties are particularly well-suited to low input and organic systems. This is the case given that there are three options available to the grower: (1) increase productivity of individual plants by planting pure stands at a low density, but this lowers gross yield; (2) reduce the severity of resource competition by increasing inputs of resources: this tends not to be an option in organically certified systems; (3) plant mixtures in systems where inputs are low and plants are at a density where plant-plant interactions are important. The case for variety diversification is strengthened further if variety mixtures can be used to contribute to a system approach for the prevention of late blight (see Phillips *et al.* 2002; Mundt *et al.* 2002).

These trials concentrate on the performance of just two varieties, Cara and Sante. However, potato varieties have different general and specific combining abilities (Phillips, 2004). Fig. 1 indicates that Cara has positive general combining abilities with other varieties and Fig. 2 shows that Sante has negative general combining abilities with other varieties. Therefore the above discussions may only be relevant to mixtures of these two or other varieties with these qualities. It may be possible to find variety combinations with other specific combining abilities: this could occur, for example, through a combination of improved performance due to plant-plant interactions and to plant-pathogen interactions with late blight.

The notion of positive specific combining ability is central to the application of variety mixtures in low input agricultural systems. The aim must be to provide as much functional heterogeneity as possible. In potato mixtures, it seems that the two main functions are reducing yield limiting intracultivar competition and the prevention of late blight. However, it is likely that it is only if components of mixtures are deliberately bred for mixture performance that the concept of heterogeneity will be properly exploited; it is too much to expect that varieties bred for monocultural production will exhibit the most useful specific combining abilities (e.g. Hill, 1996).

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Table 1 Equi-proportional variety mixtures used in 2001

Pure Stands	**Two component mixtures**	**Three component mixtures**	**Four component mixtures**
Cara (Ca)	CaNi	CaVeNi	CaVeNiRo
Nicola (Ni)	CaRo	CaVeRo	CaVeSaRo
Robinta (Ro)	CaSa	CaVeSa	CaVeNiSa
Sante (Sa)	CaVe	CaNiSa	
Verity (Ve)	NiSa	VeNiSa	
	VeNi		
	VeSa		
	VeRo		

Table 2 The treatment factors and levels in the 2002 trial examining interactions between variety frequency in mixture and planting density

Planting density (whole plot factor)	**Mixture (Split plot factor)**
450 mm (low)	100%Ca : 0%Sa
350 mm (medium)	75%Ca : 25%Sa
250 mm (high)	50%Ca : 50%Sa
	25%Ca : 75%Sa
	0%Ca : 100%Sa

Table 3 The yield (gplant^{-1}) of Cara plants in pure stands and mixtures at three planting densities.

Density	**Mixed stands**[a]	**Pure stands**	**Mean**[b]
High	521	509	515
Medium	711	671	691
Low	818	870	844
Mean	683	684	

[a] SED for interaction between planting density and mixture treatments 37.6, df=11.3.

[b] SED for mean differences between planting densities 36.4, df=10

Table 4 The performance (gplant^{-1}) of Sante plants in pure stands and in mixtures at three planting densities.

	Mixed stands[a]	Pure stands	Mean[a]
High Density	500	490	495
Medium Density	642	617	786
Low Density	763	810	630
Mean	635	639	

a Interaction not significant at P =0.05.

b Differences between planting densities were statistically significant (P<0.001, SED=13.7, df=10).

Table 5 The yield (gplant^{-1}) of Cara plants at different proportions in Cara/Sante mixtures at various planting densities.

	Proportion of mixture[a]				
	25%	50%	75%	100%	Mean[b]
High Density	971	823	716	635	786
Medium Density	1017	1005	841	814	919
Low Density	1147	1125	904	994	1042
Mean[c]	1045	984	820	814	

[a]No significant interaction between proportion of mixture and planting density.
[b]Differences significant at P<0.01, SED=54.8, df=6
[c]Differences significant at P<0.001; SED=64.2 , df=27

Table 6 The yield (g/plant) of Sante plants at different proportions in Cara/Sante mixtures planted at various densities.

	Proportion of mixture				
	25%	50%	75%	100%	Mean[a]
High Density	660	572	613	651	624
Medium Density	809	727	812	834	795
Low Density	937	879	808	1012	909
Mean[c]	802	726	744	833	

[a] Difference significant at P<0.001; SED=29.6, df=6
[b] no significant interaction between proportion of mixture and planting density.
[c] Differences significant at P<0.001; SED=30.3, df=27.

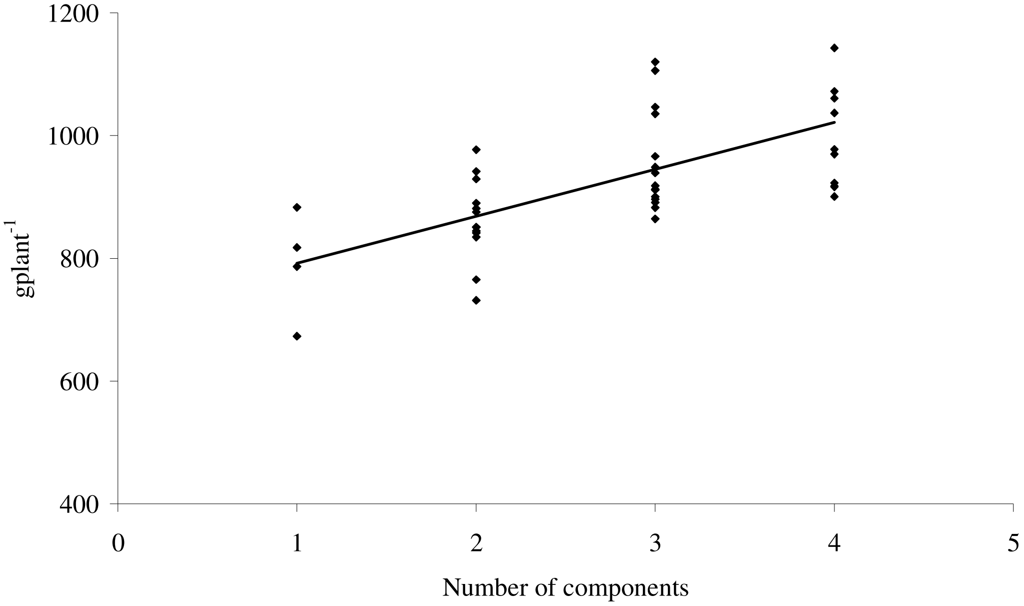

Figure 1 Tuber yields (gplant^{-1}) of Cara plants in variety mixtures in relation to the number of components. Regression equatio, SE following coefficients: yield = 715±40 + 77±14 n, P <0.001, R^{*2} 0.42

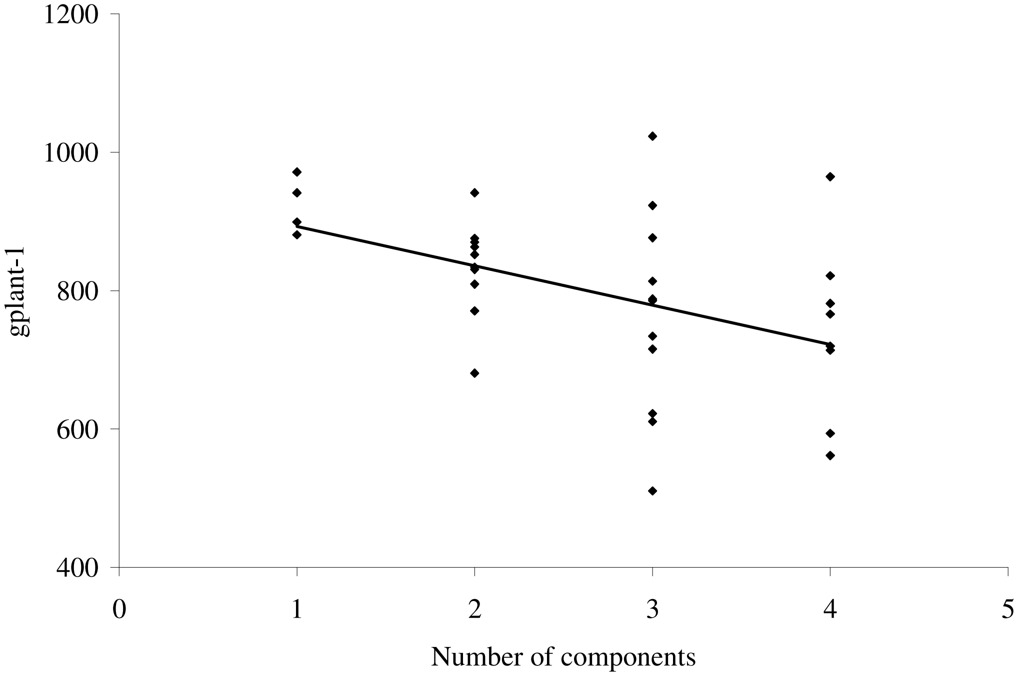

Figure 2 Tuber yields (gplant^{-1}) of Sante plants in variety mixtures in relation to number of components. Regression equation, SE following coefficients: yield = 950±56 – (57±20), P <0.01, R^{*2} 0.18.

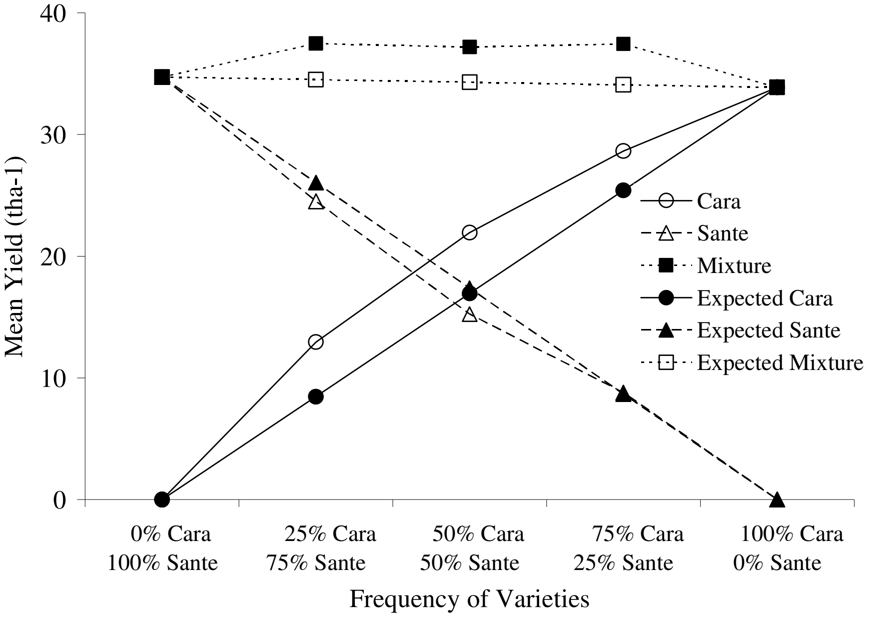

Figure 3 Mean tuber yields (tha^{-1}) for pure stands and two-way mixtures of cvs Cara and Sante (between 25% and 75% of the components) at a high planting density (250 mm between plants). (25Ca:75Sa: SED =0.92, P<0.05; 50Ca:50Sa: SED=1.5,P<0.1; 75Ca:25Sa: SED=1.19, P<0.05).

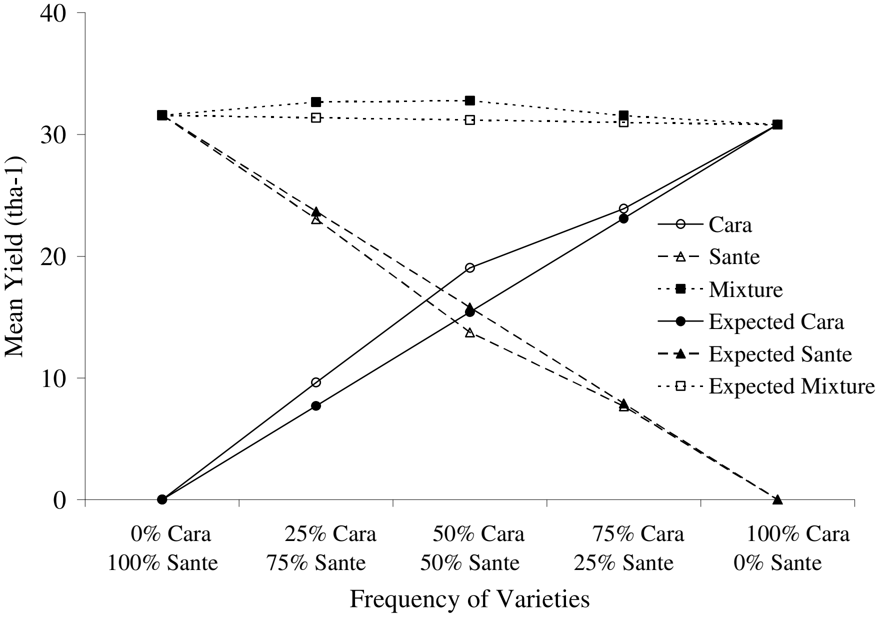

Figure 4 The mean tuber yield (tha^{-1}) for pure stands and two-way mixtures mixtures of cvs Cara and Sante (between 25% and 75% of the components), at a medium planting density (350 mm between plants).

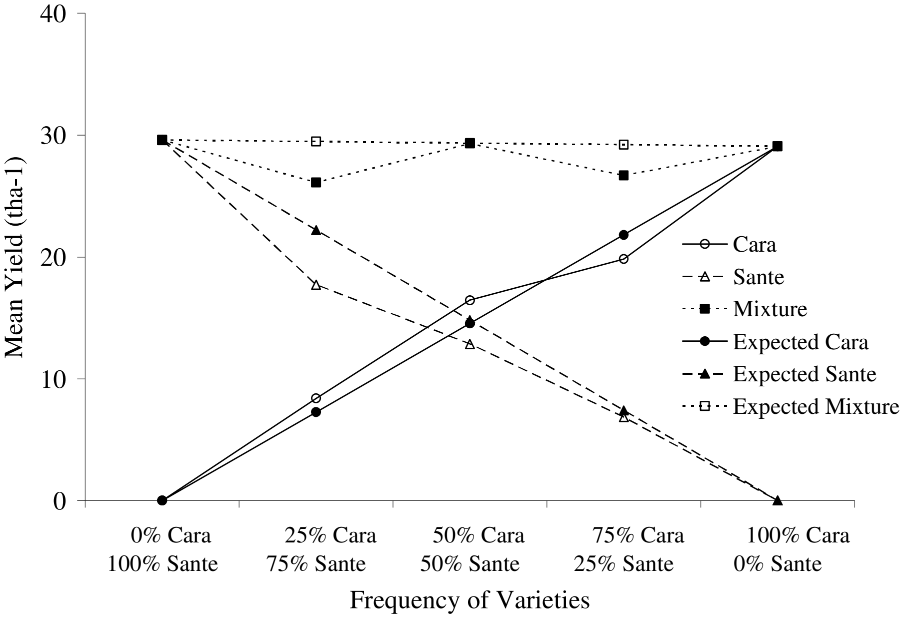

Figure 5 The mean tuber yield (tha^{-1}) for pure stands (100%) and two-way mixtures mixtures of cvs Cara and Sante, at a low planting density (450mm between plants). SED 1.28, ..df)

GERMANY (D)

Effects of cultivar mixtures and pre-crop on disease and yield of potatoes

Summary

Two white skinned (Agria and Simone) and two red skinned (Laura and Rosella) potato varieties were grown in pure stands and four mixtures of a red and white variety in 2002 and 2003. All mixtures were grown either after pre-crop grass clover or after pre-crop winter wheat in organic conditions to determine possible effects of nutrient availability on late blight (caused by *Phytophthora infestans)* and on plant-plant interactions. Disease and yield were assessed on a per cultivar basis and three sequential harvests were made to determine below-ground development.

Due to very unfavourable weather conditions in 2002 total yields were only 18.5 t/ha, half of the yields in 2003 (37.8 t/ha). Soil mineral Nitrogen availability was between 15 and 25kg/ha lower after pre-crop winter wheat than after grass-clover. Tuber yield accumulation was slowed down for all varieties and mixtures after winter wheat, resulting in consistent albeit statistically not significant mean reductions in total yield of about 18 and 7% in 2002 and 2003, respectively.

Disease pressure was high in 2002 but very low in 2003 due to very dry weather. Disease severity and disease progress rates were lower after pre-crop winter wheat than after pre-crop grass clover in all treatments in both years. Due to high spatial variation, the effect was not statistically significant, however. Mixtures had only little effect on disease in comparison to the mean of the pure stands. The greatest effects were observed in the mixture Laura-Simone, the two varieties differing most in susceptibility, the effects were stronger at the lower disease pressure after winter wheat than after grass-clover in both years.

Mixing did not result in significant changes in overall yield. However, competition reduced the amount of oversize tubers and often increased the amount of undersize tubers in the less competitive mixture component. Competitive interactions between the varieties were similar in both years and they were only little affected by the nutritional status of the crop.

Introduction

Diversity that is designed and put together to fulfil a function, here plant protection against diseases, insects and weeds, i.e. **Functional diversity,** can make an important contribution to plant protection. As well mechanistic interactions such as distance, barrier and microclimatic effect as complex eco-physiological interactions such as competition, induced resistance and allelopathy may act in diversified systems to reduce disease or insect pressure (Wolfe and Finckh, 1996). Traditionally, diversity is implemented through variety and species mixtures, reduced field size and rotations and they are of particular importance in low-input and organic management (Finckh and Wolfe, 2006). For cereals and coffee, rust and mildews are being managed successfully in different regions of the world (Finckh et al , 2000). In China, since 1998, the production of single rows of highly susceptible high value glutinous rice in between several rows of more resistant hybrid varieties has led to drastic reductions in fungicide inputs (little to no more sprays are being applied) and to a near doubling of yields of these varieties. (Youyong et al, 2000).

Important for the effectiveness of cultivar mixtures in reducing diseases is that the mixture components on the one hand differ in their resistance and, on the other hand, diversity for virulences exists in the pathogen population (Finckh and Wolfe, 2006). However, even in systems where race-specific interactions have not been found, Jeger et al. (1981a,b) and Wolfe et al. (1981) showed that differences in non-specific resistance could be beneficial in mixtures, with the disease level in the mixture often less than the mean of the components and close to that of the least susceptible component.

Within the growing season, *Phytophthora infestans* (Mont.) de Bary, the causal agent of potato late blight, is a polycyclic wind and rain splash dispersed pathogen. Similar to the rusts and mildews a strong gene-for-gene relationship exists between the host *Solanum tuberosum* and the pathogen. However, many of the commercial varieties used in Germany possess either no or no functional major resistance to late blight. Instead, they differ for their field resistance (Möller, 2002; own observations). Also, *P. infestans* populations in the area where the current research was conducted were found to be highly complex and able to attack all known resistance genes of potatoes (Bouws-Beuermann and Finckh, 2006; Butz and Finckh, unpublished). Moderate but positive effects of cultivar mixtures and alternating rows reducing disease pressure of potato late blight have been reported from the USA, South America and France (Andrivon & Lucas, 1998; Garrett et al., 1998; Garrett & Mundt, 2000).

Results from Phillips et al (see this report, WP 3.3, part 1) indicated that mixture effects on disease and yield are massively influenced by the varieties used in the mixtures and that most likely competitive interactions between plants in mixtures have a substantial effect on the outcome.

Potato development over time is very much influenced by the availability of nutrients and environmental conditions and the timing of tuber initiation depends not only on the variety but also on the nutrients available (Schulte-Geldermann et al, 2005). For example, yield of the variety Nicola under organic conditions in three years and at several sites could be modelled with a multiple regression on the amount of N_{min} in the top 60cm soil ten days after crop emergence, the temperature sum and the number of days from planting until 60% defoliation, and the percent reduction in late blight due to copper applications (Finckh et al., 2005). Thus, it is likely that competitive interactions are not constant over time and very much depend on the environmental conditions and the innate bulking and other growth parameters of varieties. Dynamic changes of competition over time were shown for cereals (Finckh and Mundt, 1996).

Following plant development not only above-ground but also below-ground can provide important insights especially when it comes to plant-plant interactions over time. In addition, while the nutritional status as influenced by the rotational position can greatly influence the timing of bulking the changes are variety specific. Therefore, it is to be expected that nutritional regime may change the outcome of mixture experiments.

The aims of this work are to quantify the effect of potato variety mixtures on late blight epidemic development and yield under organic growing conditions. The following hypotheses were addressed specifically:

(i) Disease in mixtures of varieties differing in susceptibility will be reduced in comparison to the mean of the pure stands.
(ii) Nutrient availability and competitive interactions between varieties affect disease on the varieties.
(iii) Competitive interactions between varieties are affected by the nutrient status

Details of the dynamic effects acting in mixtures over time were studied by using sequential harvesting and comparison of size class distributions and single-plant disease assessments over time. By using a split-plot design with rotational position as main effect nutrient availability was included as a factor in the study.

Materials and Methods

Experimental site: The experiment was conducted in 2002 and 2003 under organic management on the experimental farm of the University of Kassel (central Germany) 8km NW of Witzenhausen on about 250m asl. Soils are deep loess soils.
A rotation trial was set up since 1999/2000 including two four-year rotations. Rotation 1 was grass-clover; potato; winter wheat; spring cereal. In rotation 2 the position of potatoes and winter wheat were exchanged to provide for varying levels of nutrition for the potatoes. A total of 32 plots were arranged as a split plot with rotation as the main factor and subplots arranged within each main plot. The size per main plot was 22 x 60m, allowing for the arrangement of various subplots.

Plot arrangement: Each subplot was 10 rows wide by 10m. Row 1, 5 and 10 were edge rows, rows 2-4 were used for three sequential harvests and row 6-9 for final harvest (Fig. 1). Subplots were arranged in two long rows along the edges of the main plots to allow for sequential harvests from the outside in order not to disturb the parts of the plots to be used for final harvest. In 2002, all subplots were surrounded on both sides with two rows of the moderately susceptible variety Rosella. In 2003, the second surrounding row was planted to the susceptible cultivar Linda to provide for a more uniform inoculum pressure throughout the experimental area.

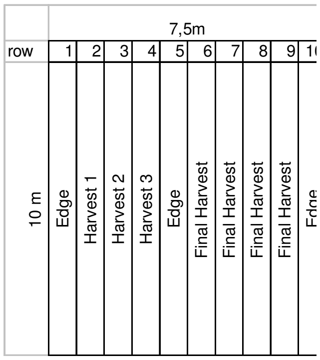

Fig. 1. Set-up of subplots within main plots.

Treatments: Two white (Agria and Simone) and two red-skinned (Laura and Rosella) potato varieties selected to be similar with respect to maturity (all middle early group, Bundessortenamt, 2002) and varying in bulking behaviour and resistance were grown as pure stands and as four two-way mixtures of a red and a white variety each (Table 1).

Table 1. Varieties and their properties used in pure stands and in four two-way mixtures with one red and one white variety per mixture

Variety/Mix	**Colour**	**Bulking**[1]	**Susceptibility**[2]
1. Agria (A)	white	middle	middle
2. Laura (L)	red	middle	high
3. Rosella (R)	red	late	middle
4. Simone (S)	white	late	low

[1] Based on Möller, 2000 and own observation (Ann rep. MOP Feb. 2002)

[2] Based on own observations in 2001 at the same site

Agronomic measures: All measures are summarised in Table 2. In 2002, with the weather relatively warm and dry in early April planting of the edge rows started on April 11 and for experimental plots on April 23. Unfortunately, unpredicted rains started in the middle of planting and lasted intermittendly for about three weeks and planting could only be continued on May 15. This resulted in two replications planted on April 23 and one on May 15, while the main plot of one replication with pre-crop grass clover was planted on April

23 and the main plot with pre-crop winter wheat on May 15. In 2003, all experimental plots were planted on April 15.

Table. 2. Agronomic measures in 2002 and 2003

2002	2003	Measure
24.10.01	15.1.	Ploughing in rotation 2 (pre-crop wheat)
16.2.	20.1.	Ploughing of grass-clover for rotation 1
11.4.	14.4.	planting of edge rows
23.4.	15.4.	planting main plots
15.5.	-	late planting main plots 2002[1]
18.5., 2.6. 26.6.	16.5.	Hoeing and hilling
2.7.	27.6.	sequential harvest 1
15.7.	08.7.	sequential harvest 2
29.7.	15.7.	sequential harvest 3
7.8.	-	sequential harvest (late planted plots 2002[2])
-	12.8	defoliation
16-19.9	25.-29.8	harvest

[1] One replication with pre-crop grass clover and two with winter wheat were planted late due to prolonged rainfalls (see text for explanations).

[2] Sequential harvests for the late planted plots in 2002 were started on the second date only.

Assessments: In 2002, soils were analysed prior to planting in mid April and five more times from early June to late July. It became clear, however, that the main mineralization phase likely had been during late April and May. Therefore, in 2003, sampling times were adjusted starting in late March and adding two assessmentsin May. Due to extreme drought no samples could be taken after June 20 in 2003. Nitrate-N (N_{min}) was determined in 0-60cm depth according to the standard assessment method of Schinner et al. (1998).

Growth stages were assessed regularly using the decimal code of Radtke et al. (2000).

Plots were checked regularly until the beginning of the late blight epidemic. After this, disease was assessed twice weekly on 5 plants per plot for the pure stands and on ten plants per plot in the mixtures (five of each variety). Percent diseased leaf area was estimated, following the key of James et al. (1971).

For each sequential harvest, plants in one row were counted and harvested. Potatoes were separated by colour and into three size classes (Table 3). In 2002, the sequential harvests for the main plots planted on April 23 were conducted on July 2, 15 and 29, for the later planted plots dates were July 15, 29 and August 7 (Table 2). For the final harvest at least four rows were harvested and sorted by colour and size classes.

Data analysis: All data were calculated using Excel and analysed with SAS (1986).

Because of the two separate planting times in 2002, in a first step, the replication data from 2002 were analysed separately for the two pre-crops to determine if there were replication effects that could be explained through planting time. Because these effects disappeared within a few weeks (see results section) the four replications were analysed together.

Cumulative disease severity was calculated as the Area under the disease progress curve (AUDC) using the following equation (Kranz, 1996):

$$AUDC = \sum_{i=1}^{n-1} \left(\frac{x_{i+1} + x_i}{2} \right) (t_{i+1} - t_i) \qquad (1)$$

where $\mathbf{x_i}$ = % infested foliage at assessment i, $\mathbf{t_i}$ = time (days) of assessment i, **n** = Number of assessments. Disease progress rates *r* were calculated based on the logistic model.

For all parameters, the absolute and relative numbers were calculated to make direct comparisons between mixtures and pure stands possible. Disease and total mixture yield were related to the mean of the pure stands. Mixtures were compared to the mean of the pure stands with linear contrasts. As in 2002 there were a few missing values due to bad plot establishment, the least squares means were calculated for the pure stands and used as reference.

Data were analysed as well on a per plot basis as on a per variety basis. For the comparisons per variety, the yields per variety in the mixtures were compared to half the yield of that variety in pure stand. As each variety was present in two mixtures only the data sets on a variety basis consisted of three treatments only. The LSmeans in 2002 and the means in 2003 were compared using the statement pdiff or LSDs, respectively.

Results

Climatic conditions

The weather was relatively cool and wet during the 2002 season and generally very unfavourable to potato development (Fig. 2). In contrast, in 2003, conditions were unusually dry and hot. From mid-May on almost every day temperatures rose above 20°C with many days above 30°C. The only substantial rain fell on May 31 when soils were already quite dry and most of the water evaporated quickly. Thereafter, there was virtually no more rain. Between April and August there was a total of only 200mm rainfall.

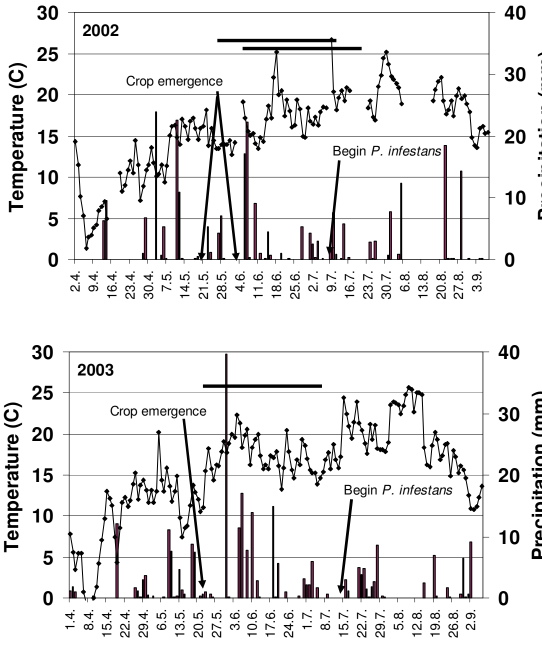

Fig. 2. Climatic conditions during the growing seasons 2002 and 2003. The date at which late blight was forst observed is indicated with an arrow. The horizontal bars indicate the first six weeks of crop development after emergence.

Soil mineral N dynamics

In both years, N_{min} contents in mid-April were similar around 50kg/ha. The two additional soil samplings in May 2003 show that during that month soil N_{min} contents peaked before declining sharply by early June (Fig. 3). After June 20 in 2002, the N_{min} levels after grass clover dropped below those of pre-crop winter wheat. No data from this period are available from 2003 because the excessive drought made soil sampling impossible.

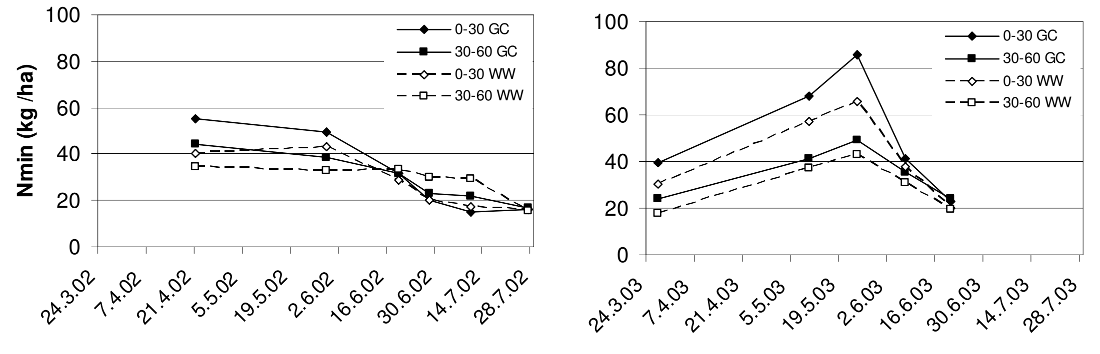

Fig. 3. Soil mineral N contents in kg/ha during spring and early summer in the main plots with pre crop Grass clover (GC, solid symbols and winter wheat (WW, open symbols) in 0-30 and 30-60cm depth in 2002 (top) and 2003 (botton).

Plant development and effects of planting time in 2002

Despite the three week break during planting in 2002, above-ground development of plants was surprisingly similar. The later planted potatoes emerged about two weeks after the early planted ones (Fig. 2) but all started flowering almost at the same time. Overall, there were no significant differences in above-ground development among the different main plots or varieties and mixtures in both years (data not shown).
Just like above-ground, below-ground the potatoes caught up rapidly in the later planted plots in 2002 (Fig. 4) and there was no effect of planting time on final yield (see below). Comparing early and late planted plots based on the respective first, second, and third sequential yields did not allow to clearly differentiate the later planted main plots based on yield.

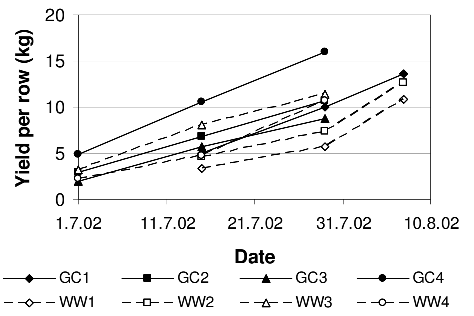

Fig. 4. Yield development (kg per 10 m row) in main plots 2002 after pre-crop grass clover (GC, solid symbols) and pre-crop winter wheat (WW open symbols). Three plots were planted late and sequential harvests were delayed.

Disease development

In 2003, late blight first was observed in the experimental plots around July 15th, about 10 days later than in 2002 (Fig. 2).

Due to the cool weather conditions disease developed relatively slowly in 2002 with the last plants dying five weeks after the first disease was observed. In contrast, in 2003, disease did not progress beyond an average of 55% diseased leaf area in the worst case by August 5th 2003 (Fig. 5). At that date, plants started yellowing due to early maturation in the hot and dry weather and disease assessments had to be stopped. Consequently, areas under the disease progress curves (AUDC) were substantially lower in 2003 than in 2002 (Table 4). In contrast to disease severity, disease progress rates (r-rates) were similar in both years (Only means are shown).

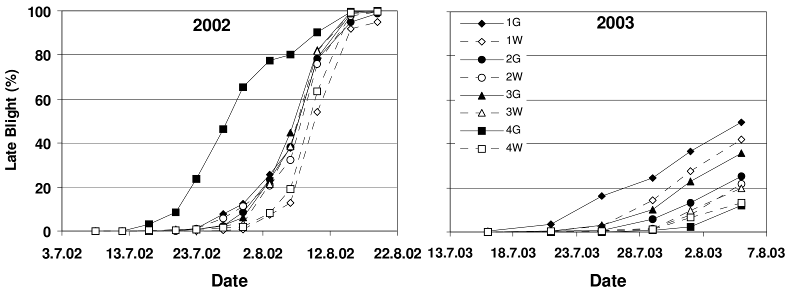

Fig. 5. Late blight development within main plots (1G = replication 1 pre crop grass clover, 1W = replication 1 pre crop winter wheat, etc) in 2002 and 2003. In 2002, 1G, 1W and 2W were planted three weeks late

In 2002, disease showed up first and progressed most rapidly in the main plot of replication four with pre-crop grass clover, resulting in consistently the highest AUDC in this plot and a strong replication effect for pre-crop grass-clover which could not be related to planting time, however. For pre-crop winter wheat there were no replication effects. In contrast, in 2003, disease started in replication 1 with the pre-crop grass clover but differences among replications were not very high due to the low disease pressure (Fig. 5).

The variable occurrence of blight over the main plots often led to non-significant differences as well between pre crop as among treatments. As rotation effects were not significant, analyses were conducted in general across pre-crop. However, the data are shown separately by pre-crop in Table 4 to show the visible trends. In both years the ranking of the varieties was the same with Laura the most susceptible and Simone the most resistant (Table 4). In 2002, Simone was heavily infested with a virus, most likely PVY, leading to an overall stunted appearance. Even without obvious viral infection in 2003, Simone remained the least susceptible variety, however.

After pre-crop winter wheat, AUDC and r-rates were lower in all treatments, however, the main effect pre-crop was not significant in both years. Mixtures also reduced disease somewhat more with pre-crop winter wheat than with grass-clover, with significant reductions in the mixtures Agria-Laura and Laura-Simone in 2002 but not in 2003. Under the low disease pressure in 2003 reductions after winter wheat were relatively greater but not significant (Table 4).

Table 4. Area under the disease progress curve (AUDC) on four pure stands and four mixtures in 2002 and 2003 after pre-crop grass-clover (GC) and winter wheat (WW). Mean diseae progress-rates (r-rates) are given for comparison.

	AUDC					
Variety	GC	Rel.[1]	WW	Rel.	Mean	Rel.
2002						
Agria	1480		1087		1283 B[2]	
Laura	2026		1614		1820 A	
Rosella	1584		1188		1386 B	
Simone	952		851		902 C	
Agria-Laura	1656	0.94	1105	0.82*[3]	1380 B	0.88
Agria-Rosella	1404	0.92	1079	0.95	1242 B	0.93
Laura-Simone	1550	1.04	1058	0.86(*)	1304 B	0.95
Rosella-Simone	1315	1.04	1052	1.03	1183 B	1.03
Mean 2002	**1496**	**0.98**	**1129**	**0.91**	**1312**	**0.96**
r-rate 2002	**0.365**		**0.346**			
2003						
Agria	113		41		77 B	
Laura	393		350		372 A	
Rosella	195		154		175 B	
Simone	53		31		42 B	
Agria-Laura	285	1.12	142	0.73	214 AB	0.93
Agria-Rosella	183	1.19	38	0.39	112 B	0.80
Laura-Simone	180	0.81	104	0.55	142 B	0.68
Rosella-Simone	111	0.90	57	0.61	84 B	0.75
Mean 2003	**189**	**1.00**	**115**	**0.57**	**152**	**0.79**
r-rate 2003	**0.392**			**0.353**		

[1] Rel.: disease in mixtures relative to the mean of the pure stands.

[2] Means followed by different letters are significantly different from each other ($P<0.05$, LSD).

[3] *, (*), Mixtures differ significantly from the mean of the pure stands at $P<0.05$ or 0.1, respectively, linear contrasts.

In general, disease severity on the varieties in the mixtures changed little. On Laura and Rosella disease was reduced in mixtures in both years, however, this effect was only significant on Laura in 2002. In contrast, r-rate reductions on Laura in mixture with Simone were significant in both years (Fig. 6).

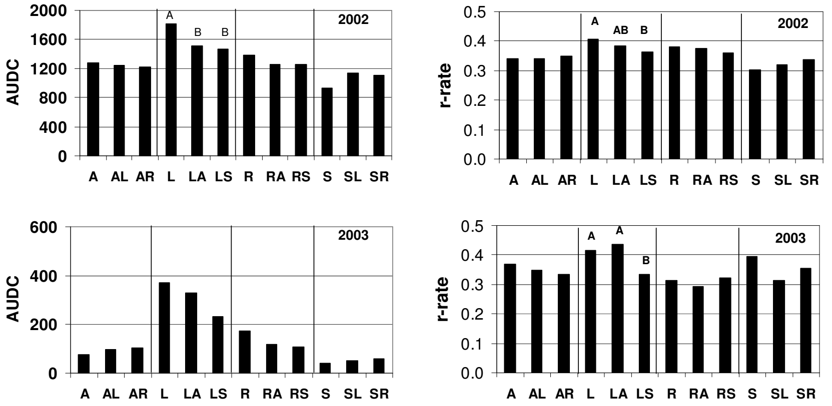

Fig. 6. Area under the disease progress curve (AUDC), left and disease progress rate (r-rate) right on the varieties Agria (A), Laura (L), Rosella (R), and Simone (S) in 2002 and 2003 in pure stands and mixtures. Data for the different varieties are separated by vertical lines. Columns within group marked with different letters differ significantly at *P*<0.05 (LSD). Scales for the two years are different for AUDC.

Yield and size classes

All varieties accumulated their tuber mass slower afterpre-crop winter wheat than after grass-clover in both years (Data not shown). These effects were stronger in 2002 than in 2003, in general.

Total yields in 2002 were low with 20.1 and 16.7 t/ha after grass clover and winter wheat, respectively (Table 6). This reflected the generally unfavourable conditions for potato in 2002. The differences between pre-crops were not statistically significant although they were marked and consistent. There were no significant differences in gross yield of the varieties or mixtures either. The mixtures did not behave consistent over the two pre-crops, however, the effects were not significant. Most markedly, the mixture Rosella-Simone overyielded by about 10% after both pre-crops while the three other mixtures yielded the same or less than the mean of the pure stands. There were significantly fewer tubers in the undersize class in the mixtures with Simone after grass clover and in the Rosella-Simone mixture more oversize tubers. In contrast, in the Agria-Rosella mixture the amount of oversize tubers was reduced significantly after grass clover. There were no significant changes in size class distribution after winter wheat in 2002 (Table 6).

Table 6. Final yield and size classes (t/ha) by pre-crop[a] in 2002 and 2003. LSMEANS are reported.

Pre-crop	Variety	2002	*Rel*	2003	*Rel*
2002					
GC	Agria	22.0		38.4	
GC	Laura	20.4		35.3	
GC	Rosella	21.3		45.2	
GC	Simone	21.1		36.5	
GC	AL	18.9	0.89	36.8	1.00
GC	AR	18.8	0.87	41.3	0.99
GC	LS	19.8	0.96	38.6	1.07*
GC	RS	23.4	1.10	42.1	1.03
GC	Mean	20.7	0.95	39.3	1.02
WW	Agria	16.9		36.5	
WW	Laura	16.1		32.7	
WW	Rosella	19.5		39.2	
WW	Simone	15.6		34.5	
WW	AL	16.3	0.99	34.7	1.00
WW	AR	17.0	0.93	39.3	1.04
WW	LS	15.0	0.95	34.5	1.03
WW	RS	19.4	1.11	39.3	1.07*
WW	Mean	17.0	0.99	36.4§	1.03

[a] GC = pre crop grass-clover, WW = winter wheat

**, *, (*) Mixtures differ significantly from the mean of the pure stands with $P<0.01$, 0.05, 0.1, respectively (linear contrasts)

$^{\$}$ Effect of pre-crop was significant at $P<0.1$

In 2003, overall yields were almost twice as high than in 2002 with 37.8t/ha in comparison to 18.5t/ha, respectively. The effect of pre-crop was in 2003 like in 2002 with yields after grass-clover higher than after winter wheat. After grass-clover the mixture Laura-Simone and after winter wheat the mixture Rosella-Simone yielded significantly more than the mean of the pure stands. In both cases, the overyielding was due to the production of significantly more oversize potatoes in the mixture than in the pure stands (Table 6). Like in 2002, there were no other significant changes in size class distribution after winter wheat in 2003while after grass clover, there was a significant increase of undersize tubers in the mixtures with Agria.

In both years, mixture yields were not composed of equal amounts of each variety indicating strong competitive interactions in mixtures. Each variety interacted differently with the two mixture partners and the interactions were relatively symmetrical (Table 7). For example, Agria suffered when grown in mixtures with Rosella, reducing total yield significantly by 18% in both years while it overyielded when mixed with Laura (+7 and 15% in 2002 and 2003, respectively). On the other hand, Rosella yielded 18% more when mixed with Agria in2003 but not in 2002 and Laura yielded 15 and 17% less in 2002 and 2003, respectively. Thus, whatever one variety gained in the mixture was lost by the partner variety.

The way competition works in the mixtures can be further determined by analysing the size class distributions (Table 7). Here it becomes clear, that especially the oversize tuber classes change. Both Rosella and Agria have a tendency to produce oversize tubers. Rosella increases this tendency in mixtures with Simone and Agria. For Agria it is decreased in mix with Rosella and increased when mixed with Laura. Overall, there were especially more regular size potatoes in the Agria-Rosella mixture than in the pure stands, fewer oversize potatoes but somewhat more undersize potatoes resulting in a more favourable overall size distribution in the mixture (Table 7).

The competitive interactions between the different potato varieties can be visualised best in a competition graph (Fig. 7). Here the performance of each variety alone relative to its pure stand is depicted with the position of the data points relative to the perpendicular lines. The performance of the mixtures relative to the mean of the pure stands can be read directly from the positon of the mixture relative to the diagonal line.

Table 7. Effects of companion cultivar on relative[1] amount of tubers produced in three size classes of four potato varieties at final harvest in 2002 and 2003.

Variety	**mixed with**	**<35mm**	**35-60mm**	**>60mm**	**Total**
2002					
Agria	Agria	1.00	1.00 A[2]	1.00 A	1.00 A[2]
	Laura	1.19	1.07 A	0.92 A	1.04 A
	Rosella	0.94	0.84 B	0.63 B	0.82 B
Laura	Laura	1.00	1.00	1.00	1.00
	Agria	0.88	0.83	1.05	0.85
	Simone	1.01	1.06	1.05	1.05
Rosella	Rosella	1.00	1.00 B	1.00 AB	1.00 B
	Agria	1.04	1.00 B	0.91 B	0.98 B
	Simone	1.16	1.28 A	1.42 A	1.31 A
Simone	Simone	1.00	1.00	1.00	0.97
	Laura	0.85	0.87	0.63	0.86
	Rosella	0.84	0.89	0.75	0.88
2003					
Agria	Agria	1.00	1.00 B	1.00 B	1.00 B
	Laura	1.15	1.12 A	1.20 A	1.15 A
	Rosella	0.98	0.98 B	0.60 C	0.82 C
Laura	Laura	1.00	1.00 A	1.00 A	1.00 A
	Agria	1.31	0.87 B	0.51 B	0.83 B
	Simone	0.95	0.97 A	1.15 A	0.99 A
Rosella	Rosella	1.00	1.00 B	1.00 C	1.00 C
	Agria	1.32	1.15 A	1.24 B	1.18 B
	Simone	1.20	1.13 A	1.58 A	1.31 A
Simone	Simone	1.00	1.00 B	1.00 A	1.00 B
	Laura	1.01	1.11 A	1.09 A	1.11 A
	Rosella	0.98	0.79 C	0.47 B	0.74 C

[1] The numbers are expressed relative to the mean produced in pure stands.

[2] Numbers within group followed by different letters are significantly different at $P<0.05$ (LSD).

The symmetry of the competitive interactions in 2003 becomes quite visible in Fig.7 as the mixtures fall very near the diagonal line. The deviation from the perpendicular lines shows which variety was doing better than expected and which worse. In contrast to 2003, in 2002, competitive interactions were not symmetrical; resulting in mixture yields quite different from the means of the pure stands (Fig. 7, left).

In both years, Rosella was a very good competitor against Simone while it out-competed Agria in 2003 but not 2002. There had been an interaction between pre-crop and performance of Agria in mixture with Rosella in 2002. This interaction was not apparent in 2003. The performance of Laura was quite similar in both years. Simone appeared to do quite a bit better in 2003 than in 2002. In view of the heavy virus infection of Simone in 2002 this is not surprising, however.

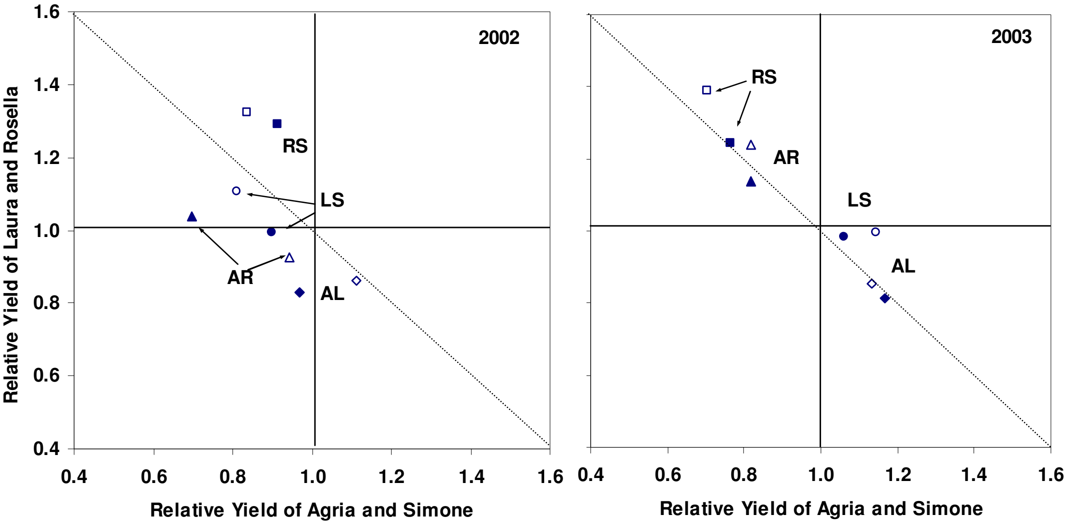

Fig. 7. Competitive interactions in four different two-way mixtures of the varieties Agria (A) Laura (L) Rosella (R) and Simone (S) when grown after the pre-crop grass-clover (open symbols) and after winter wheat (closed symbols) in 2002 and 2003. The relative yields of the varieties as compared to the same variety in pure stand are plotted. If the data fall on the diagonal line, the yield of the mixture equals the mean of the pure stands. If mixtures fall to the right of the diagonal they overyield, if they fall to the left they yield less than expected. The relative position of the data points to the perpendicular points indicate how each of the two mixture components performs relative to the same variety in pure stand, the perpendicular lines show the relative yield per variety in pure stands (1.0).

No complete ranking in competitive ability can be made as not all possible two-way combinations were planted. However, when relating the varieties in the different columns from Table 7 to each other a picture emerges when placing the variety with the two mixing partners to the right and to the left:
Rosella>Agria>Laura
Agria>Laura>=Simone
Agria<=Rosella>Simone
Laura>=Simone<Rosella.
Rosella emerges as the best competitor followed by Agria, then Laura and then Simone.

Discussion

Disease severity in mixtures was not reduced substantially. However, reductions were greater after pre-crop winter wheat than after grass clover indicating that mixtures work better at lower disease pressure. Despite the large differences in environmental conditions with a cool and wet year in 2002 and a hot and dry year in 2003, the interactions among varieties with respect to disease, yield and yield components were surprisingly similar in both years.

The greatest differences in susceptibility between varieties were in the Laura-Simone mixture, followed by Rosella-Simone and Agria-Laura. Accordingly, based on the initial hypothesis, the greatest disease reductions should be expected in the mixture of Laura and Simone. In 2003, relative disease reductions in that mixture were greatest while in 2002 disease was only reduced after winter wheat (Table 4), however when considering only the disease severity on Laura in the mixture it was significantly reduced in mixtures with Simone in 2002 and disease progress rates were reduced in both years (Fig.6). It could be that the interactions in mixtures with Simone were affected by the virus infestation. The virus infestation of Simone in 2002 did not reduce its yielding ability relative to the other varieties in pure stands (Table 6), however, it resulted in poor growth and overall a very poor competitive ability in the mixtures resulting in large reductions in all size classes in both mixtures. In contrast, in 2003, Simone overyielded in mixtures with Laura and only the amount of tubers in the large size classes was reduced in mixtures with Rosella. In addition to yield reductions, above ground the Simone plants were considerably smaller than plants of the other varieties in 2002. Thus, considerably more than half the leaf area in the mixed stands was produced by Laura and the epidemiological effects of Simone were likely reduced in 2002 in comparison to 2003. A similar pattern can be observed in the Rosella-Simone mixtures when comparing the two years.

The overall differences in yield between the two years could be due to a combination of effects. While it is likely that soil N_{min} dynamics during May were similar in both years as temperature and precipitation conditions were similar in this period this cannot be determined due to the lack of soil samples in May 2002. The excellent water holding capacity of the deep loess soils at the experimental site became obvious in 2003, as the crop did not suffer of a lack of water at any time despite the fact that the top soil layers appeared completely dry while in 2002 on several occasions the field was excessively wet possibly also causing nutrient leaching as indicated by higher N_{min} levels late in the seasons in 30-60 cm depth than in 0-30 cm (Fig. 3). In addition, in 2003, there were likely no effects of late blight on yield in contrast to 2002. This was shown for the variety Nicola grown at the same site in both years with and without copper applications (Finckh et al, 2005).

The necessity to assign rotation effect to the main plots on the one hand, and the variability in disease pressure among the main plots, on the other hand explains the lack of statistical significance of rotation effects on disease. The consistently lower AUDC and disease progress rates in all treatments in both years indicate that this effect may still be real.

An open question is if the different effects of the nutrient availability on disease are due to differences in crop susceptibility or to the fact that above-ground growth was much denser after grass clover making the micro-climate more conducive to disease. To test this hypothesis, leaves of the varieties Laura, Nicola and Simone were collected from the field in late June 2003 before the start of late blight in the field and spray-inoculated with two different isolates of late blight.

There were clear interactions between variety, isolate and pre-crop (Fig. 8). Both isolates were equally aggressive on Nicola independent of pre-crop. In contrast, Laura was significantly less susceptible to isolate 1 than to isolate 2 ($P<0.01$). Again, there was no effect of pre-crop. A different picture emerged for Simone. The variety was equally susceptible to isolate 1 independent of pre-crop. When inoculated with isolate 2, however, Simone was significantly more susceptible after pre-crop grass-clover than after winter wheat. The interaction was statistically highly significant.

The results show that susceptibility is not only influenced by the growing conditions of the host but also by the specific genetic interactions between each pathogen isolate and each crop genotype. Thus, while the nutritional status

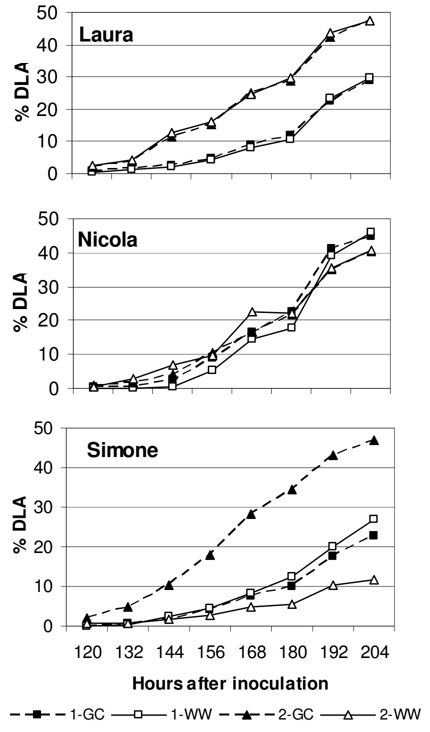

Fig. 8. Effects of pre-crop grass-clover (GC) and winter wheat (WW) on the susceptibility of three potato varieties to two isolates of *P. infestans* (1 and 2). Leaves were detached and spray-inoculated in the laboratory and assessed every 12 hours from day five (=120h) for percent diseased leaf area (DLA).

of the crop does influence the host-pathogen interactions, the influence is crop and pathogen genotype dependent.

There was no evidence that the competitive interactions between the varieties were affected by the nutrient levels provided in our experiments, however, overall nutrient availability was low when comparing to regular conventional growing conditions. Thus, it is well possible that under higher nutrient input conditions competitive interactions may change in general due to the earlier canopy closure and generally higher competition for light.

The fact that significant disease reductions in mixtures occurred if at all after winter wheat, i.e. when disase pressure was lower is in line with observations by Garrett et al. (2001) who observed that mixture effects were generally greater under moderate natural inoculum pressure than under high natural inoculum pressure. Similarly, when planting potatoes in alternating rows, the best results were obtained for the slowest epidemics (Andrivon et al., 2003). While management options that reduce disease pressure are of interest to growers in general, the reductions in yield due to reduced nutrient availability are generally not desirable. The decision as to the rotational position of potatoes in organic farming depends on many factors with yield expectations being dominating. Under the climatic conditions of the experimental site it has been shown that nutrient availability and not *P. infestans* is the dominating yield limiting factor (Finckh et al., 2005; Schulte-Geldermann et. al, 2005) similar to results from the southern part of Germany (Möller, 2000a).

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Section 3: Intercropping

GERMANY (D)

1) Effects of strip intercropping on late blight severity and potato tuber yields in field experiments, 2000-2002

Summary

The effects of strip cropping of potatoes with neighbour cereals or grass-clover on late blight severity and yield of two potato varieties were studied from 2000 to 2002 in large-scale field experiments. Plots were arranged in and perpendicular to the main wind direction. Potato plot size was 3 x 10 m in 2000 and 6 x 18 and 6 x 36 m in 2001 and 2002. From the initial late blight start, percent diseased leaf area was assessed twice weekly in 12 to 18 sections per plot. Tubers were harvested from the same sections.

There were no effects of strip cropping on disease in 2000. However, there were strong spatial patterns within and between plots indicating interplot interferences. When plot size and distance were increased, area under the disease progress curve (AUDPC) was significantly reduced by 9-20 % in 2001 and 4-12 % in 2002. Reductions were highest in plots planted perpendicular to the wind neighboured by grass-clover. The most important mechanism leading to disease reduction is loss of inoculum outside of the plots due to the distances between strips and the barrier function of neighbouring non-potato hosts. The effects of the cropping strategy did not interact with variety. However, yields in the (edge) potato rows directly neighboured by cereals were significantly reduced in all three years.

A maximum of 20 % of the variation in yield could be explained by AUDPC depending on variety and year but there were no effects of competition by cereals on the yield loss relationship. In 2000, the yield of the more resistant but late bulking variety was affected three times stronger than the yield of the susceptible variety indicating that early bulking might be more important than resistance. The lack of correlation between AUDPC and yield in 2002 can be explained by the very dry spring which impeded the mineralization of organic matter in the soil resulting in much reduced nutrient supply to the crop. Thus, nutrient limitation rather than disease were responsible for the reduced yields.

Introduction

Phytophthora infestans (Mont) de Bary, the causal agent of late blight is the most devastating disease in conventional and organic potato production systems. The only effective fungicides against late blight in organic farming are copper based with a maximum application of 3 kg pure copper per ha and season allowed in organic farming in Germany, (Anonymous, 2005). Copper only is effective when applied preventively. However, in Germany prophylactic treatments are not allowed in organic farming and before application the presence of late blight in or near the field has to be confirmed by an advisor. While copper may have a significant impact on late blight epidemics (Hein, 1998; Meinck, 1999; Oxley and Mc Creath, 1995; Schüler et al., 1999; van Bol et al., 1993), these are not always translated into yield increases with results varying from no increase to up to 30% (Meinck, 1999; Möller, 2002; Schüler et al., 1999). On the other hand, copper is a heavy metal that remains in the environment (Brümmer et al., 1986; Filius, 1993) and only 200-300 g per ha and year are taken up by plants as a micronutrient (Filipinsky, 1989; Karalus, 1992). Therefore, copper fungicides are altogether prohibited in the Netherlands and Scandinavia and the EU is aiming at prohibiting copper by 2008 and there is a need for alternatives to copper in organic farming (Phillips et al., 2002; Tamm et al., 2004).

So far, alternative treatments such as plant or compost extracts or mineral based products have failed to reliably reduce the disease under conducive natural field conditions (Hein, 1998; Schmitt, 1997; Stephan et al., 2003). However, when disease pressure was lower, some alternative treatments have been reported to stabilize potato yields (Kürzinger, 1995; Monkos and Gruber, 1998). These results suggest that, if it were possible to reduce disease pressure with preventive strategies, alternative treatments might become more reliable (Kessel et al., 1993).

The most effective strategy for late blight reduction is the use of resistance resulting in later and less severe epidemics (Bouws-Beuermann et al., 2002; Darsow, 2002; Meinck and Kolbe, 1999; Möller et al., 1999; Schmittke et al.1998; Stöppler et al., 1990; Tamm et al., 2004). Unfortunately, resistant varieties often are late maturing (Colon et al., 1995; Umaerus and Umaerus, 1994; Umaerus, 1983; Thurston, 1971). In addition, the market may demand other varieties thus limiting the varietal choice (Dierauer and Speiser, 1998; Möller et al.1996; Tamm et al., 2004).

Besides resistance, field hygiene and the removal of infected seed tubers before planting and especially good microclimatic conditions in the field are important as *P. infestans* is very sensitive to weather and climatic conditions, respectively (Croxall and Smith, 1973; Harrison, 1992; Hirst and Stedman, 1960; Johannes, 1953; Ullrich and Schrödter, 1966). It is generally recommended to plant the crop in the main wind direction to allow for better drying of plants. However, several researchers pointed out that wind is also mainly involved in the spread of sporangia of the pathogen (Aylor et al., 2001; Harrison and Lowe, 1989; Waggoner, 1952). The change from 0.60 m to 0.75 m row distance (i.e. around 40,000 plants*ha^{-1}) in the 1970s has resulted in less favourable microlimatic conditions for *P. infestans* in the fields (Schöber, 1976). However, varying within-row planting densities between 38,000 to 59,000 plants per ha did not affect late blight epidemic development (Karalus, 1998). Only when reducing density to 10,000 plants*ha^{-1} leaving one haulm per plant, a significant late blight reduction has been observed (Rotem, 1973).

Diversity of crops and varieties is known to reduce many air-borne diseases and pests (Finckh and Wolfe, 1997), for example, cultivar mixtures are highly effective in cereals (Finckh et al., 2000). However, with respect to late blight in potatoes results are not straight forward. When growing differentially resistant potato varieties either as mixtures or in alternating rows, higher disease severity on the more resistant component and lower severity on the susceptible one is commonly reported (Andrivon et al., 2003; Beuermann et al., 2000; Finckh, et al., 2003; Garrett and Mundt, 1999; Phillips, 2004; Pilet, 2003) with little or no overall reduction in disease. To avoid such negative effects on resistant varieties it might therefore be more effective to diversify the system by using different crops and arranging them in strips alternating with potatoes. Planting potato strips perpendicular to the main wind direction should lead to loss of inoculum being blown outside the field (Waggoner, 1952) and reduce the chance of inoculum spreading along the rows (Harrison and Lowe, 1989; Waggoner, 1962). When considering strip cropping, the choice of the neighbouring crop will be important. Generally, taller crops might provide a barrier to incoming inoculum. However, shading and turbulence effects (van Arsdel, 1967) may also play a role. Thus, inoculum loss, reduced spread within rows, and an overall reduced density of potatoes through strip intercropping might altogether reduce inoculum pressure within the farming system.

The primary objective of this study was to determine if strip intercropping could be used as a strategy to reduce late blight pressure within an organic farming system. From 2000 to 2002, field experiments were conducted at the organic experimental farm of the University of Kassel, in central Germany. Plots of different varieties were planted in and perpendicular to the main wind direction and with different neighbour crops to address the following questions: (**i**) What are the effects of intercropping of potatoes with crops of different architecture (cereals or grass-clover) on late blight epidemics and tuber yield? and (**ii**) Are there interactions between varietal resistance, plot size and planting direction and late blight? As the final goal of this work is to contribute to stable yields special attention was paid to possible effects of the neighbour crops on potato yields and the interactions of these effects with yield losses due to disease.

Material and Methods

General conditions at the experimental site

Field experiments were conducted at the experimental organic farm of the University of Kassel which is located 10 km north of Kassel in central Germany. The experimental site is characterized by deep rich loamy silty soils. The annual mean precipitation is around 720mm and mean air temperature is 8.1 °C.

Temperature, humidity, solar radiation and wind direction were recorded at the experimental site in 2001 and 2002 with a datalogger of type Combilog 1020 (T. Friedrichs & Co). In 2000, data were taken with semi-automatic devices and completed with data obtained from the German weather service station 5km away (http://www.dwd.de/FundE/Klima/KLIS/daten/online/nat/ausgaben_monatswerte.html).

For all experiments fields with a legume as pre-crop were chosen. Soils were ploughed after the pre-crop in early spring and the seed beds prepared shortly before planting. Hilling (as mechanical weed control) was performed two times before canopy closure. Depending on the year and field conditions planting dates varied by three weeks (Table 1).

Table 1. Field operations and pre-crops of intercropping experiments, 2000-2002.

Field parameters	**2000**	**2001**	**2002**
pre-crop	faba beans	peas	grass-clover
planting of potatoes	April 26	May 6	May 16
Hilling	June 14/June 25	June 20/July 3	June 25/July 9
tuber harvesting	2nd week Sept	3rd week Sept	1st week Sept

Experimental design

All experiments were arranged as split-plot trials with four replications. In 2000, planting direction was assigned to the main plots to simplify the field operations. Variety and neighbour were assigned to the subplots (Table 2). Two potato varieties, Secura and Simone (susceptible and moderately resistant against late blight, respectively) were planted with four different neighbour crops: spring oats, potatoes, mulched and unmulched grass-clover.

Table 2. Experimental design of potato strip intercropping experiments, 2000-2002.

Factorial design	**2000**	**2001/2002**
main factor	planting direction	potato variety
subplot factors	variety x neighbour	neighbour x (plot size x direction)
planting direction	East-West (= in) and North-South (= perpendicular to wind)	
suceptible variety	Secura	Linda
resistant variety	Simone	Agria
neighbour crop	potatoes spring cereals grass-clover, mulched	
	grass-clover, unmulched	--
plot size [1]	3 m x 10 m	6 m x 36 m (in wind) 6 m x 18 m (perpendicular)
distance between blocks [2]	6 m grass-clover + 6 m cereal	12 m grass-clover + 12 m cereal
total no. of plots	64	56

[1] In 2001 and 2002, the smaller plots planted perpendicular to the main wind direction were arranged crosswise as downwind and upwind plots (see Figure 1).

[2] Blocks were arranged perpendicular to the main wind direction: from upwind (replication I) to downwind (replication IV) in 2000 and 2001 and as two by two pattern in 2002.

Subplots were arranged in four long rows in north-south direction, each row accommodating one replication. The rows were separated by 12 m consisting of 6 m of spring barley with 3 m of regularly mulched grass-clover on either side. Subplots were 10 m long with a central plot of four rows (3 m) of potatoes and two 3 m wide plots of the respective neighbour crop on either side. If the neighbour crop was potatoes, 3 m of grass-clover were inserted before the next subplot to ensure that the distance between potatoes in the subplots was always 6 m. The experimental size was in total 1.5 ha (220 m x 72 m).

As the spatial distribution of disease within and between plots in 2000 suggested that interplot interference was large, especially between the two varieties and that the plots were too small to detect patterns within plots, in 2001 and 2002, variety was assigned as main plot factor and plot sizes and distances were increased (Table 2). As this resulted in a total potato area of about 1.2 ha the susceptible variety Linda and the moderately resistant Agria were chosen to match the varieties grown on the organic farm to avoid logistical problems. Also, the certified seed potatoes were produced and stored on the farm, ensuring consistent quality. The number of neighbour crops was reduced to three with potatoes, spring wheat and mulched grass-clover. Plot size was 6 m x 36 m, however, the plots with neighbour spring wheat and grass-clover that were arranged perpendicular to the main wind direction were split in two and arranged crosswise so that one half of each treatment was located upwind, the other downwind of the other (Figure 1). Also, the treatment with neighbour potato perpendicular to the wind had to be omitted for space reasons.

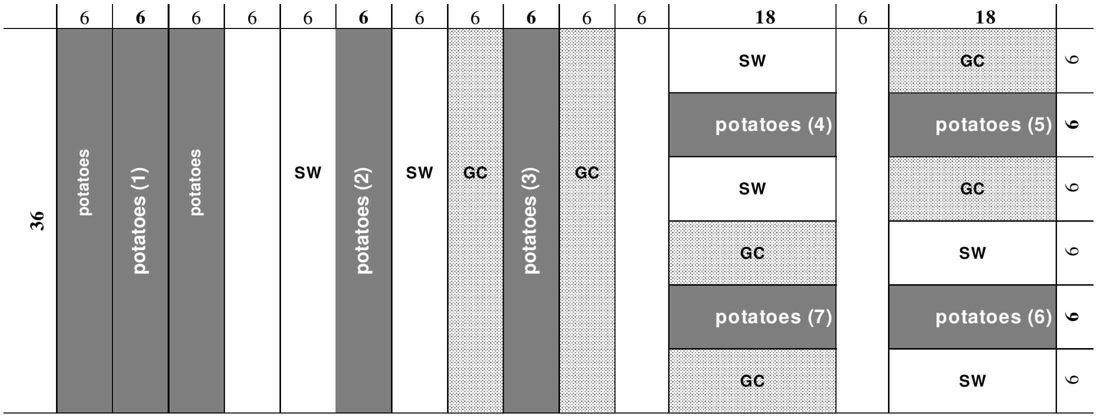

Figure 1. Schematic arrangement of experimental treatments in 2001 and 2002, representing one variety. Neighbours were either potatoes (dark grey), mulched grass-clover (GC, light grey), or spring wheat (SW, white). The main wind direction was along the long plots (treatments 1-3), while perpendicular planted plots were arranged upwind (4 and 5) and downwind (6 and 7), respectively. Plot sizes and distances are marked in m.

Subplots were again arranged in long rows in north-south direction with 12 m distance between potatoes. In east-west direction, the distance was increased to 24 m with 12 m spring wheat in the centre. To avoid a low-lying section of the field in 2001, plots were arranged in four rows. In 2002, a long enough field was available to allow for two long rows of plots. Experimental size was 4.5 and 4.2 ha in 2001 and 2002, respectively.

Assessments

Late blight was assessed as percent diseased leaf area (DLA) following the scheme given by James (1971). In 2000, each potato row was divided into 3 equal sections (12 assessments per plot) which were individually assessed every 3-4 days once the disease had started. In the following years, assessments were done in 6 m sections. In 2001, each plot was divided into the left and right half with four potato rows each, resulting in a total of 12 and 6 observations for the large and small plots, respectively. In 2002, the edge rows of the plots were assessed separately and the four central (inner) rows of each plot together, resulting in a total of 18 and 9 observations in the large and small plots, respectively. After detection of first late blight lesions in at least one experimental plot, assessments were made 12, 11 and 11 times in 2000-2002, respectively.

Harvest and sorting of tubers

In 2000, potato tubers were harvested per row. In 2001 and 2002, tubers were harvested from the same 6 m sections where late blight had been assessed. From each plot, the two outer potato rows and two of the inner rows (selected randomly) were harvested. The tubers were separated into the size classes (i) <30 mm, (ii) 30-60 mm (marketable yield) and (iii) >60 mm and weighed separately.

Data processing and analysis

Each field experiment was analysed separately. The area under the disease progress curve (AUDPC) was calculated according to Kranz (1996). AUDPC was determined for each assessment location within the plots and the mean per plot calculated. Because late blight assessments in 2002 were carried out for the outer rows separately, for the calculated plot mean the values of the assessments of the inner rows were weighted each three fold in contrast to the single outer rows.

Tuber yields were calculated in $t*ha^{-1}$. As a high proportion of edge rows are a result of strip cropping, their yield was included in the yield analysis. With two inner and two outer rows in 2000, the means of all four rows was used. In 2001 and 2002, mean tuber yield was calculated out of one-fourth outer and three-fourth inner rows.

All statistical analyses were carried out with SAS software (SAS Institute, Cary, NC). In 2000, late blight and yield were analysed with the GLM procedure as a split plot with wind as main-plot and variety by neighbour crop as subplot. If significant effects were detected, means were compared with DUNCAN-test.

With the increased plot size and distance in 2001 and 2002 the spatial distribution of disease across the experimental area became very uneven. In a separate study, the spatial patterns of disease development were modelled by the integration of polynomial trends into the MIXED models used for analyses. Inferences for treatment effects could then be made adjusted for spatial trends in the field (Büchse, et al. 2004).

Modelling analyses of the large-scale trends of the experiment revealed that the block effect could be neglected in both years. However, errors due to main factor variety, subplot, and autocorrelation among neighbouring assessment points were included into the final ANOVA model (equation 1) on AUDPC data. The SAS procedure used was Proc MIXED (LSMeans/pdiff).

$$y_{ijh} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + f_{ih} + g_{ihk} + \gamma\ (\mathrm{NS,OW}) + \lambda_1\ (\mathrm{OW\_P}) + \lambda_2\ (\mathrm{OW\text{-}P})z + e \qquad (1)$$

with α_i = main effect of i^{th} variety
β_j = main effect of j^{th} treatment (i.e. planting pattern x neighbour crop)
$(\alpha\beta)_{ij}$ = interaction of variety x treatment
f_{ih} = main error (variety x block)
g_{ihk} = subplot error (variety x subplot x block)
γ (NS,EW) = large-scale trends of the field in North-South and East-West direction
λ_1 (EW_p) = East-West gradient within a potato plot
λ_2 (EW_p)z =East-West gradient among treatments (s_plot and ss_plots, see below)
e = error of each individual assessment point with spatial variance-covariance matrix (for more detail see Büchse et al., 2004).

AUDPC in the eastern most and western most sections within treatments were compared as a two-factorial design with treatment x section with GLM (comparison-wise t-tests). Variety x planting direction x neighbour were included in the factor treatment. The upwind and downwind plots in 2001 and 2002 with the same neighbours were combined for the analysis (i.e. a total of 12 and 10 different treatments in 2000 and 2001/2002, respectively, see Table 5).

Potato tuber yields were also analysed with Proc MIXED. The SAS code was as following:

```
Proc MIXED;
Class  rep  variety  treatment  size  s_plot  ss_plot;
Model tha= rep  variety  treatment  variety*treatment / ddfm= satterth;
      random variety*rep  variety*rep*s_plot;
      repeated variety*rep*ss_plot/group=size;
LSmeans  variety  treatment  variety*treatment / pdiff;
```

By using repeated, the error variance is calculated separately for the different plot sizes. The plot error is divided into two parts: s_plot (subplot) error (large versus small plots) and ss_plot (subsubplot) error (small plots arranged crosswise upwind and downwind to each other). The large plots have one s_plot and one ss_plot each, while the two crosswise small plots of same neighbour crop share one s_plot but have different ss_plots.

For estimation of competition effects of the neighbour crop on tuber yield the yield data of inner (central) and outer (edge) potato rows were analysed with factors neighbour by row (inner/outer) by either i) wind or ii) variety. As no interactions were detected, the model of GLM procedure was reduced to neighbour x row. In case of significant differences, LSMeans were compared pair-wise with t-tests. Correlation and simple linear regression analyses (Proc CORR and REG) were used to determine the relationship between late blight severity (AUDPC) and potato tuber yield.

Results

General conditions at experimental site

Most climatic differences among years were due to monthly precipitation and radiation period, respectively. The characteristics of each year were as following: Months June to August in 2000 were cool and rainy with mean 14.9 °C and 102 mm rainfall in July (Table 3). Due to continuously severe rainfall events in July late blight destroyed the susceptible plots within two weeks (Figure 2a). While June 2001 was relatively cool and cloudy, April and July were relatively dry (Table 3). After rainfall events around July 10th late blight started a few days later in 2001 and in 2002 (Figure 2).

Table 3. Monthly mean air temperature (°C), precipitation (mm) and radiation (h) at the experimental site from March to August in 2000-2002.

	2000			**2001**			**2002**		
Month	° C	mm	*h*	° C	mm	*h*	° C	mm	*h*
March [1]	6.0	72	*83*	**4.0** [2]	90	***44***	5.6	**41**	*114*
April	10.7	34	*161*	7.5	49	***116***	7.6	**13**	*167*
May	15.0	47	*229*	14.2	**25**	*252*	**13.2**	49	***129***
June	16.9	83	*224*	**14.9**	79	***151***	16.5	**43**	*181*
July	**14.9**	102	-	18.4	**59**	*223*	16.9	106	***133***
August	17.8	**71**	*209*	18.6	48	*205*	18.5	**35**	***171***

[1] Measurements were recorded in intervals of ten seconds and means were calculated per day (data not shown) and per month.

[2] Bold marked values within rows (months) point out extreme values of climatic parameters either max. or min. compared among years. Stroke indicate missing data.

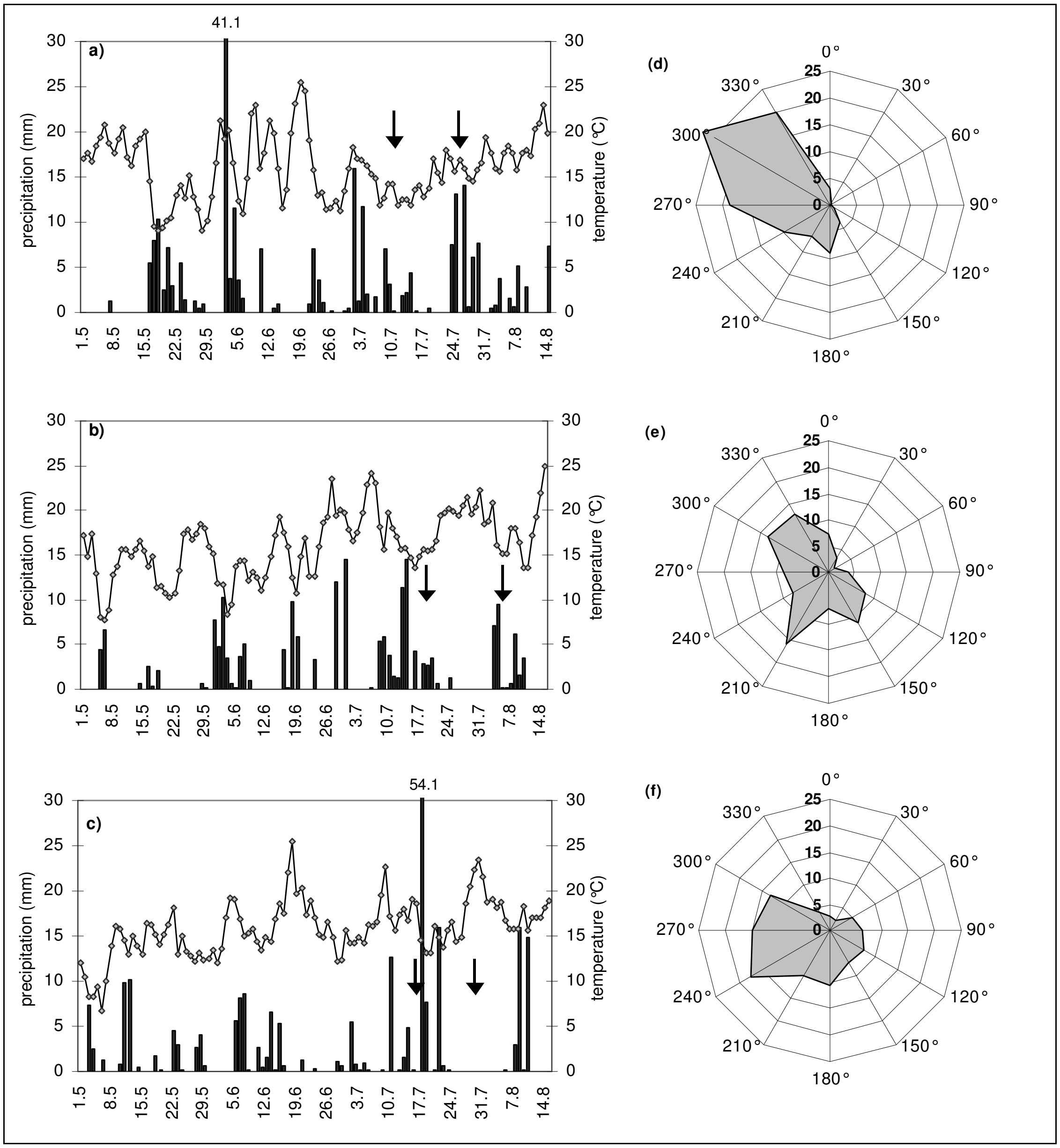

Figure 2. Daily precipitation (mm) and mean air temperature (°C in 2 m above ground) at the experimental site from 1st of May to 15th of August in 2000 (a), 2001 (b) and 2002 (c).

Relative distribution (%) of wind direction (North=0/360°, 90°=East, 180°=South and 270°=West) in 2000 (d), 2001 (e) and 2002 (f) during late blight epidemic, i.e. the period from 1 to 75 % DLA (as indicated by the black arrows in the respective figures on the left)

In 2002, because of wet weather in early May (Figure 2c), planting and thus emerge of potatoes was delayed. While May and July were cool and cloudy the overall precipitation was low until June in this year. In all three years, during July and August south-western to western winds prevailed (Table 3).

Disease development

While first lesions in the experimental plots were detected around the 11th of July in 2000 and 2002. As a result of severe rainfall events in mid-July 2000 late blight progressed fast in susceptible Secura resulting in around 75 % DLA within only one week (Figure 3a) and the highest AUDPC among varieties and years (Table 4). In contrast, on the moderately resistant Simone exponential disease increase started 10 days later in late July and it progressed more slowly (Figure 3a). In 2001 first late blight symptoms were found sporadically in the 1st decade of July, however, 1 % DLA was noticed around the 20th of July 2002.

Generally, around 1 % DLA was observed for both varieties Agria and Linda at the same date in both 2001 and 2002. Due to long-term hot and dry weather conditions from mid-July to mid-August 2001, late blight development in 2001 was slow and before 100 % DLA were reached on Agria the crop started senescing by mid-August (Figure 3b). Agria was killed by late blight in 2002, however (Figure 3c).

Consequently, AUDPC on Agria in 2002 was nearly twice that of 2001 (Table 4). As a result of regular rainfall events in mid-July 2002 (Figure 2b), disease severity increased rapidly from 25th to 30th of July resulting in an increase from 25 to about 80 % DLA on Linda within five days (Figure 3c)

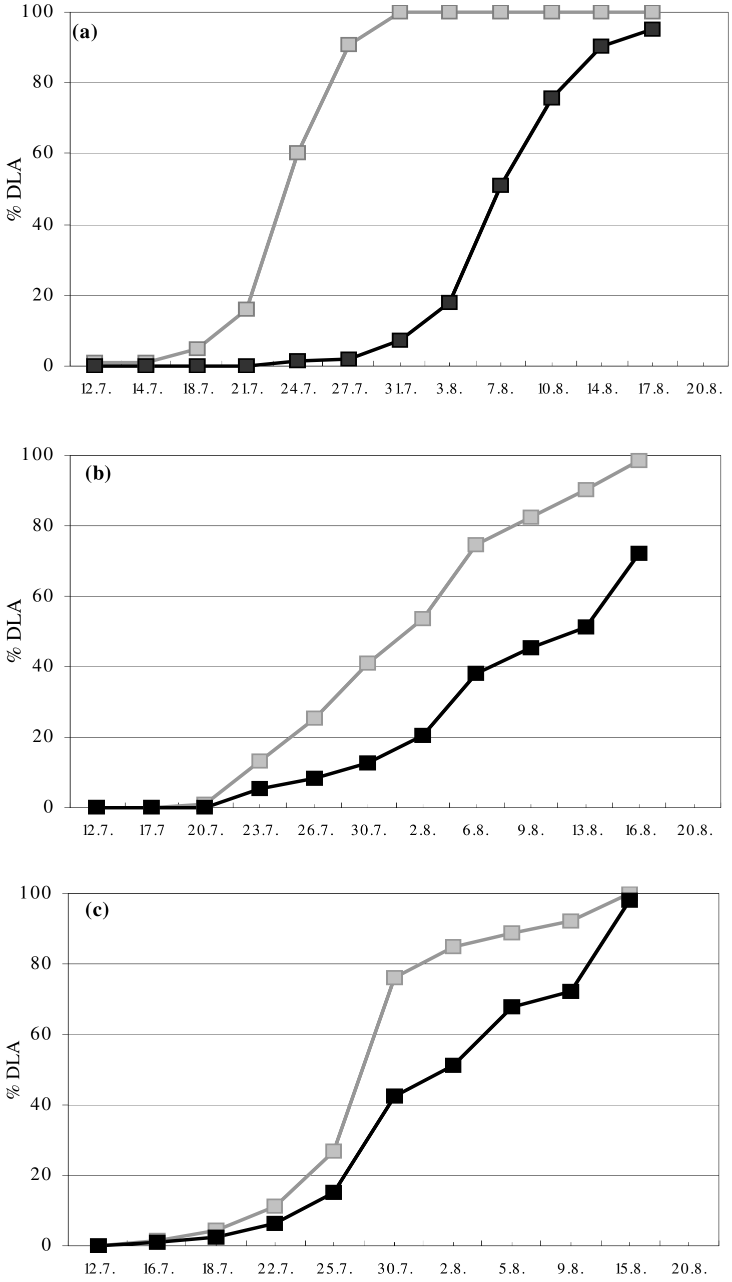

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Figure 3. Mean late blight severity (% diseased leaf area) over time in potato plots planted with the susceptible variety Secura in 2000 and Linda in 2001 and 2002 (grey) or the moderately resistant variety Simone in 2000 and Agria in 2001 and 2002 (black) 2000 (a), 2001 (b) and 2002 (c).

Table 4. Effects of potato variety, planting pattern and neighbour crop on area under disease progress curve (AUDPC) of late blight epidemics, 2000-2002.

potato variety		**2000**	**2001**	**2002**
susceptible		2471 A[1]	1459 A	1767 A
moderately resistant		1053 B	723 B	1308 B
planting direction x **neighbour crop**[2]				
in wind	potatoes	1786 a	1264 a	1654 a
	cereals	1813 a	1076 b	1564 bc
	grass-clover, mulched	1751 a	1105 b	1513 cd
	grass-clover, unmulched	1773 a	-	-
perpendicular	potatoes	1756 a	-	-
	cereals (upwind)	1766 a	1025 b	1519 bcd
	cereals (downwind)		1005 b	1588 ab
	gr.-clover, mulched (upwind)	1747 a	1016 b	1475 de
	gr.-clover, mulched (downwind)		1147 ab	1449 e
	gr.-clover, unmulched	1703 a	-	-

[1] Data for 2001/2002 are cleared of spatial trends (Büchse et al., 2004). Different letters within columns indicate significant differences between mean values of varieties or treatments, respectively ($P<0.05$, GLM and Duncan in 2000, Proc Mixed with pdiff in 2001 and 2002, respectively).

[2] In 2000, all plots were 3 m x 10 m long while plot size varied between 6 x 36 and 6 x 18 m in 2001 and 2002, when planted in or perpendicular to the prevailing wind direction, respectively (see Figure 1).

Due to the arrangement of replications as two by two blocks the epidemic pattern was less variable in 2002 than in the two years before. While there were highly significant replication effects in 2000 and 2001 ($P<0.001$) with increasing AUDPC from upwind to downwind lying blocks, no such effects were observed in 2002.

Overall, interferences between plots and blocks were larger in 2000 than in 2001 and 2002. In 2000, a random accumulation of Secura plots in the north-western corner of the experiment promoted late blight development in the neighbouring plots of Simone. With main factor potato variety in 2001 and 2002, interferences between varieties were reduced. As barrier strips between plots and blocks were expanded in 2001 and 2002, interferences between blocks and plots were much reduced as shown by geostatistical analyses and semivariograms (Büchse et al., 2004; Finckh et al, 2004).

Overall, there were no significant differences in disease severity between treatments in 2000. However, there was a pattern discernible across the experimental area: In the first replication located upwind, AUDPC in susceptible plots neighboured by potatoes was 37-44% higher than in plots neighboured by other crops. The differences between treatments became successively smaller while overall disease increased in the downwind located replications two to four (data not shown).

In 2001 and 2002, the highest AUDPC was observed in the large potato plots which were planted in wind direction and neighboured by potatoes (Table 4). In both years, AUDPC in the large plots planted in wind was significantly higher than in the small plots perpendicular to the wind located upwind (linear contrasts, $P=0.03$). While in 2002, disease was lowest in plots with mulched grass-clover as neighbour crop (relative AUDPC between 0.88 and 0.91) in 2001 reduction was greatest with neighbour cereals (rel. AUDPC 0.80-0.85). There were no significant differences in AUDPC between the upwind and downwind plots with the same neighbour in both years.

There were strong disease gradients from west to east in ten out of the twelve 36 m long plots planted in wind direction in 2001 and 2002. No clear gradients were observed in 10 m long plots in 2000 that were planted with the wind (Table 5). In the plots planted perpendicular to the wind with neighbour grass clover disease severity was higher in the east than in the west row except for the Simone plots in 2000 in which overall severity was quite low. In contrast, when neighboured by cereals, the gradients were reversed in 2000 and 2002. In 2001, assessments were not carried out in the edge rows separately. While the differences were clearly visible in the field, unfortunately only data of the left and right half of the plots were taken. These do not show these differences.

Table 5. Area under the disease progress curve (AUDPC) of upwind (west) or downwind (east) located rows/sections within potato plots[1]. Two different varieties were planted in and perpendicular to the main wind direction and arranged with different neighbour crops, 2000-2002.

	2000		**2001**		**2002**	
neighbour x planting direction	west	east	west	east	west	east
Suceptible variety	Secura		Linda		Linda	
potatoes – **in wind**	2449	2438	1550	**1802**	1654	**1850** **
grass-clover – in wind	2510	2525	1400	**1521**	**1762**	1636
cereals – in wind	2560	2522	1245	**1450**	1657	**1862** **
potatoes – **perpendicular**	2426	2482	-		-	
grass-clover – perpendicular	2273	**2520** $^{(*)}$	1408	**1531**	1597	**1670**
cereals – perpendicular	**2568**	2350	1355	1352	**1870** $^{(*)}$	1721
moderately resistant variety	Simone		Agria		Agria	
potatoes – **in wind**	1140	1120	748	**853** $^{(*)}$	1264	**1404** $^{(*)}$
grass-clover – in wind	977	1039	562	**997** $^{(*)}$	1169	**1311** $^{(*)}$
cereals – in wind	1105	1074	681	615	1261	**1355**
potatoes – **perpendicular**	1068	1063	-		-	
grass-clover – perpendicular	990	980	672	757	1180	**1238**
cereals – perpendicular	**1130**	1034	679	699	**1344**	1219

[1] Sections along rows were 3 m in 2000 and 6 m in 2001/2002. Assessments were carried out separately in the two edge rows of the plots in 2000 and 2002. In 2001, only the left and right half of each plot were assessed separately. Thus, the western or eastern sections for the in wind planted plots represent the 3 m sections of 10 m long plots in 2000 and the 6 m sections of 36 m plots in 2001 and 2002, respectively. For the plots planted perpendicular to the wind data for the edge rows are presented for 2000 and 2002. For 2001, only the two halves of the plots could be compared.

(*), ** indicate that differences between eastern and western sections were significant at $P<0.10$ or $P<0.01$, respectively (t-test)

Potato tuber yields

Total tuber yields were on the same level in 2000 and 2001, while yield in 2002 was only half that of the years before. In 2002, the proportions of non-marketable small tubers (<30 mm) were 44 % and 20 % of the total yield of Linda and Agria, respectively (Figure 4). In the following, marketable tuber yields are presented.

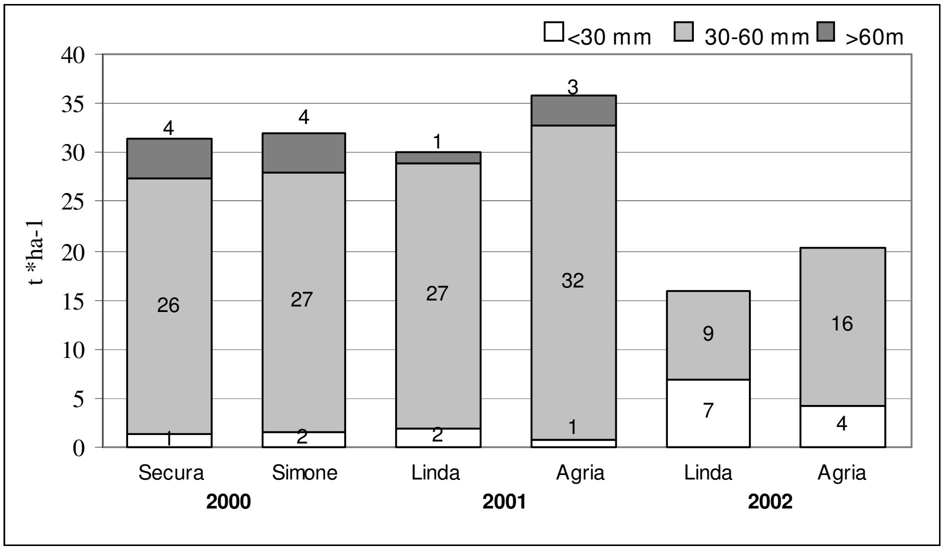

Figure 4. Total yield (t*ha^{-1}) subdivided into different tuber size classes ranging from <30 mm to >60 mm of potato varieties Secura and Simone (2000) and Linda and Agria (2001 and 2002), respectively.

Table 6. Effects of potato variety, planting pattern and neighbour crop on marketable potato tuber yield [1] (t*ha^{-1}) of intercropping experiments, 2000-2002.

potato variety		**2000**	**2001**	**2002**
susceptible		26.1 A [1]	27.0 B	8.9 B
moderately resistant		26.5 A	32.0 A	15.6 A
planting direction x **neighbour crop** [2]				
in wind	potatoes	25.8 a	29.9 ab	12.5 ab
	cereals	25.3 a	29.5 abc	10.7 c
	grass-clover, mulched	26.2 a	29.8 ab	12.8 ab
	grass-clover, unmulched	27.1 a	-	-
perpendicular	potatoes	26.7 a	-	-
	cereals (upwind)	26.0 a	30.3 a	12.1 b
	cereals (downwind)		27.7 c	12.1 b
	gr.-clover, mulched (upwind)	26.4 a	30.6 a	12.4 ab
	gr.-clover, mulched (downwind)		28.6 bc	13.3 a
	gr.-clover, unmulched	26.7 a	-	-

[1] Different letters within columns and year indicate significant differences between means of varieties or treatments, respectively ($P<0.05$, GLM and Duncan in 2000, pdiff in Proc Mixed 2001 and 2002).

While there were no differences in marketable yield between Secura and Simone in 2000, marketable yield of Agria was significantly higher than that of Linda in 2001 and 2002 (Table 6). In 2002, marketable yield of Linda was only one third of 2001 and that of Agria was half (Table 6). In all three years, the highest yields were obtained in one of the treatments with neighbour grass-clover, while the lowest yields were observed in plots neighboured by cereals. Overall, these differences were rarely significant, however (Table 6).

A more detailed analysis comparing the yields of the potato rows neighboured by the different crops (outer rows) with the yields of the central rows in the plots revealed that differences between the whole plots were mostly due to significant yield reductions in the rows neighboured by cereals (Table 7).

Table 7. Total tuber yield ($t*ha^{-1}$) of potato plots planted with different neighbour crops in strip intercropping experiments, 2000 to 2002.

	2000		**2001**		**2002**	
Neighbour crop	inner rows [1]	outer rows [1]	inner	outer	inner	outer
potatoes	30.8	29.9 b [3]	33.4	33.5 a	18.1	17.9 a
grass-clover, mulched	30.4	32.1 a	33.5	32.7 a	18.9	18.3 a
cereals [2]	30.7 **	28.0 c	33.6**	28.7 b	19.0**	15.7 b

[1] Total tuber yield ($t*ha^{-1}$) of inner (central) and outer (edge) potato rows, i.e. in direct contact to the neighbour crop.
[2] Spring oats (2000) and spring wheat (2001-2002) were sown as cereal neighbour crop.
[3] Numbers within columns followed by different letters are statistically different at $P<0.01$ (LSMeans/pdiff).
** differences between inner and outer potato rows were significant at $P<0.01$ (linear contrast).

Yield loss relationships

As yield loss relationships in the literature are commonly calculated for total tuber yield (James, et al., 1972; Rotem et al., 1983) the same was done here. The effects of disease on total tuber yield varied between variety and years (Figure 5). About 20 % of the variation in yield in 2000 and 2001 could be related to AUDPC but not in 2002. Although in 2000 AUDPC was much higher for Secura, the slope of the regression line was much shallower
(-0.06) than for Simone (-0.19). Slopes were more similar for Agria and Linda in 2001.

The negative effects of the cereals on yield in the edge rows could be due to competition or to increased disease in the edge rows. In addition, competition could lead to changes in yield-loss relationships due to additional stress on plants already suffering from disease. When considering the mean of both outer rows, on average, disease in inner and outer rows was not different in the cereal plots (data not shown). Also, comparing the regressions of yield on AUDPC between rows neighboured by potatoes versus cereals or grass-clover did not result in a clear pattern (Table 8). While in 2000, with cereals the slope became somewhat steeper for the susceptible Secura and R^2 increased, the pattern was reverse for the moderately resistant Simone. With susceptible Linda and moderately resistant Agria the patterns in 2001 were again different.

The R^2 values were similar for the correlation between AUDPC and marketable yield of susceptible/moderately resistant varieties: 0.16/0.17 (2000), 0.23/0.13 (2001) and 0.00/0.11 (2002), respectively.

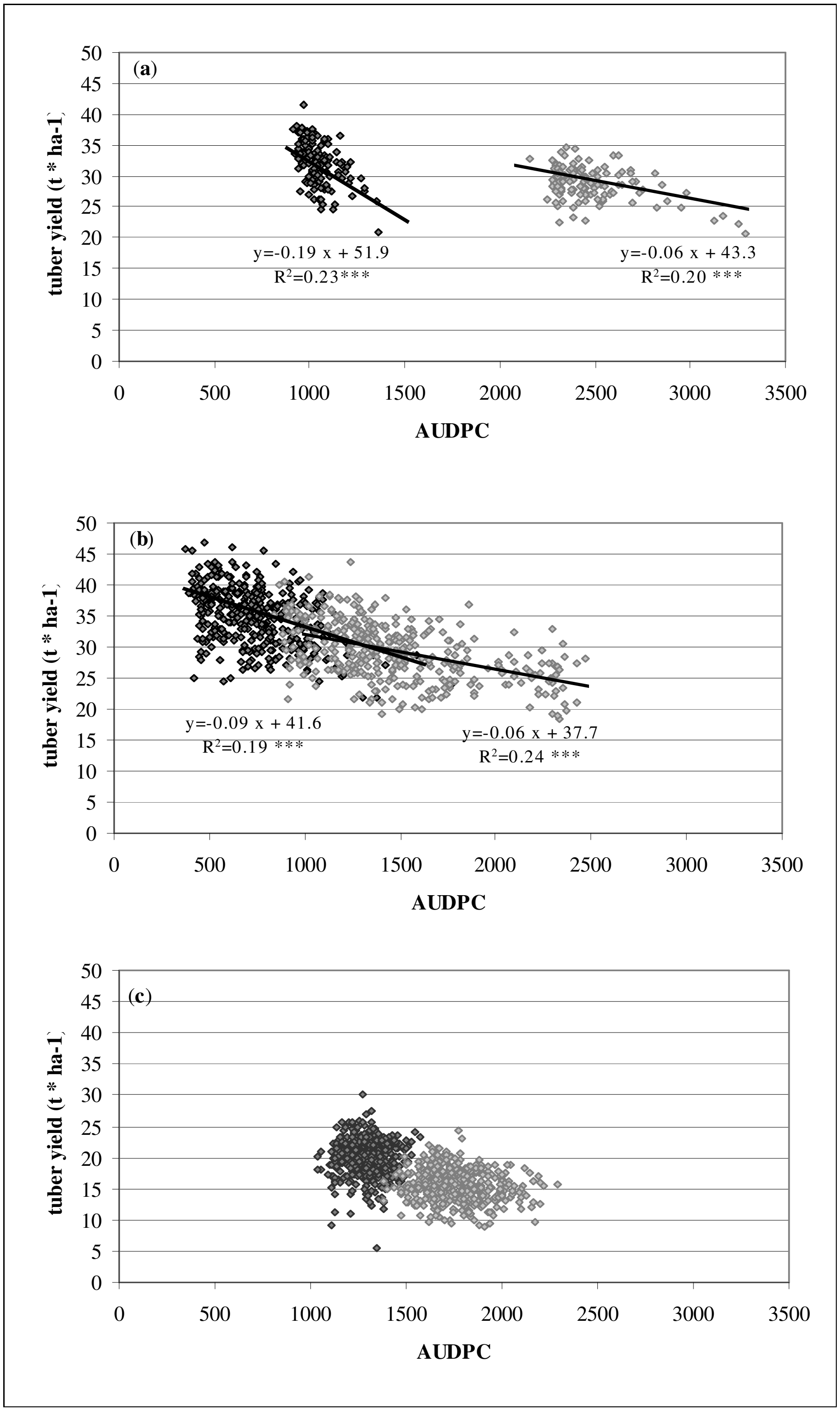

Figure 5. Correlation of area under disease progress curve of late blight (AUDPC) and tuber yield ($t*ha^{-1}$) in potato plots planted with susceptible varieties Secura in 2000 and Linda in 2001 and 2002 (grey) or moderately resistant varieties Simone in 2000 and Agria in 2001 and 2002 (dark) 2000 (a), 2001 (b) and 2002 (c).

Table 8. Parameters of linear regression[1] and correlation of tuber yield with AUDPC of susceptible and moderately resistant potato varieties [2] neighboured by different crops, 2000-2002.

neighbour crop x row [3]	**susceptible**			**moderately resistant**		
2000	**b**	**y_o**	**R^2**	**b**	**y_o**	**R^2**
potatoes	-0.005	42.1	**0.15** **	-0.022	51.4	**0.30** **
cereals	-0.007	44.0	**0.73** **	-0.003	32.0	0.02
grass-clover	-0.007	47.7	0.23	-0.042	76.0	0.22
2001						
potatoes	-0.006	40.3	**0.39** **	-0.011	44.8	**0.33** **
cereals	-0.005	33.7	**0.14** **	-0.009	37.3	**0.33** **
grass-clover	-0.008	41.3	**0.50** **	-0.011	43.6	**0.31** **
2002						
potatoes	+0.001	18.8	0.01	+0.000	14.8	0.05
cereals	-0.002	17.3	0.03	-0.003	21.1	0.01
grass-clover	-0.002	20.1	0.01	+0.006	12.7	0.06

[1] Linear regression function y=bx+yo with b=slope of regression function and intercept y_o. Correlation coefficients R^2 are shown. ** indicate correlation was significant at $P<0.001$.
[2] In 2000, Secura and Simone and in 2001 and 2002, Linda and Agria were planted as susceptible and moderately resistant potato varieties, respectively.
[3] Tuber yield of inner potato rows (neighbour potato) in contrast to outer rows with non-potato host as neighbour, i.e. cereals or grass-clover.

Discussion

When tested in large experimental plots in 2001 and 2002, strip intercropping generally reduced late blight disease pressure as well with grass clover as with cereals as neighbour. Planting the strips perpendicular to the wind in smaller plots in most cases led to further disease reductions. No such effects could be observed in 2000, when the plots had been much smaller. The neighbour crops greatly affected the spatial patterns in disease development when the crop was planted perpendicular to the wind. While disease was most severe in the downwind parts of plots when neighboured by grass clover the opposite pattern was observed in plots neighboured by wheat. While varietal resistance was the strongest factor affecting disease, cropping pattern and neighbour crop did not interact with the varieties. Overall yield levels were strongly affected by year and variety and there were clear competitive effects of cereals as neighbouring crop on potatoes. Depending on the year late blight had moderate to no effect on yield.

The effects of strip intercropping especially with grass-clover on late blight can at least be partially explained by reduced inoculum interchange between potatoes planted in strips. Geostatistical analyses of Büchse et al. (2004) show that the replication effects in the first year led to interference between plots reaching up to more than 12 m. Semivariograms indicate, however, that the influence between points in a field becomes negligible at distances of around 20 m within the prevailing wind direction and about 12 m perpendicular to the wind (Büchse et al. 2004). Therefore, clear disease gradients were visible in the downwind sections of the 36 m long plots in 2001 and 2002 but not in the short plots of 10 m length in 2000. Thus, growing strips of potatoes perpendicular to the wind separated by at least 12 to 20 m should decrease the overall epidemic pressure in comparison to a regular potato field. These findings confirm the studies and predictions of Waggoner (1952; 1962) which indicated that planting in wind direction promoted late blight spread into the field, whereas perpendicular arrangement of generally narrower plots resulted in disease reductions.

Besides loss of inoculum, disease reduction in smaller potato plots might also be due to an overall reduced density of potato plants, i.e. genotype unit area (GUA) (Garrett and Mundt, 2000) and barrier effects. While the intercropped neighbour (barrier) crops may catch or filter out some of the inoculum, the chances of incoming inoculum to fall onto a plot are also reduced. In an earlier experiment comparing disease dynamics in two-row potato plots with four-row potato plots that were separated by at least 40 m within a large cereal field, disease was reduced in the small potato plots. The reductions were mostly due to delayed onset of the epidemic in the small plots while disease increase was almost the same in all plots once it had started (Beuermann, et al. 2000). Similar observations were made by Phillips (2004) who found that disease in larger potato plots was significantly higher than in smaller plots.

The barrier effects of cereals and grass-clover in general might be similar as indicated by the fact that disease severity in the small plots lying upwind and downwind did not differ significantly. The relatively large difference between the respective grass-clover plots in 2001 might be explained by the fact that grass clover was mulched down relatively early and thus its barrier function was removed allowing for free entry of spores into the potatoes.

The opposite spatial patterns within the potato plots perpendicular to the wind when neighboured either by grass-clover or cereal neighbour may be due to a combination and interaction of mechanisms such as microclimatic changes and turbulences as influenced by architecture of the neighbour crop. The duration of conducive conditions within the canopy (i.e. minimal 90 % relative humidity and 15 to 19 °C) during the period from 14th of July to 16th of August 2001 in plots neighboured by cereals was 156h in the upwind rows compared to 134 h in the downwind rows. When neighboured by grass-clover the overall number of conducive hours was lower and the pattern reversed with 114 h upwind and 130 h downwind (Finckh et al., *in preparation*). Generally, the same tendencies were observed in both potato varieties. As cereals are taller than potatoes it is possible that turbulences will arise causing incoming air currents to possibly fall faster into the crop than would be expected. That could lead to an increased deposition of spores in the upwind row. Effects of plants of different heights on spore deposition were observed with white pine blister rust along forest edges (van Arsdel, 1967). Such effects would be synergistic with the more conducive microclimatic conditions in the upwind rows neighboured by wheat.

The data show that varietal resistance clearly remains the most important strategy for managing late blight. While the varieties used cannot be compared directly, when comparing the reactions of the varieties in 2000 and 2002, the two years with relatively high epidemic pressure it can be assumed that probably Simone is overall more resistant than Agria, and Secura is more susceptible than Linda, respectively. The epidemic on Secura lasted 13 days (from 1% to 75% DLA) compared to around 20 days for Linda and around 26 days for Agria each year, respectively. While blight resistance is an important criterion for selecting a variety (Meinck, 1999; Möller et al., 1999; Tamm et al., 2004; Phillips, 2004) the lack of differences between Secura and Simone shows that it may not necessarily lead to higher yields.

The low yields in 2002 and the high percentage of tubers below 30 mm diameter were mostly due to climatic conditions. Because in organic farming no soluble mineral fertilisers can be applied, the potato cropping system depends very much on warm soil temperatures with adequate moisture that allow for mineralization of organic matter (Möller, 2001; Neuhoff, 2000). The low precipitation from March to June combined with the cool temperatures in May apparently resulted in low nutrient availability. The effects were probably exacerbated by the delayed planting. In a parallel experiment about 40 km east of our experimental site, soil nitrogen-dynamics below normal were recorded in 2002 also resulting in very low tuber yields at that experimental site (Schulte-Geldermann et al., 2005). Similar conditions were reported to be responsible for low tuber yields in experiments of Rotem et al. (1983). These findings support the theory of Möller (2001), that often, nitrogen supply is the limiting factor in organic potato crops, whereas the role of late blight may often be overestimated.

Only few data on the effects of late blight on yield are available for conventionally grown potato crops (James et al., 1972; Large, 1952; 1958; Rotem et al., 1983). When comparing sprayed and non-sprayed plots with DLA between 12 and 65 % Rotem et al. (1983) reported an R^2 of 0.91 between disease and yield.. However, the correlation was based on the yield of only 10 single plants per plot. In the present study the correlations were based on hundreds of data points. Multiple regression of yield on weekly disease increments in plots subjected to different fungicide spray schedules resulted in estimated yield losses ranging between 23 to 55 % compared to 24 to 52 % actually measured yield loss over nine years (James et al., 1972). Based on the assumption that tuber production stops when 75 % of the foliage is affected by late blight (Large, 1952), the equation modelling method of James et al. (1972) predicted zero to maximal 23 % yield losses due to late blight. In conventional systems potato tuber growth duration usually lasts until late August if the crop is not killed by disease. In contrast, in organic farming, usually final yields are reached by early to mid August due to limited N-supply (Möller, 2002; Schulte-Geldermann et al., 2005). With blight levels over 75 % occurring only late in July in our experiments the lack of strong yield effects is to be expected. Clearly, yield loss relationships from conventional systems cannot be simply transferred to organic farming.

While nitrogen supply and availability probably is most important in limiting yields, the varietal properties with respect to timing of tuber initiation are likely second most important. This could be seen in 2000, when comparing the effects of late blight on Simone and Secura. Although much less diseased, Simone suffered relatively more yield loss through late blight than Secura as indicated by the three times steeper negative slope in the regression (Fig. 5). This discrepancy can be explained by the fact that Simone is later bulking than Secura and thus Secura had a chance to accumulate much of its tuber yield before the onset of heavy disease. Similar results were obtained and documented with sequential harvests in parallel experiments in 2002 and 2003 when varieties of different bulking behaviour were compared (Schulte-Geldermann et al., 2005). Because of such effects, recommendations in organic farming stress the importance of early bulking varieties (Kölsch and Stöppler, 1990; Meinck, 1999; Möller, 2000; Reents, et al,. 1997) and of chitting (Böhm and Haase, 2003; Karalus, 1996; Meinck, 1999; Pagel and Hanff, 1997; Schüler, 1999). While competition of wheat led to significantly reduced yields in the edge rows of the plots, the influence of this additional stress was not translated into greater effects of disease on yield. This is a further indication that overall yields were only partially limited by late blight in the experiments. Thus, in both organic and conventional farming systems, several parameters such as e.g. individual seasons, varietal and site properties will affect yield loss relationships (Rotem et al., 1983).

The results of this study indicate that growing of potatoes in narrow strips perpendicular to the wind and separated by non-potatoes may in general reduce epidemic pressure within the farming system. Grass-clover as neighbour crop appears to be a more useful choice than cereals due to competition in the edge rows. It would be interesting, however to test other crops e.g. legumes such as faba beans or peas, for example, as intercrops.

While late blight often appears very severe in organic farming, still nutrient availability may be of greater importance than commonly thought. Selection of potato varieties with moderate resistance against late blight and/or early tuber initiation is therefore of prime importance for organic farmers. As additional preventive strategy chitting of seed tubers should allow for the selection of diseased tubers reducing initial inoculum. It will also lead to earlier emerge of potato plants and partial escape from damage by late blight. Such a strategy might on first sight be more attractive to small farmers as their fields are already smaller and smaller amounts of seed potatoes will need to be chitted. However, on larger farms it might be easier to divide fields in the right direction and possibly arrange for a rotation that accommodates alternating strips of potatoes and grass-clover in a field over two years.

Acknowledgements

We like to express our grateful thanks to Günther Kellner and Dana Preiss for excellently dealing with the tricky large-scale field experiments and for assistance in late blight assessments. Many thanks to a great number of students of University of Kassel for helping during harvest and sorting of tubers. We benefited very much from helpful advise on statistics by Hans-Peter Piepho and Andreas Büchse (University of Hohenheim).

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2) Effects of cropping history and origin of seed potatoes on population structure of Phytophthora infestans (Mont.) de Bary

Summary

The effects of origin of seed potatoes and the cropping history on the phenotypic structure of *Phytophthora infestans* populations was studied in northern Hessia, Central Germany from 2000 to 2002. Populations originating from fields with a history of potato cropping (old fields) with only short or no rotation were compared with populations from new fields, i.e. where no potatoes had been grown for at least 30 years and seed potatoes were either imported from breeders or produced on-farm (certified). Isolates were characterized for mating type, virulences and rep- (repetitive extragenic palindromic) PCR fingerprints.

Among a total of 639 isolates sampled from 31 sites, both mating types co-existed in all three years in 60 to 92 % of the habitats. Over all three years, 53 different pathotypes were detected in a subsample of 272 isolates. Thirty-six different rep-PCR fingerprints were detected among 281 isolates from which 22 were unique while four occurred in all three years and in many sites. Comparison with representatives of US lineages US-1, US-6, US-7 and US-8 revealed nearest Nei's genetic standard distances to the US-1 isolate (0.24) followed by the US-7 (0.24), US-8 (0.32) and at last the US-6 isolate (0.47). Isolates originating from the new fields had significantly higher frequencies of the virulences v1, v2, v3, v6 and v7, respectively, indicating effects of seed introduction into a new region in general. Populations from old fields were more diverse and 14 of the 22 rep-PCR types occurred there among 132 isolates tested in comparison to 6 in the new fields (n=140 isolates) and 2 among the 6 isolates from volunteers. The results suggest that both sexual and asexual reproduction play a role.

Introduction

Late blight, caused by *Phytophthora infestans* (Mont) de Bary is one of the most important diseases affecting potato production worldwide. The first introduction of the fungus into Europe in the 1840s via infected seed potatoes (Goodwin, 1997) lead to the great potato famine in Ireland in 1845 (Bourke, 1964; Burt, 1995). With the almost worldwide appearence of the new populations after 1980, late blight research has intensified again.

While the old clonal population consisted only of mating type A1, the new immigrants were of both, A1 and A2 allowing for sexual reproduction and oospore formation as new inoculum source. The new populations are characterized by more complex pathotypes (Drenth et al., 1994; Goodwin et al., 1995b; Rullich et al., 2002), higher genetic diversity (Brurberg et al., 1999; Drenth et al., 1994; Fry et al., 1993; Gavino et al., 2000; Goodwin et al., 1994) and also increased aggressivity in comparison to the old clonal lineage (Day and Shattock, 1997; Medina et al., 1999; Miller et al., 1998; Peters et al., 1999; Tooley et al., 1996). This has lead to earlier and more severe late blight outbreaks (Deahl et al., 1993; Drenth et al., 1993b; Goodwin et al., 1998; Sujowkski et al., 1994; Turkensteen et al., 2002) and an increase of costs for crop protection and yield losses (Johnson et al., 2000; Guenther et al., 2001).

Seed potatoes have played a key role in the worldwide migrations of *P. infestans* as well in the 1840s as during the 1980s (Goodwin, 1997; Niederhauser, 1991; Fry et al., 1993) and it appears that in countries or regions with only low or no import of seed potatoes such as China, the Philippines, Taiwan (Koh et al., 1994), Siberia and the Far East (Elansky et al., 2001), Ecuador (Forbes et al., 1997) and Brazil (Reis et al.,

2003) *P. infestans* populations are characterized by only a few (dominating) clonal lineages. Even in South Africa the old US-1 lineage is still dominant (Mc Leod et al., 2001; Ochwo et al., 2002).
Infected tubers can result in plant infestations under favourable conditions (Bäßler et al., 2002) and just a few percent infected tubers could be responsible for the initiation of late blight epidemics in a potato field (Andrivon, 1994; van der Zaag, 1956). However, other researchers have reported no transmission of *P. infestans* via infected tubers over several years (Platt et al., 1998; Jenkins and Jones, 2003) suggesting that latent tuber infections either destroy their host (Hirst and Stedman, 1960; Miller et al., 2002) or the fungus dies of starvation so that the sprouts do not emerge or a healthy potato plant results, respectively. However, there may be much more infections in seed potatoes as previously thought as demonstrated by diagnosis through PCR (Adler, 2001).

The main factors influencing the frequency of latent infections are the tuber blight susceptibility of varieties and the climatic conditions during the epidemic, ripening and harvest, respectively (Flier et al., 2003). Favourable weather conditions resulted in infection rates of up to 50 % infested potato tubers in Sweden (Andersson et al., 1998) and the evaluation of climatic and epidemic data of more than 50 years revealed a higher incidence of tuber infections, the more severe the late blight epidemics were (Croxall and Smith, 1976).

The importance of latent tuber infections for *P. infestans* epidemics probably differs between organic and conventional production. While no seed-borne below-ground foci were detected in conventional potato fields in the Netherlands (Zwankhuizen et al., 1998), the authors expected infested seed to be a more important inoculum source in organic potato production because no seed treatment with protective fungicides is practised there. On the other hand, the generally faster death of organic potato canopies results in shorter epidemics compared to sprayed crops reducing the chances for tuber infections via the soil.

The *P. infestans* populations that have been characterised so far in Europe can be divided into two groups based on the overall diversity (Day et al., 2004). Phenotypic and genetic diversity is high in the Netherlands (Drenth et al., 1993a; Fry et al., 1991; Zwankhuizen et al., 2000) and Poland (Sujkowski et al., 1996), i.e. countries that frequently import seed potatoes from Mexico. Similarly, high diversities of pathogen populations were found in Scandinavia (Andersson et al., 1998; Brurberg et al., 1999; Hermansen et al., 2000) and the former German Democratic republic (Daggett et al., 1993). These countries import much of their seed potatoes from the Netherlands and Poland, respectively. Sexual reproduction also appears to contribute to the genetic diversity within *P. infestans* populations in these regions. First studies indicated that oospores function as inoculum source in European potato crops even under natural field conditions in Norway and Finland (Brurberg et al., 1999) and oospores have been detected in plant tissue in Sweden (Andersson et al., 1998) and the Netherlands (Drenth et al., 1993b; Turkensteen et al., 2000; Turkensteen et al., 2002). While both mating types have been reported from the more homogeneous populations with low to moderate diversity of *P. infestans* populations in France, United Kingdom and Switzerland, there, the A2 mating type appears to be rare on potato (Andrivon, 1994; Carlisle et al., 2001; Day and Shattock, 1997; Day et al., 2004) and more common on tomato (Knapova and Gisi, 2002; Lebreton and Andrivon, 1998) and it is unclear if sex occurs commonly in those regions.

In Western Germany, the region in between the clearly sexual northern and eastern region and the Swiss and French region it appears that the old *P. infestans* population has been successively displaced by the new populations as revealed by mt-DNA haplotype analysis (Rullich and Schöber-Butin, 2000) or AFLP analysis (Flier et al., 2000). The A2 mating type and complexer pathotypes have been found with increasing frequency among sporadically sampled German isolates since the early 1980s (Rullich et al., 2002; Schöber, 1983).

For the molecular characterization of *P. infestans* populations various methods have been employed such as isoenzymes (Tooley et al., 1985), RFLPs (Goodwin et al., 1992), AFLPs (Vos et al., 1995), microsatellites (Knapova and Gisi, 2001) or RAPD-PCR (Maufrand et al., 1995). The allozymes Gpi and Pep are used to define and compare different clonal (US)-lineages (Forbes et al., 1998; Goodwin et al., 1995b). In addition, RFLP fingerprinting with probe RG57 resulting in about 25 DNA bands functions as an international standard fingerprinting method for use in the Global Marker Database on *P. infestans* (Forbes et al., 1998) allowing for worldwide comparison of isolates and the determination of migration patterns of the pathogen (Drenth et al., 1993a; Goodwin et al., 1992; Sujkowski et al., 1994). A higher number of identifiable polymorphisms can be obtained through AFLPs with 50 to more than 100 loci (Abu-El Samen et al., 2003; van der Lee, 1997; Zwankhuizen et al., 1998). However, both, RFLP and AFLP techniques are labour-intensive and expensive to generate because the fragments are detected by silver staining, fluorescent dye or radioactivity or require special sequencing equipment.

In contrast, RAPD-PCR technology requires only small amounts of DNA (15-25 ng) and is a nonradioactive and cost-effective assay, that can be performed within only several hours. PCR using different RAPD primers each of 10-mer length produces about 10 to 30 polymorphic DNA fragments allowing to reveal genetic diversity among *P. infestans* isolates (Carlisle et al., 2001; Mahuku et al., 2000; Punja et al., 1998) and to differentiate isolates belonging to the same clonal lineages (Ghimire et al., 2002). Rep- (repetitive extragenic palindromic) PCR was originally developed to distinguish between different bacterial strains (Versalovic et al., 1991). The rep-primers are sequences of 15 to 18 bp length occurring throughout the genome which are highly conserved repetitive elements in many organisms (Versalovic et al., 1994). Rep-PCR has been used successfully to discriminate among closely related phytopathogenic bacterial species and to reveal diversity within species among isolates (Louws et al., 1999; Rademaker et al., 2000; Scortichini and Rossi, 2003). Initial applications of this technology on *P. infestans* using one pair of primers revealed about 15 polymorphic DNA fragments of a good reproducibility (Bouws-Beuermann and Finckh, *unpublished*). These results, the cost-effectiveness, simple protocols and speed were the main motives to establish and optimize rep-PCR technology in our lab at the University of Kassel.

When in 1999, potato production was taken up on the organic 300 ha experimental farm of the University of Kassel in central West Germany in fields where no potatoes had been grown for over 30 years and in a region where potatoes are a minor crop only, this offered a unique opportunity to study the establishment and evolution of *P. infestans* populations from the start. All inoculum in the ”new fields” on the farm had to originate either from the seed potatoes or from the surrounding region. Own observations on the spatial dynamics of the disease occurrence across the farm, the main wind direction and spore catches in 1999 suggested that the inoculum arrived from potato sites situated west of the farm which were infested about 10 days earlier (Bouws-Beuermann and Finckh, *unpublished*).

From 2000 on, *P. infestans* isolates were sampled in fields and experimental plots of the farm with seed potatoes originating either from northern German breeders or from the certified seed production on the experimental farm. Isolates were also collected from the surrounding potato crops where farmers and gardeners traditionally grow potatoes with short or no rotation, commonly from their own propagation and for own comsumption. In the years 2000 to 2002, a total of 639 isolates were characterized for mating type. Subsets of isolates were tested for virulences and molecular rep-PCR fingerprints in order to determine the phenotypic and haplotypic diversity among the *P. infestans* populations addressing the following questions:

i) Do *P. infestans* populations on the experimental farm originate from the surrounding area or from the seed potatoes?
ii) Are there differences between *P. infestans* populations due to the cultivar of origin or to side effects?
iii) How much do *P. infestans* populations change in the transition from the seed potato crop to the main crop?

Materials and methods

Collection of isolates

A total of 95,315 and 229 *P.infestans* isolates were collected in the years 2000, 2001 and 2002, respectively, from a 15 x 20 km region, situated in northern Hessia, Central Germany, including the experimental farm of the University of Kassel (Figure 1).

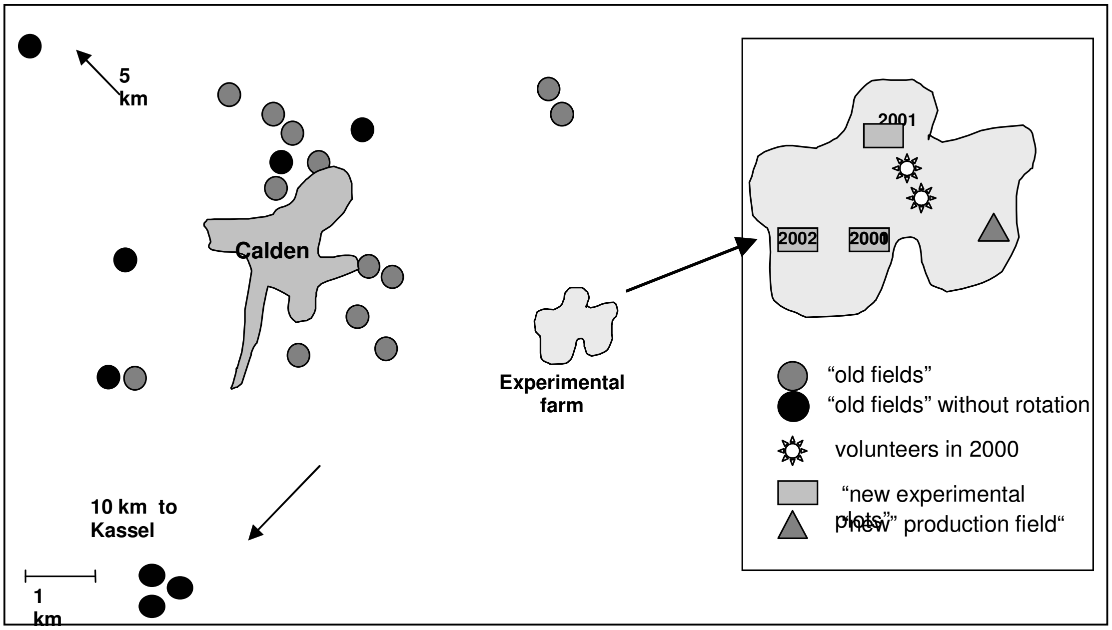


Figure 1. Schematic map of 15x20 km^2 region in northern Hessia, Central Germany where *P. infestans* isolates were sampled from old and new fields (i.e. with and without a potato cropping history, respectively) from 2000 to 2002. Fields without rotation indicate that potatoes were grown in two or three consecutive years.

About a third of the isolates was sampled from so-called ”old fields” (mostly up to 1 ha) from the western surroundings of the experimental farm (Table 1). The majority of the isolates originated from potato fields and plots which were planted on the experimental farm. In 2000, isolates were collected from nine 9x10 m^2 experimental plots distributed within a 150x300 m field. Plots were separated by other crops by at least 9 m. Isolates were collected from six plots of the variety Secura (susceptible) and three plots of Simone (moderately resistant). Seed potatoes of both varieties originated from growers in northern Germany.

Table 1. Origin and number of *P. infestans* isolates characterized for mating type and frequency of A2 in 2000 to 2002.

	2000		**2001**		**2002**		**total**
origin	**n (sites)** [1]	**A2**	**n (sites)**	**A2**	**n (sites)**	**A2**	**n**
volunteers	6 (1)	0.50					6
refuse pile			4 (1)	0.25			4
experimental plots [2]	59 (9)	0.49	116 (2)	0.00	110 (2)	0.38	285
large fields (> 2 ha) [3]	5 (2)	0.40	105 (2)	0.14			110
”old fields”	25 (6)	0.46	90 (10)	0.30	119 (9)	0.24	234
total	95 (18)	0.46	315 (15)	0.13	229 (11)	0.31	639

[1] n= number of isolates tested for mating type. Number of different sites per origin given in parentheses
[2] In 2000, experimental plots were planted with seed potatoes obtained from northern German breeders. Certified potato seed produced on-farm was used in 2001 and 2002.
[3] Large seed production fields were planted with breeders‘ seed in 2000 and 2001. Seed potatoes from these fields (2001) were planted in the plots in 2002.

In 2001 and 2002, two experimental plots of 18x36 m^2 (24 rows x 36 m) size planted with the varieties Linda (susceptible) and Agria (moderately resistant) were sampled intensively. Certified seed potatoes produced on-farm were used in these plots. In 2001, isolates were also sampled from larger seed potato production fields (> 2 ha) of the same two varieties on-farm (Table 1). Seed potatoes for these fields came from the respective breeders. Isolates were sampled from mid-June to mid-August in 2000 and 2001 and from mid-July to mid-August in 2002, respectively. They were collected in diagonal transects from potato fields or by regular sampling every 3 m (in 2000) or 6 m (in 2001 and 2002) along two separate rows within the experimental plots. For comparison, isolates of the four most investigated clonal lineages US-1, US-6, US-7, US-8 (kindly provided by W. E. Fry, Cornell University, Ithaca, New York) were included in the molecular analysis with rep-PCR.

Isolation and culturing of the isolates

For the isolation of the fungus, tubers of potato cultivar Atica (early maturing, without any known R-gene) were surface sterilised, cut into 1 cm thick slices and pressed dry on filter paper. Sandwiches with two tuber slices and a 1 cm^2 piece of the sporulating leaflet in between were prepared. The potato sandwiches were placed on filter paper in plastic boxes with transparent lids and kept at 17 °C with 16 h daylength, starting with 16 h darkness. After five to seven days, sporulating mycelium emerging on top was harvested with sterile tweezers and plated on 1.5 % pea agar (125 g frozen peas l^{-1} H_2O) containing 100 mgl^{-1} Ampicillin, 30 mgl^{-1} Rifamycin, 10 mgl^{-1} Benomyl and 0.4 ml^{-1} Pimaricin. Pure cultures of *P. infestans* were transferred on 1.5 % pea agar without antibiotics after 5 to 7 days. The agar plates were stored at 17 °C in the dark and controlled weekly. Success rates with this method were about 85 %, 94 % and 75 % in the years 2000 to 2002, respectively. For long-term storage, the isolates were transferred into test tubes, containing 1.5 % pea agar, overlaid with sterilised mineral oil, and stored at 17 °C in darkness.

Mating type testing

For mating type determination each isolate was paired on pea agar with a known A1 and a known A2 strain, respectively which were kindly provided by D. Andrivon (INRA, Rennes, France). The petri dishes were incubated for 10 to 18 days at 17 °C in the dark and examined microscopically for the presence of oospores. An isolate was considered to be mating type A1, if oospores were found when paired with a known A2 strain and vice versa. Test isolates producing oospores with both A1 and A2 were scored as A1A2, probably self-fertile, but this was not determined.

Virulence tests

Virulence tests were conducted with Black's single R-gene differential set (Black et al., 1953; Malcolmson and Black, 1966), each possessing one of the race-specific resistance genes R1, R2, R3, R4, R5, R6, R7, R8, R10, R11 plus one line without a known R-gene R0 (kindly provided by D. Andrivon, INRA, Rennes, France). Potato plants were cultivated in 13 cm diameter plastic containers in a sand-peat-compost mixture (each 33 %) in the glasshouse at 18 °C/15 °C day/night, with 14 h daylength. The virulence tests were carried out with plant material of about seven week old plants. Only fully expanded leaves were used for the tests. The leaflets were detached with a scalpell and deposited abaxial face up on moist filter paper in square petri dishes (14 x 14 cm), each differential host set replicated twice. For the inoculation suspension, mycelium of two week old cultures on pea agar was washed down with 4 ml sterilized water. Sporangia were counted with a "Fuchs Rosendahl" haemacytometer and the suspensions were then stored at about 8 °C for 3 to 4 hours, to allow hatching of the zoospores. All sporangial suspensions were adjusted to a concentration of 5 x 10^4 sporangia/ml. Each leaflet was inoculated on the abaxial side with two separate 20 µl drops of sporangial suspension. The petri dishes were incubated for 7 days at 18 °C/15 °C day/night, the first day in the dark and then 16 h daylength. In order to avoid drying of the leaflets, the lids of the petri dishes were moistened with distilled water every two days. After an incubation period of 7 days, an isolate was considered virulent to a given differential host if at least two of the four inoculations resulted in sporulating lesions and if the R0-line was infected the same way.

Genomic DNA extraction

To obtain pure mycelium of *P. infestans*, the fungus was grown in 50 ml Erlenmeyer flasks containing sterilised liquid pea medium (125 g frozen fresh peas l^{-1} destilled H_2O, filtered and autoclaved twice at 120

°C for 20 min, with 100 mg/l ampicillin added). Mycelium of 10 to 14 day old cultures was rinsed with sterilised water and harvested by vacuum-filtration. The mycelium was frozen at –20 °C and freeze-dried in 2.0 ml Eppendorf tubes. For DNA extraction, 15 to 20 mg of the freeze-dried mycelium was ground with a little bit of sterile quarz sand, 700 µl extraction buffer (1M Tris-HCl, 0.5 M EDTA, 10 % SDS, pH 8.0) was added and the tubes were incubated for 1 hour in a water bath at 65 °C. After adding 50 µl RNase A (1 mg/ml) and 50 µl Proteinase K (10 mg/ml) the tubes were incubated for 1 h in a water bath at 37 °C. The DNA was purified two times with phenol-chloroform-isoamylalcohol (25:24:1) followed by centrifugation at 15,000 and 13,000 r.p.m. for 15 and 10 min, respectively. A tenth of the supernatant quantity was added as 3 M NaOAc (usually 20-50 µl) plus 1 ml Isopropanol (100%) and the tube was centrifugated again with 13,000 r.p.m for 6 min. At last, the probe was mixed with 500 µl Ethanol (70 %) and centrifugated with the same speed for 5 min. The DNA pellet in the Eppendorf tubes was dried in an exsiccator and the DNA was dissolved in 100 µl 0.1 M TE buffer (1 M Tris-HCl, pH 7.6; 0.5 m EDTA). The probes were stored at 4 °C until dilutions were made, stock solutions of DNA were then stored at –20 °C. For estimation of the DNA content, 5 µl of each DNA probe, mixed with 5 µl of ultra pure water and 2 µl of loading buffer was loaded on a 0.8 % agarose gel. As DNA standard, 10 µl of a λ-DNA, which was cut with Hind III and Eco R1 was loaded with 2 µl loading buffer per lane. The agarose gel was run at room temperature for 45 min at 100 V. After coloration in a water bath containing ethidium bromide (100 µl of ethidium bromide 10 mg/ml added to 800 ml of destilled water) for about 20 min, the gel was photographed on a UV transilluminator (INTAS, Germany). The DNA content was quantified visually by comparing each DNA probe with the fragments of the DNA standard.

Rep-PCR analysis

For rep-PCR amplification reactions were carried out in 0.2 ml microtubes with volumes of 25 µl mix, containing 3 ng DNA, 200 µM each dNTP, 1 U taq polymerase (Peqlab, Germany), 50 pM primer rep-I and 50 pM primer rep-II, 2.5 µl 10-fold concentrated reaction buffer (200 mM Tris-HCl, pH 8.55, 160 mM $(NH_4)_2SO_4$, 20 mM $MgCl_2$) and 13.3 µl sterile ultra pure water.

The sequences of the primers (Life Technologies, UK) were as follows:

rep-I:	5‘-III ICG ICG ICA TCI GGC-3‘
rep-II:	5‘-ICG ICT TAT CIG GCC TAC-3‘

Amplifications were performed in a Biometra T 1 Thermocycler (Biometra, Germany) with one cycle at 95 °C, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 40 °C for 1 min and extension at 65 °C for 5 min, a final cycle at 65 °C for 15 min, and a final soak at 4 °C. Fifteen µl of the amplification products with 3 µl loading buffer per lane were separated by gel electrophoresis in 1.5 % agarose gel in 1x TBE buffer. As DNA standards, a 1 KB and/or a 100 bp ladder (Peqlab) were added to the gel, which was run at room temperature for about 6 h at 70 V. The gels were stained and photographed as described above. Each probe was repeated two or more times.

Data analysis

Rep-PCR fingerprint patterns were analysed with INTAS PCI Gel-Imager software (INTAS, Germany) with manual correction for faint bands and exclusion of controversial bands. A binary matrix for the presence (1) or absence (0) of reproducible bands was constructed and used for further analysis. Only the polymorphic bands were included in the analysis. POPGENE and TFPGA software which are available cost-free via internet (http://www.ualberta.ca/~fyeh/ and http://bioweb.usu.edu/mpmbio/index.htm), respectively were used. For further analysis of molecular data, polymorphic bands plus mating type were treated as one locus each in a haploid dominant data set. Dendrograms were constructed from a Nei’s genetic standard distance (Nei, 1972) with the unweighted pair-group method of averages (UPGMA) with 1,000 permutations of bootstrapping using TFPGA software. Population differentiation was estimated with an exact test (Raymond and Rousset, 1995) on in our case haplotypes instead of genotypes (see equation (1), according to Goudet et al. (1996).

$$Pr(S)= \frac{\prod_{i=1}^{r} N_{i}!\prod_{j=1}^{Ng} N_{.j}!}{N_{..}!\prod_{i=1}^{r}\prod_{j=1}^{Ng} N_{ij}!} \qquad (1)$$

where $Pr(S)$=population probability, N_{ij}=number of individuals of haplotype j in population i, N_i=sample size of population i, $N_{.j}$=total number of individuals with haplotype j, $N_{..}$=total number of individuals sampled, r=number of populations and N_g=number of haplotypes.

Within an exact test, all possible tables given the marginal haplotype counts according to the value of a particular statistic are ranked and the probabilities $Pr(S)$ of the tables with more extreme ranks are summed-up, the resulting sum is the P-value of the test. Under the null hypothesis of absence of population differentiation defined contrasts of *P. infestans* populations (e.g. seed origin or cropping history of fields from which isolates originated) were contrasted with 1,000 dememorization steps with 10 batches and 2,000 permutations per batch using TFPGA software.

Statistical analysis of virulence frequencies was performed with the FREQ procedure of SAS software (SAS Institute, Inc.) using the two-tailed Fisher's exact test. The cumulative frequency of virulences, i.e. the mean complexity per isolate was compared pair-wise with t-tests (GLM procedure). The Shannon diversity index H_o (Shannon and Weaver, 1949) was calculated for defined populations using the following equation :

$$H_o= - \sum_{i=1}^{s}(P_i) * \ln(P_i) \qquad (2)$$

where P_i = frequency of isolates of the i^{th} phenotype within a sample or population and s = number of different phenotypes within a sample.

Then, normalized Shannon statistics (Sheldon, 1969) were calculated. As this statistic takes into account the total number of tested isolates the normalized Shannon indices H' present the fraction of the maximum diversity per sample thus ranging from 0 to 1. The normalized Shannon index was considered adequate as the potential number of genotypes was much larger than the sample sizes (Grünwald et al., 2003). Diversity indices were calculated based on pathotypes and also for multilocus haplotypic data (mating type plus binary data of polymorphic rep-PCR bands) of isolates of different populations or sampling sites.

Results

Late blight epidemics in 2000 to 2002

In 2000, first lesions were observed on June 20th in three old potato fields about 2 to 4 km northwest of the experimental farm and also on volunteer plants within a pea field located downwind of the plots on the experimental farm. After heavy rainfall in the last days of June, the epidemic started in mid-July in the plots at the experimental farm, while the production fields on the farm were already up to 15 % infected, sometimes with foci of 90 % disease severity. Due to high disease pressure the susceptible crop in the experiment was destroyed within two weeks (Table 2).

Table 2. Dynamics of late blight epidemics in a 15 x 20 km^2 region in northern Hessia, Central Germany, 2000 to 2002.

start of late blight symptoms	**2000**		**2001**		**2002**	
in surroundings ("old fields")	June 20		July 10		July 18	
in plots ("new fields")	July 11		July 12		June 28	
disease pressure ^{1}	+++		+		++	
AUDPC ^{2}	Simone	Secura	Agria	Linda	Agria	Linda
	1053	2471	740	1476	1281	1746

death of cultivar [3]	14/8	27/7	25/8	13/8	15/8	5/8
sampling of isolates	11/7 to 12/8		12/7 to 9/8		18/7 to 12/8	

[1] +, ++ and +++ indicating low, moderate and high disease pressure per season, respectively
[2] AUDPC = Area under the disease progress curve. Means were calculated from 28-32 experimental plots per variety and year.
[3] More than 90 % late blight severity

In 2001, first symptoms were found on potato plants in a refuse pile, adjacent to the experimental plots on 20th of June which was immediately destroyed. The first surrounding potato fields were infested on 10th of July and two days later, late blight started in the susceptible potato plots. With weather conditions prolonged hot and dry until the first week of August, disease development was slow and plants died in mid-August. In 2002, late blight in the experimental plots started about two weeks earlier, on 28th of June but with a moderate epidemic increase. Due to continuous heavy rainfall in mid-July, the susceptible plants in the plots died within three weeks.

Mating types

Mating types were determined successfully for 639 *P. infestans* isolates. Both mating types were detected in all three years, with A2 varying in frequency from 0.13 to 0.46 (Table 1). If the isolates from the experimental plots in 2001 (n=116), that were uniformly A1 are excluded, the frequency of mating type A2 in 2001 amounts to 0.21. Both mating types co-existed within the same habitat in 65 %, 60 % and 92 % of the different sites sampled from 2000 to 2002, respectively. The other sites were either dominated by A1 or A2 isolates. In 2000, an interesting pattern was observed in the experimental plots. Within an area of about 150 by 300 m the A2 types were concentrated in the north to northwest and the A1 in the southeast region, with an area in the center where both mating types co-existed within the plots. In 2001, both mating types were found in the large new fields that had not been planted to potatoes for at least 30 years, while only A1 was found in the experimental plots also located in a new field. Finally, in 2002 both mating types occurred side by side throughout the examined experimental plots with both potato cultivars.

Pathotypes

In total, 79, 114, and 79 isolates of the collections of 2000 to 2002 were tested successfully for virulences. Over all three years, 53 different pathotypes were detected with 26, 36 and 22 different pathotypes in 2000, 2001 and 2002, respectively (Table 3). Eight pathotypes were found in all three years: 1.3.4.7 (n=15), 1.3.4.7.11 (n=17), 1.3.4.7.8.10.11 (n=48) and 1.3.4.7.10.11 (n=74), 3.4.7.10.11 (n=8), 1.2.3.4.6.7 (n=5), 1.3.4.7.8.11 (n=4) and 1.3.4.5.7.8.10.11 (n=4). In total, four isolates originating from the volunteers 2000 (n=1) and the new plots 2002 (n=3) were able to overcome all tested virulence genes. No general association of pathotype and either A1 or A2 mating type was observed. However, the majority of the isolates of the two most common pathotypes 1.3.4.7.10.11 and 1.3.4.7.8.10.11 were of A1 mating type (82 % and 92 %, respectively).

Table 3. Number of different pathotypes, mean complexity and normalized Shannon diversity of *P. infestans* populations collected early and late in late blight epidemics 2000 to 2002.

	number of different pathotypes [2]						**total**	
no. of virulences [1]	**2000 (n)**		**2001 (n)**		**2002 (n)**		**tested**	**A2**
2		(0)	3	(7)	1	(2)	9	1
3	2	(2)	6	(9)	3	(3)	14	6
4	3	(10)	6	(12)	4	(9)	31	13
5	6	(9)	7	(22)	4	(11)	42	13
6	4	(29)	8	(34)	4	(27)	90	20
7	3	(15)	3	(25)	3	(21)	61	10
8	4	(7)	3	(5)	1	(2)	14	4
9	3	(6)		(0)	1	(1)	7	2
10	1	(1)		(0)	1	(3)	4	4
total	26	(79)	36	(114)	22	(79)	272	73
complexity [3] early	6.1	(47)	5.0	(60)	6.0	(26)		
complexity late	6.3	(32)	5.9	*** (54)	5.9	(53)		
H'	0.59		0.62		0.54			

[1] Number of virulences per isolate.

[2] Number of different pathotypes with same mean complexity. The number of tested isolates is given in parentheses.

[3] Mean complexity, i.e. mean number of virulences per isolate from populations sampled early and late during the epidemics. *** indicate that differences between early and late sampled isolates were highly significant at $P<0.001$ (t-test).

The mean number of virulences per tested isolate was 6.2, 5.4 and 5.9 from 2000 to 2002, respectively. In 2001, but not in 2000 or 2002 the mean complexity of isolates increased significantly within the growing season (Table 3) with an increase of 4.3 to 5.7 in the old fields and 5.2 to 6.2 in the new fields. Virulence genes v2, v5 and v6 were rarest, however with significantly higher frequencies among isolates belonging to the A2 mating type (Figure 2). On the other hand, v8, v10 (both with $P=0.057$) and v11 ($P<0.01$), were more frequent among A1 isolates. The virulence gene v2 was significantly more frequent among isolates which were collected early in the three late blight epidemics ($P=0.02$). Also, virulence genes v5 and v6 occurred more frequently among the early collected isolates, however, the differences were not significant. Interestingly, v2 occurred only in combination with either v5 or v6 or both whereas v5 and v6 also occurred alone. All three virulences were present only in isolates with at least 5 or more virulences.

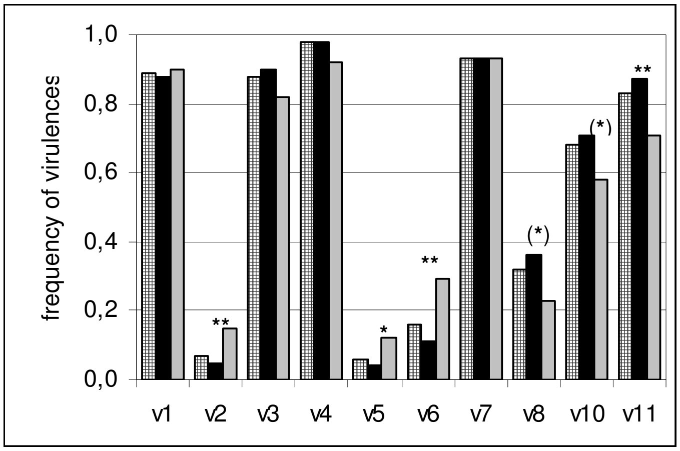


Figure 2. Frequency of virulences for all tested *P. infestans* isolates (n=272, caro) and separately for mating type A1 (n=195, black) and A2 (n=73, grey). (*), * and ** indicate that virulence frequencies of A1 and A2 isolates differed significantly at (*P*=0.06); *P*<0.05 and *P*<0.01, respectively (two-tailed Fisher's exact test).

The frequencies of virulence genes v1, v5, and v10 were significantly more rare within the collection 2001 than in 2000 and 2002. Virulence genes v2 and especially v6 (*P*<0.001) were most frequent among isolates of the collection 2000 (Figure 3).

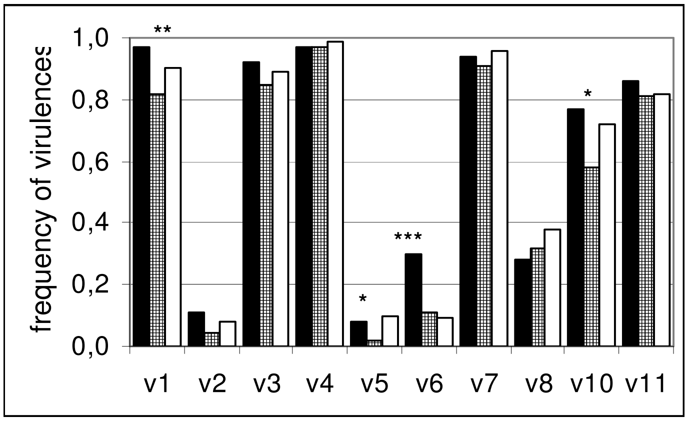
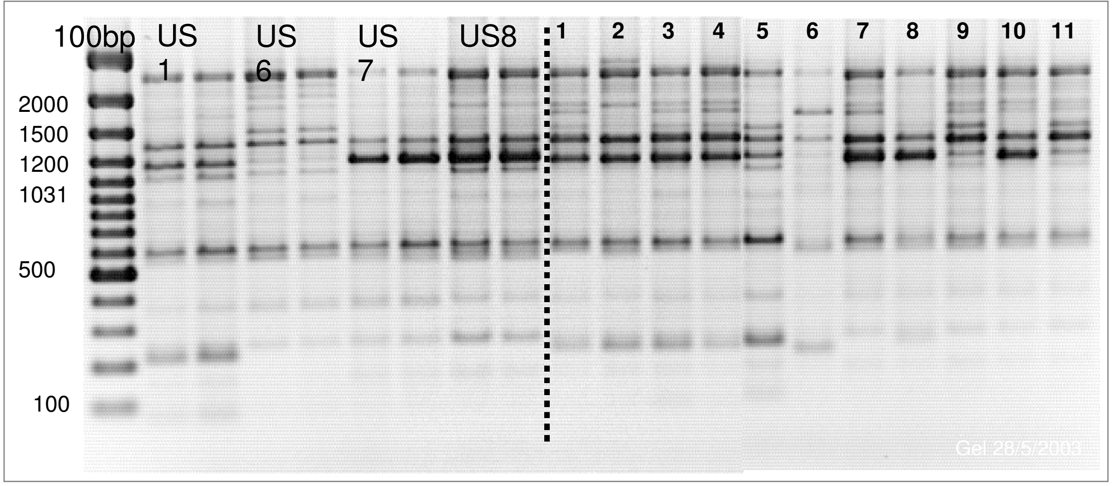


Figure 3. Frequency of virulences of *P. infestans* isolates collected in 2000 (n=79, black), 2001 (n=114, caro) and 2002 (n=79, white), respectively. *, ** and *** indicate that virulence frequencies of isolates differed significantly over years at *P*<0.05, *P*<0.01 and *P*<0.001, respectively (two-tailed Fisher's exact test).

With respect to the cropping history of potato fields, i.e. the comparison of new fields (n=159) versus old fields (n=113), isolates from the former ones had significantly higher frequencies of the virulence genes v1 (0.92 / 0.83), v2 (0.11 / 0.03), v3 (0.94 / 0.81), v6 (0.20 / 0.10) and v7 (0.97 / 0.88) (all contrasts with *P*<0.05). Within the new fields and plots on the farm, *P. infestans* virulence frequencies of isolates originating from sites planted with introduced seed (n=82) or on-farm certified seed (n=70) did not differ significantly except for v1 (*P*=0.02) which was more common for isolates originating from sites with introduced seed. Generally, no association of virulence frequency with potato variety or host population size (e.g. plots versus large fields from which the isolates originated) was found.

Rep-PCR fingerprints



Overall, 44, 137 and 100 different isolates of the populations of 2000 to 2002 plus one isolate each of the US-lineages US-1, US-6, US-7, US-8 were analysed successfully for their rep-PCR fingerprints.

Figure 4. Rep-PCR fingerprints each of two *P. infestans* isolates belonging to clonal US-lineages in comparison with 11 north hessian isolates originating from an old field in 2002 (left and right of pointed line, respectively). Isolates of lineages US-7, US-8 and probes 5 and 8-10, respectively were A2 mating type.

Seventeen and 15 out of 24 reproducible DNA fragments were polymorphic concerning all (i.e. US-isolates included) or only the north Hessian isolates, respectively. Rep-PCR of isolates belonging to the US-lineages revealed somewhat different fingerprint patterns from the north Hessian collections (Figure 4).

Besides the US isolates, 36 different rep-PCR fingerprints were identified from which 22 were unique and 28 were found only once during the three years in northern Hessia (Table 4). Most of the unique rep-types occurred in the old fields (n=14), followed by the experimental plots (n=5), the volunteers 2000 (n=2) and the seed production field 2001 (n=1). Combination of mating type with DNA fingerprinting pattern resulted in 51 different multilocus phenotypes. Four rep-PCR fingerprints occurred over all three years with fingerprints rep-1 and rep-2 dominating the populations with 32 and 30 %, respectively. These most common rep-types were of both mating types and they were found in all categories of origin. Moreover, within each of these common rep-types several pathotypes were found (Table 4) revealing no obvious association of molecular type with virulences.

Table 4. Number of different and unique rep-PCR fingerprints and the banding pattern of in total 281 *P. infestans* isolates collected in northern Hessia from 2000 to 2002 plus each one isolate of the clonal lineages[1] US-1, US-6, US7 and US-8.

no.[2]	**rep-PCR fingerprint** [3]	**2000**	**2001**	**2002**	**total**	**A2**	**pathotypes (n)** [4]
1	0 1 1 1 0 1 0 1 0 0 0 1 0 0 0 0 1	14	52	24	90	24	21 (60)
2	0 1 1 1 0 1 0 1 0 0 0 1 0 0 0 1 0	10	52	23	85	26	20 (46)
3	0 1 1 1 0 1 1 1 0 0 0 1 0 0 0 1 0	3	6	20	29	15	10 (18)
4	0 1 1 1 0 1 1 1 0 0 0 1 0 0 0 0 1	2	5	11	18	6	6 (10)
5	1 1 1 1 0 1 0 1 0 0 0 1 0 0 0 0 1		6	8	14	5	5 (6)
6	0 1 1 1 1 1 0 1 0 0 0 1 0 0 1 0 1	4			4		2 (4)
7	1 1 1 1 0 1 0 1 0 0 0 1 0 0 0 1 0		1	3	4	3	2 (2)
8	0 1 1 1 0 1 0 1 0 0 0 1 0 1 0 1 0		3		3		n.d.
9	0 0 1 1 0 1 0 1 0 0 0 1 0 0 0 0 1		2		2	1	n.d.
10	0 1 0 1 0 1 0 1 0 0 0 1 0 0 0 1 0		1	1	2	1	1
11	0 1 1 1 0 1 0 1 0 0 0 1 0 1 0 0 1		2		2		2 (2)
12	0 1 1 1 0 1 0 1 0 1 0 1 0 0 0 1 0		1	1	2	1	1
13	1 1 1 1 0 1 1 1 0 0 0 1 0 0 0 0 1			2	2	1	n.d.
14	1 1 1 1 0 1 1 1 0 0 0 1 0 0 0 1 0			2	2	1	1
15	0 1 1 1 0 0 0 1 0 0 0 1 0 0 0 0 1		1		1	1	
16	0 1 1 1 0 0 0 1 0 0 0 1 0 0 0 1 0		1		1		
17	0 1 1 1 0 1 0 1 0 0 0 0 1 0 0 0 1			1	1		
18	0 1 1 1 0 1 0 1 0 0 0 0 1 0 0 1 0			1	1		
19	0 1 1 1 0 1 0 1 0 0 0 0 1 0 1 1 0	1			1	1	
20	0 1 1 1 0 1 0 1 0 0 0 1 0 0 1 1 0	1			1	1	
21	0 1 1 1 0 1 0 1 0 0 0 1 1 0 0 1 0	1			1		
22	0 1 1 1 0 1 0 1 0 1 1 0 1 0 0 1 0	1			1		
23	0 1 1 1 0 1 1 1 0 0 0 0 1 0 0 0 1	1			1		
24	0 1 1 1 0 1 1 1 0 0 0 1 0 1 0 1 0		1		1		
25	0 1 1 1 1 0 0 1 0 0 0 1 0 0 0 1 0	1			1		
26	0 1 1 1 1 1 0 1 0 0 0 0 1 0 1 1 0	1			1		
27	0 1 1 1 1 1 0 1 0 0 0 1 0 0 1 1 0	1			1		
28	0 1 1 1 1 1 0 1 0 1 0 1 0 0 1 0 1	1			1		
29	0 1 1 1 1 1 0 1 0 1 0 1 0 0 1 1 0	1			1		
30	1 0 1 1 0 1 0 1 0 0 0 1 0 0 0 0 1		1		1		
31	1 1 1 0 0 0 1 1 0 0 0 1 0 0 0 0 1			1	1		
32	1 1 1 0 0 1 0 1 0 0 0 1 0 0 0 0 1			1	1		
33	1 1 1 0 0 1 0 1 0 0 0 1 0 0 0 1 0			1	1		
34	1 1 1 1 0 0 0 1 0 0 0 1 0 0 0 1 0		1		1		
35	1 1 1 1 0 1 0 1 0 1 0 1 0 0 0 1 0	1			1		
36	1 1 1 1 1 1 0 1 0 0 0 1 0 0 0 0 1		1		1		
US-1	0 1 1 1 0 1 0 1 1 0 0 0 1 0 0 1 1			(1) [1]	1		
US-8	0 1 1 1 0 1 1 0 1 0 0 1 0 1 0 1 0			(1)	1	(1)	
US-7	0 1 1 1 0 1 1 1 0 0 1 1 0 1 0 1 0			(1)	1	(1)	
US-6	1 1 1 1 0 1 0 1 1 1 1 0 1 0 0 1 0			(1)	1		
	isolates tested in total [5]	44	137	100	281		

different rep-types [5]	16	17	15	36		
unique rep-types over all years [5]	11	6	5	22		
polymorphic bands [5]	12	11	11	17		
H' [5]	0.40	0.27	0.33			

[1] Isolates of the four US-lineages were tested with the collection 2002 resulting in a total of 104 tested. isolates. Each US-strain revealed one of the overall unique rep-PCR types resulting in a total of 40 different unique rep-PCR patterns.

[2] Rep-PCR fingerprints were sorted by frequency and then assigned a number.

[3] Polymorphic DNA fragments of 2,900; 2,000; 1,650; 1,500; 1,250; 1,150; 1,100; 1,050; 1,020; 950; 870; 850; 800; 520; 450; 250; 220 bp size are listed.

[4] Number of different pathotypes within rep-PCR type. The number of isolates tested for virulences is given in parenthesis. n.d.= pathotype was not determined.

[5] Only data of the north Hessian collections were considered. For estimation of the normalized Shannon diversity H' only the polymorphic bands per collection were considered.

Among years and origin the sampled *P. infestans* populations were highly similar with genetic distances (Nei, 1972) of 0.05 (Figure 5). With respect to the four US-lineages the nearest genetic standard distance was to the US-1 isolate (Nei distance=0.24), followed by the US-7 (0.22-0.25), US-8 (0.30-0.33) and at last the US-6 isolate (0.47), respectively. The reference isolates US-7 and US-8 clustered together at 0.18 Nei distance.

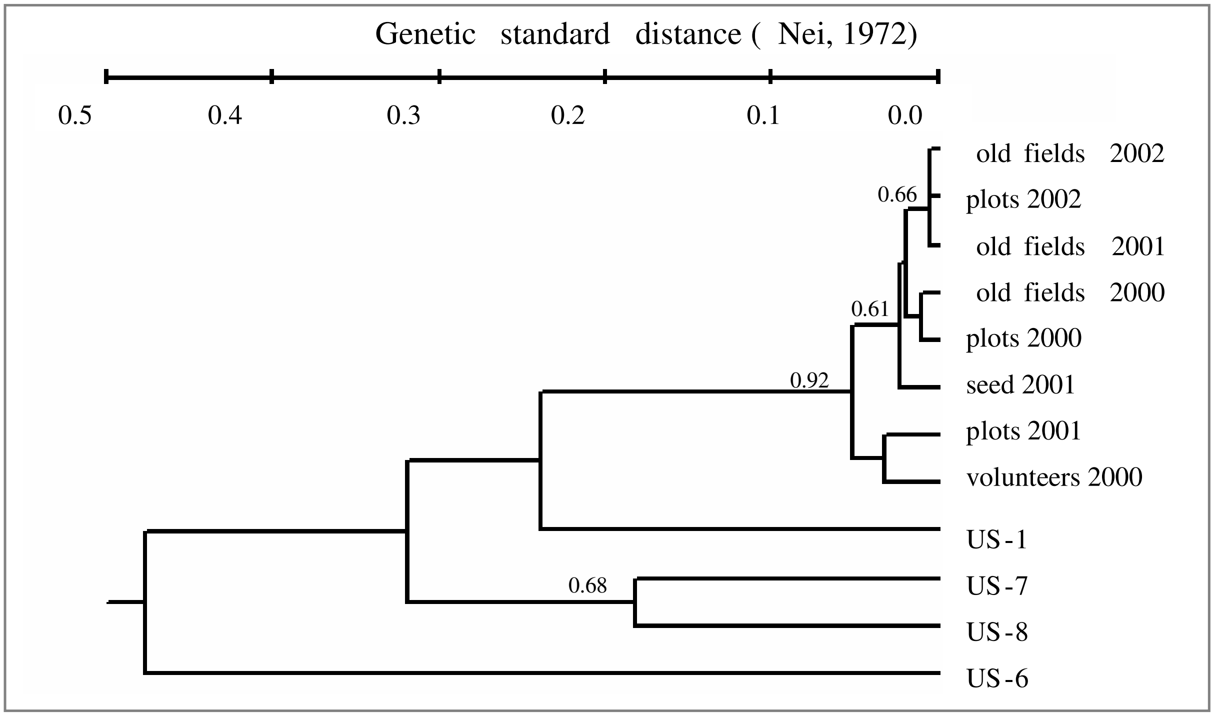


Figure 5. UPGMA dendrogram of multilocus haplotypes (Rep-PCR fingerprints plus mating type) of *P. infestans* isolates collected from different sites in Northern Hessia, 2000 to 2002 compared to single isolates of four clonal US-lineages. Cluster analysis was based on a genetic standard distance matrix (Nei, 1972) with 1,000 permutations of bootstrapping and proportions of >0.50 similar replicates are marked at the nodes.

Generally, the *P. infestans* populations sampled from old fields were highly diverse with normalized Shannon diversity indices of H'=0.91, 0.60 and 0.65 in 2000, 2001 and 2002, respectively (Table 5).

Overall, the populations originating from fields with and without rotation could not be differentiated. However, most of the unique rep-PCR types from the old fields came from fields where potatoes had been cultivated in successive years. Some isolates from the new fields were also unique except for the populations originating from the experimental plots 2001. There, the isolates all shared the most common rep-PCR fingerprints rep-1 and rep-2 representing only one and six pathotypes, respectively, setting the populations clearly apart from the old field populations (Table 5).

Table 5. Population differentiation of *P. infestans* isolates (Chi^2-statistics, Raymond and Rousset, 1995) with different origin based on multilocus haplotypes.

population 1	**population 2**	**n_{loci}** [1]	**n_{test}** [2]		**Chi^2**	P-*value*	*H'* [3]	
potato cropping history								
old fields with rotation	no rotation (2000 to 2002)	15	42	90	21	0.87	0.66	0.78
old fields (2000)	new plots (2000)	11	14	27	13	0.93	0.91	0.57
old fields (2001)	new plots (2001)	11	68	33	51	<0.001	0.60	0.16
old fields (2001)	new large fields (2001)	11	68	30	29	0.12	0.60	0.48
old fields (2002)	new plots (2002)	11	50	50	18	0.25	0.65	0.61
origin of seed potatoes in new fields [4]								
Simone	Secura (plots 2000)	10	16	11	14	0.82	0.59	0.59
Agria	Linda (plots 2001)	2	18	15	7	0.14	0.09	0.13
Agria	Linda (large fields 2001)	6	14	16	22	0.04	0.40	0.60
own certified plots 2001	breeders seed fields 2001	6	33	30	83	<0.001	0.16	0.48
Agria	Linda (plots 2002)	7	25	25	15	0.39	0.76	0.62
seed production field 2001	plots 2002	8	30	50	47	<0.001	0.48	0.61

[1] n_{loci} = number of polymorphic loci (i.e. rep-bands plus mating type) between populations. The resulting degrees of freedom are $df = n_{loci} * 2$.

[2] n_{test} = number of isolates tested per population 1 and 2, separated with a vertikal stroke.

[3] Normalized Shannon diversity H' calculated on multilocus haplotypes per population.

[4] Potato plots 2000 and seed production fields 2001 were planted with two cultivars of introduced seed potatoes, while the plots of 2001 and 2002 were planted with certified seed potatoes produced on-farm.

The seed potatoes for Simone and Secura in the plots 2000 and for the seed production fields of Agria and Linda in 2001 all originated from different growers in northern Germany. This different origin affected the *P. infestans* populations as reflected by the UPGMA dendrogram (Figure 6) and a significant population differentiation between Agria and Linda fields in 2001 (P=0.04, Table 5). In contrast, the populations from the plots 2001 and 2002 with the seed originating from the experimental farm clearly paired together (Figure 6). The effects of origin of seed potatoes also became clear when comparing populations derived from plots which were planted with own on-farm certified seed potatoes in 2001 with the populations in the larger fields grown with breeders seed (P<0.001, Table 5). While there were clear effects of origin of seed potatoes, no prediction as to the population composition over time could be made. Thus, the populations in the plots 2002 grown with potatoes produced on-farm in 2001 were again highly dissimilar from the 2001 populations (Table 5).

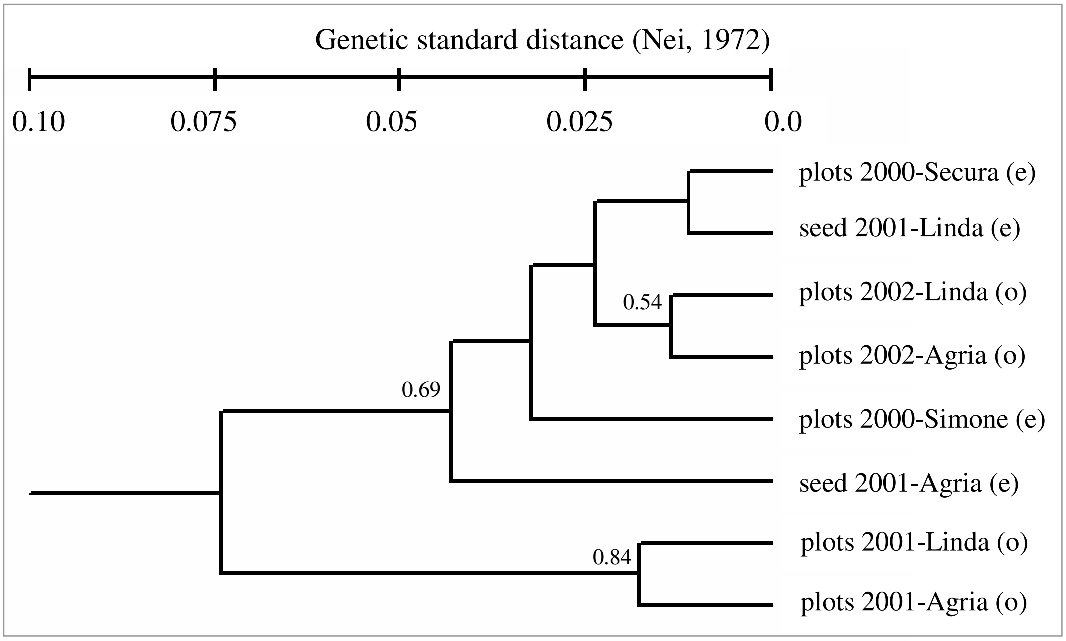


Figure 6. UPGMA dendrogram of multilocus haplotypes of *P. infestans* isolates collected from new plots and seed production fields planted with two different potato cultivars in 2000 to 2002. The origin of the seed potatoes was either external (e) from various growers in northern Germany or from own certified seed production on-farm (o). Cluster analysis was based on a genetic standard distance matrix (Nei, 1972) with 1,000 permutations of bootstrapping and proportions of >0.50 similar replicates are marked at the nodes.

Discussion

Overall, the studied *P. infestans* populations were highly diverse with respect to pathotypes and rep-PCR fingerprints. While two rep-PCR fingerprints dominated the population in all three years, representing on average 62 % of the isolates, clearly, these were not clones as isolates with the same fingerprint were of different mating type and pathotype. There were clear effects of the origin of seed potatoes and year on the population composition and/or virulence frequencies while effects of potato varieties and cropping history of fields were ambiguous.

The rep-PCR fingerprints were well reproducible and readily detected polymorphisms among the *P. infestans* isolates. The results for the reference isolates from the US were similar to SSR microsatellite analysis (Knapova and Gisi, 2002) and to RFLP results using probe RG-57 (Forbes et al., 1998). In these studies, lineage US-7 and US-8 always grouped closest together and clearly separated from US-1 (Knapova and Gisi, 2002) or US-1 and US-6 lineages (Forbes et al., 1998). In contrast, RAPD-PCR and AFLP analysis either grouped US-1 with US-7 or with US-8, respectively (Ghimire et al., 2002; Knapova and Gisi, 2002).

The greater similarity of the north Hessian *P. infestans* populations to the reference isolate US-1 in comparison to isolates of the other US-lineages possibly suggests that some members of the pre-1980 population might still be around. However, isolates of the old German populations would be necessary to allow for clear conclusions. Other recently characterized German populations were dominated by individuals belonging to the new populations (Flier et al., 2003; Rullich et al., 2002). Generally, the similarity of our isolates with the US-lineages was low like in the study of Knapova and Gisi (2002) comparing French and Swiss isolates with isolates of the US-lineages indicating that the European populations may have evolved independently from the populations in the US. This is supported by results of characterization studies on composition and diversity of *P. infestans* populations in different European countries (Day et al., 2004; Drenth et al. 1994; Fry et al., 1991; Sujkowski et al., 1996).

The overall diversity among the 281 isolates that were analysed with rep-PCR and two primers (H'=0.64) was similar to a Canadian study employing RAPD analysis. There, with six RAPD primers 77 polymorphic bands were detected, resulting in high Shannon diversity (H'=0.76) within the *P. infestans* populations (Mahuku et al., 2000). However, the results are highly primer dependent as 11 primers resulted in only 26 polymorphic bands in a study in Nepal (Ghimire et al., 2002). While diversity indices H'>0.70 are commonly reported for *P. infestans* populations it is important to keep in mind the scale at which the collections were sampled. For example, the normalized Shannon diversity of populations from Norway and Finland was H'>0.75 (Brurberg et al., 1999). In contrast, our samples originated from a relatively small 15 by 20 km region in northern Hessia in Central Germany. An even higher diversity could be expected if *P. infestans* populations from a larger region such as whole Germany including regions with high potato cropping intensity were sampled.

With increasing sampling intensity more rare phenotypes will be picked up (Day et al., 2004). Thus, if there were no difference, a lower diversity should be expected in the old fields where sampling intensity was lower than in the new fields. In contrast, the number of different and unique rep-PCR types and the Shannon diversity indices among the isolates from the old fields were considerably higher than from the new fields. This might be a result of the growing practices at those sites. Very similar to allotment gardens, potatoes were commonly grown with short or no rotation and the farmers usually planted their own potato seed. Other researchers also reported higher genetic diversity of *P. infestans* populations derived from allotments than from conventional potato fields (Cooke et al., 2003, Zwankhuizen et al., 1998). Because of early infections, severely infested foci and the common co-existence of both mating types within most of the old fields, it is likely that oospores did provide some inoculum in these sites. However, the sampling intensity in the old fields was much lower than in the new areas. The association of the rarer virulences v2, v5 and v6 with mating type A2 and the significant differences in virulence frequencies between new and old fields on the other hand suggest that asexual reproduction and migration through seed potatoes were at least as important in shaping the *P. infestans* populations studied.

The effects of the origin of the seed potatoes were clearly evident in the large fields of Agria and Linda in 2001 and also when comparing with the plots grown from own seed. The lack of significant population differentiation between the populations on Simone and Secura in 2000, in contrast, might be due to differences in initial population size and also plot arrangement. The Agria and Linda fields in 2001 were about 2 ha each while the size of the Simone and Secura plots was only 700 m^2 per variety combined over all plots. Thus, most likely, the initial *P. infestans* population sizes from the introduced seed potatoes were much smaller in 2000 than in 2001. In a 100 m^2 plot the likelyhood of all seed potatoes being healthy is much higher than in a 2 ha field and thus some of the plots in 2000 might have been infected by incoming inoculum while the inoculum from within the fields might have dominated in the production fields in 2001. Waggoner (1962) expected lower late blight infection probability in smaller potato fields, especially with planting pattern in wide strips perpendicular to the main wind direction. As our own field experiments revealed reduction of the infection frequency and blight epidemic progress depending on plot size, planting direction and neighbouring species (Bouws-Beuermann and Finckh, 2001) an influence on the population structure in such diversified smaller plots can be expected**.**

The changes of the *P. infestans* populations from the seed potato fields in 2001 to the plots in 2002 that were grown from these seeed potatoes may be due to genetic bottlenecks through which a predominantly asexually reproducing population has to go each winter (Drenth et al., 1994; Fry et al., 1991). These may have been very narrow for our studied populations due to low field size and also low tuber infections due to faster death of the unsprayed potato canopy and relatively unfavourable weather. The occurrence of only a few genotypes in successive years is also suggestive of bottlenecks which have been detected and inferred in many other cases (Drenth et al., 1994; Ghimire et al., 2001; Goodwin et al., 1995a; Jaime-Garcia et al., 2000; Punja et al., 1998; Zwankhuizen et al., 2000). If only a few advantageous genotypes survived the winter this would explain that the populations on Agria and Linda plots were more similar to each other than to the populations in the original seed potato fields. A more detailed study of the isolates originating from the plots 2002 indicated cultivar-specific adaptation which was already apparent at the beginning of the epidemic (Bouws-Beuermann and Finckh, *unpublised*).

It is unclear, if the results of this study are influenced by the organic growing practices at the experimental farm. In organic farming, seed potatoes are often presprouted, a process during which most infected tubers should become visible and consequently discarded. This will contribute to late blight control. As the frequency of latent infested tubers and also the transmission rates via seed-borne inoculum are not finally clarified, more intensive screenings of seed tuber lots are needed when studying field populations. It would be interesting to compare the influence of migration via seed potatoes in organic versus conventional farming, when tubers are treated. Therefore, *P. infestans* populations from different locations/fields grown from the same lots of seed potatoes (and varieties) should be characterized to better understand the role of seed potatoes on the inter- and intraregional population dynamics over time.

Acknowledgements

Many thanks to Roselyne Corbière and Didier Andrivon (INRA, Rennes, France) for providing molecular training in their lab and to Bill Fry (Cornell University, US) for providing the isolates of the clonal US-lineages. Hanna Stolz, Johanne Kossmann and Evelyn Geithe provided technical assistance in the lab at University of Kassel. The field experiments 2001 and 2002 on intercropping of potatoes are funded by the EU Blight-MOP Project.

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3) Cultivar-specific adaptation of *P. infestans* isolates to different potato hosts as revealed by tests of aggressiveness

Summary

A total of 90 *Phytophthora infestans* isolates originating from two naturally infested potato cultivars Agria and Linda (moderately resistant and susceptible to late blight, respectively) were tested in two laboratory experiments for parameters of aggressiveness such as infection efficiency (IE), sporulation capacity (SC), maximal growth rate (MGR) and Area under lesion expansion curve (AULEC). Each isolate was inoculated on leaflets of both test cultivars, Agria and Linda. There was no effect of sampling time (early and late in the epidemic) on aggressivity of the isolates. Overall, A2 isolates grew faster and produced larger lesions but had a lower sporulation capacity than the A1 isolates. Also, A2 isolates originating from the moderately resistant host Agria resulted in significantly higher IE and AULEC. The implications for resistance deployment strategies in potato production are discussed.

Introduction

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary is considered to be one of the most devastating potato pathogens. Since the introduction of the new populations including the A2 mating type in the 70‘s or 80‘s of the 20th century to Europe and the US, *P. infestans* populations have changed considerably, increasing in variability (Dorrance et al., 1999; Drenth et al., 1995; Elansky et al., 2001; Gavino et al., 2000; Goodwin et al., 1995; Sujkowski et al., 1996; Therrien et al., 1993; Zwankhuizen et al,. 2000) and reproducing sexually as indicated by the occurrence of oospores under natural field conditions (Andersson et al., 1998; Brurberg et al., 1999; Chycoski & Punja, 1996; Turkensteen et al., 2004).

Along with this general change in genetic make-up, the newly introduced populations have been reported to be fitter than the populations present before the 1980s (Day & Shattock, 1997; Miller et al., 1998; Peters et al., 1999). Also, in some cases, especially in the US, an increase of fungicide resistance was observed (Cooke et al., 2003; Daayf et al., 2000; Davidse et al., 1989; Goodwin et al., 1992; Marshall-Farrar et al., 1998; Wangsombondee et al., 2002). While insensitivity against the systemic fungicide Metalaxyl of most of the new immigrants has been implied as a reason for the rapid displacement of the old population (i.e. US-1 lineage) which had been sensitive (Goodwin et al., 1992), even under unsprayed conditions Metalaxyl resistant strains have been reported among the new populations (Fraser et al., 1999; Goodwin et al., 1995; Goodwin et al., 1998; Chycoski & Punja, 1996; Holmes & Shannon, 1984).

Apart from the use of fungicides processes of variation and adaptation of *P. infestans* populations towards its host may be influenced by many factors such as the frequency of sex, environmental conditions, the production intensity, the cropping frequency of potato (varieties) and the cultivation of hosts with different late blight resistance background. Adaptation processes of *P. infestans* populations and distributional patterns of clonal lineages especially in the absence of sex often are host-related. Different genotypes or clonal lineages were reported to prefer either potatoes or tomatoes, respectively (Fraser et al., 1999; Hammi et al., 2002; Knapova & Gisi, 2002; Lebreton & Andrivon, 1998; Reis et al., 2003; Suassuna et al, 2004; Wangsombondee et al., 2002). Often, a few genotypes dominate in an area, varying from region to region and from year to year, especially in North America indicating that sex may be absent or rare (Daayf & Platt, 2000; Goodwin et al. 1995; Goodwin et al., 1998; Punja et al., 1998;). In contrast, in most European countries sexual reproduction of the pathogen contributes to high variability and genotypic diversity among

P. infestans populations in successive years (Bouws-Beuermann & Finckh, *submitted*; Brurberg et al., 1999; Hermansen et al., 2000; Drenth et al., 1993; Sujkowski et al. 1994; Zwankhuizen et al., 1998; Zwankhuizen et al., 2000).

Cropping intensity and the frequency of popular potato varieties has led to an adaptation of *P. infestans* populations on many occasions when race-specific resistances had been used (Graham et al., 1959; Janssen, 1973; Malcolmson, 1969; Schick et al., 1958; Shattock, 1974; Shattock et al., 1977) resulting in the typical boom and bust cycles known for so many pathosystems such as potato - *P. infestans* (Pilet, 2003; Turkensteen, 1993; van der Plank, 1971), cereals and many of their pathogens (Brown, 1995; Johnson, 1961; Kiyosawa, 1989; McDonald et al, 1989; Wolfe & McDermott, 1994). Because of the common breakdown of race-specific resistances of potatoes against *P. infestans,* the focus in breeding has been on durable non race-specific resistance and there are several cultivars that are still cultivated with no apparent resistance loss after 50 to 70 years of cultivation (Colon et al., 1995; Turkensteen, 1993; van der Plank, 1971). Several newer breeding programs dealing with wild *Solanum* species as e.g. *S. bulbocastanum, S. berthaultii, S. chacoense* among others have resulted in highly resistant potato clones expressing durable resistance (Colon & Budding, 1988; Grünwald et al., 2002; Wastie, 1991). While some authors have promoted the cultivation of highly resistant potato varieties as an effective strategy also enabling the reduction of maximum fungicide dosages (Colon et al., 1995; Fry, 1978; van Oijen, 1991), this strategy is expected to increase the selection for increased fitness within *P. infestans* populations and to thus finally also lead to a breaking of relatively durable resistances when those varieties are widely grown (Latin, 1981).

An example for such a breakdown may be the cultivar Agria, a multi-purpose variety with moderate resistance against foliage late blight which was introduced in 1985 in Germany (Erbe & Lüthke, 2004; Schuhmann, 2004). Because of these properties Agria has become popular especially in German (Erbe, 2004; Möller et al., 1996; Möller et al., 1999; Stöppler et al., 1990), organic production systems However, in recent years, its resistance has been broken especially in Switzerland and it is listed as a susceptible variety there. Also, in our own field experiments since 1999 we have observed an apparent increase in susceptibility of Agria (Finckh & Bouws-Beuermann, unpublished).

Cultivar-specific adaptation of *P. infestans* populations in general to varieties without major race-specific resistance has been reported (Caten, 1974; Jeffrey et al., 1962; Paxman, 1963) with some studies indicating adaptation only to the more resistant varieties such as Pimpernel (Bjor & Mulelid, 1991). In contrast, others have found that fitness of *P. infestans* isolates decreased with increasing resistance level of potato varieties (Berggren et al., 1988; Caten, 1974; Day & Shattock, 1997). A preliminary study in 2001 comparing *P. infestans* isolates originating from the moderately resistant Agria and the highly susceptible Linda (both do not possess any of the known R1 to R11 genes, own unpublished results) revealed that isolates sampled late in the epidemic were more aggressive than early sampled isolates and a tendency for cultivar-specific adaptation towards Agria was observed (Bouws-Beuermann et al., 2003).

Most of the studies on fitness of *P. infestans* populations discussed above have been conducted with isolates originating from commercial sprayed potato fields. Therefore, selection was not only for adaptation to the hosts of origin but also for fungicide resistance. As more and more fungicides are being lost from the market and public pressure for reduction in their use is intense, disease resistance will play a more and more important role in the future. For the most effective use and management of resistances it is important to know if different cultivars select in different directions. This would allow a farmer to reduce disease pressure within years by growing varieties selecting for differentially aggressive populations and also over years by strategic changes of potato varieties or at least the fields in which certain varieties are grown.

The objectives of this study were therefore to determine in the absence of the selective force of fungicides if *P. infestans* populations adapt to potato host cultivars with different resistance level over the course of an epidemic. Isolates were sampled from naturally infected well separated large experimental plots (18 m x 36 m) which were managed organically without any fungicide application. Isolates were collected at the beginning and peak of the epidemic 2002 from two potato hosts Agria and Linda (moderately resistant and susceptible to late blight, respectively) and tested under controlled laboratory conditions on leaflets of both test cultivars Agria and Linda. Aggressiveness parameters such as infection efficiency, growth rate, sporulation capacity and Area under the lesion expansion curve (AULEC) were determined to investigate i) if *P. infestans* populations adapt to their potato host of origin over the course of an epidemic. As the first experiment revealed an effect of mating type on the fitness parameters, in the second experiment mating type was included as a factor to determine ii) if mating type influences the aggressiveness of isolates and iii) if mating type and the two host cultivars with different non race-specific resistance level do interact.

Materials and Methods
Experimental conditions and late blight epidemic 2002

Potato plots from which *P. infestans* isolates originated were part of a large field experiment dealing with different plot sizes, neighbour crops and field geometry (Bouws-Beuermann & Finckh, 2002; Finckh et al., submitted; Das muß je nach Zeitpunkt der Submission rausgenommen werden. Bouws-Beuermann, 2005 thesis) conducted at the organic experimental farm of University of Kassel situated in northern Hessia, Central Germany. No chemical fertilizers or pesticide sprayings were used. The moderately resistant and susceptible potato varieties Agria and Linda, respectively, were planted on 16th of May 2002.
After appearance of first blight symptoms the percentage infested canopy per plot was assessed every 3 to 4 days until crop death and the Area under disease progress curve (AUDPC) was calculated. Agria was infested around two weeks later than Linda (10th of July and 28th of June 2002, respectively). Due to continuous heavy rainfall in mid-July, the epidemic progressed very fast and severity reached up to 80 % infected canopy in Linda plots within two weeks (Bouws-Beuermann & Finckh, 2002). The epidemic pressure was significantly lower on Agria than on Linda (AUDPC=1281 and 1746, respectively).

Collection and selection of *P. infestans* isolates

P. infestans populations were sampled in two 24 row (=18m) wide by 36 m long potato plots of either Linda or Agria, respectively. Isolates were collected at the initial stage and peak of the epidemic (around 5 % and 75 % diseased leaf area, respectively) and time intervals between early and late sampling were two and three weeks for Linda and Agria isolates, respectively. Isolates were picked up in a regular pattern: From six out of 24 rows an isolate was collected every 6 m, i.e. five per row resulting in 30 isolates per variety (n=2) and sampling date (n=2) amounting to a total of 120 isolates. Isolation procedures are described in detail elsewhere (Bouws-Beuermann and Finckh, submitted). All isolates were tested for their mating type and subsets were analysed for their virulence and molecular types, respectively (Bouws-Beuermann & Finckh, submitted).
As laboratory tests of aggressiveness are very labour-intensive only about 60 isolates could be handled in one experiment. For each sampling date and variety 15 isolates were selected randomly and tested immediately after their isolation in September 2002, i.e. test 1 (Table 1). While mating type did not play a role in isolate selection in test 1 it appeared to be of importance and was therefore included as experimental factor in test 2 in March 2003 (Table 1). There, thirty isolates of test 1 were included plus 30 additional well growing isolates to obtain a relatively even balance for mating type and host of origin.

Cultivation of plants and inoculations

The potato cultivars Agria and Linda were cultivated in 13 cm diameter plastic containers in a 1:1:1 sand-peat-compost mixture in the glasshouse at 18 °C/15 °C day/night, 14 h daylenght. Only the four lateral leaflets of fully expanded leaves of ca 7 week old plants were used for the tests. Leaflets were deposited adaxial face up on moist filter paper in square plastic dishes (14 cm x 14 cm) with four leaflets per dish.

During the whole experimental time the isolates were kept on 1.5 % pea agar (125 g fresh frozen peas per litre water) at 17 °C in darkness transferring them onto new medium every three weeks. Mycelium grown for two weeks on agar plates was scraped and washed down with 4 ml of sterilized water. All sporangial suspensions were adjusted to a concentration of 5×10^4 sporangia/ml with a “Fuchs Rosendahl” haemacytometer and stored at 8 °C for 3 h to allow emergence of the zoospores. Each leaflet was inoculated with a 20 µl drop of the prepared sporangial suspension and leaflets were incubated at 18°C/15°C day/night with 16 h photoperiod with the first 12 h in darkness. To prevent drying of leaflets and to enhance fungal development, a high relative humidity was maintained in the petri dishes throughout the experiments by moistening the lids of the petri dishes every two days. Each isolate was tested on leaflets of both potato cultivars Agria and Linda. The number of pseudo-replicates for the combination of isolate by test variety was eight and 12 for the two tests, respectively (Table 1).

Assessments

The infection efficiency (**IE**) was calculated as the proportion of inoculated spots that developed sporulating lesions. From the 4th to 7th day after inoculation the percentage of diseased leaf area was assessed visually. Length and width of the leaflets were measured and leaf area was calculated as an ellipse. The absolute lesion size (LS) was calculated by multiplying percentage of diseased leaf area by leaf area. Area under the lesion expansion curve (**AULEC**) was calculated using the following equation (Kranz, 1996):

$$\text{AULEC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) \left(t_{i+1} - t_i \right) \qquad (1)$$

where y_i = % diseased leaf area at assessment i, t = time (days) of assessment i,
n = number of assessments

Due to some variance in leaf size which could influence also lesion size, the AULEC was calculated for the time interval day 4 to day 6 after inoculation, because no isolate had reached 100% diseased leaf area before day seven. Disease progress curves were generated for each successful inoculation and the curves were fitted to the Logistic and Gompertz growth models (Campbell & Madden, 1990). The Gompertz model fitted best for moderate disease progress at the beginning of lesion expansion, whereas the Logistic growth model led to higher maximum growth rates, but a reduced fit to the actual AULEC curves. From the Gompertz model the maximal growth rate (**MGR**) was calculated as the first derivative of the fitted curve at the point of inflexion.

At the 7th day after inoculation the leaflets were placed in test tubes, overlaid with 4 ml sterile distilled water and frozen at –20°C. Gradually, the tubes were heated in a water bath of about 40 °C, vortexed strongly and the sporangia were counted with a haemacytometer. The total number of sporangia produced per lesion was divided by the lesion area (day 7) to obtain the sporulation capacity per cm^2 lesion (**SC**).

Statistical analysis

Statistical analysis was performed with SAS software (SAS Institute, Cary, NC). Only those isolates resulting in at least two or more sporulating lesions on both test cultivars (n= 54 and n=50 in test 1 and 2, respectively) were analysed the following way. Normality of raw data was confirmed with Shapiro-Wilk-Test (UNIVARIATE procedure). For further statistical analysis of the parameters IE, AULEC, SC and MGR, means were calculated from the sporulating pseudoreplicates for each combination of isolate x test cultivar (i.e. n=108 and 100, respectively). These combined mean data were analysed with the GLM procedure as a three-factorial design with host by sampling by test cultivar (test 1 only) and host by mating type by test cultivar (both tests separately), respectively.

To evaluate different behaviour of the isolates in the two tests, the data for the 30 isolates, which were used in test 1 (immediately after isolation) and replicated in test 2 (same isolates after 6 months of storage) were compared for an influence of the factor test. Also, these replicated isolates were analysed with GLM as mentioned above. Generally, if significant effects were detected, means were compared pairwise with t-tests, based on Least Square Means.

Pearson's correlation coefficients for the calculated fitness parameters were estimated with CORR procedure. Correlations were determined for each test separately (data not shown) and in a joint analysis of both tests, i.e. combinations of isolate by test cultivar (n=208) together.

Results

Inoculation success and reproducibility of tests

The inoculation success for all inoculation spots was 0.66 and 0.42 in test 1 and 2 (n=960 and n=1440 inoculated leaflets), respectively. Fiftyfour and 50 isolates resulted in at least two lesions on each of thetwo tested cultivars. Infection efficiencies presented are based on these isolates only. All fitness parameters were significantly higher in test 1 when the "fresh isolates" were inoculated (P<0.01). The 30 isolates that were replicated in test 2 revealed highly significant isolate effects (P<0.001). Also, they had significantly reduced IE and AULEC in comparison with the corresponding parameters of test 1 (P<0.001). In total, 27 out of 30 isolates had higher IE, AULEC, MGR and SC in test 1 than in test 2, indicating an effect of storage time on fitness in general (data not shown). There appeared to be a specific effect of storage on the SC of A2 isolates. While the sporulating capacity of the 15 A1 isolates was nearly identical in both tests (17.2 and 18.0 respectively), that of the 15 A2 isolates was reduced from 17.1 to 8.7, resulting in a significant interaction of test by mating type (P<0.01).

Effect of sampling date

Test 1 did not reveal any significant difference in fitness parameters between early and late sampled isolates (data not shown). Because the number of early and late sampled isolates was not balanced in test 2 (see Table 1) sampling time was not included as a factor.

Effect of host of origin, test cultivar and their interaction

In test 1, the infection efficiency of isolates originating from Agria was significantly higher than that of Linda isolates, while there was no difference in test 2 (Table 2). Also, Agria isolates resulted in greater AULEC than Linda isolates in test 1 with the same tendency in test 2, but was not significant (Table 2). Infection efficiency, AULEC and MGR were nearly the same on both test cultivars within each test (data not shown). In test 1, all isolates originating from either host resulted in higher sporulation capacity when they were tested on the susceptible test variety Linda (Table 2) however, the effects of this test cultivar on the SC of isolates was not significant (P=0.10).

Both tests showed a tendency towards highest AULEC for Agria isolates tested also on Agria (96.8 and 45.3, respectively) followed by the Agria isolates tested on Linda (85.9 and 34.6. respectively), while the other combinations of Linda host by test cultivar revealed no trend (Table 2). While all isolates sporulated best on susceptible test variety Linda in test 1 (i.e. mean SC on Linda = 22.9 versus 19.6 on Agria), only the Linda isolates resulted in highest SC on Linda in test 2, however not significant.

Effect of mating type and interaction with potato host of origin

The A2 isolates that were included in test 1 had a significantly higher MGR than the A1 isolates (P=0.01) and the same trend was observed in test 2 (Table 3). Also, A2 isolates resulted in higher AULEC than the A1 isolates in both tests. While, the IE of the "fresh tested" isolates did not differ among mating types in test 1, the A2 isolates infected significantly more frequently than the A1 isolates (P=0.01) in test 2. In contrast, the SC of the A2 isolates was significantly reduced in comparison to A1 isolates in test 2 (Table 3). The same trends were observed when considering separately the 30 isolates that had been used in both tests.

The differences of the fitness parameters of A1 and A2 isolates were also affected by the host of origin of isolates. The infection efficiency of the A2 isolates originating from host Agria was significantly higher than that of the A1 isolates from Agria in both tests (Figure 1a). No significant differences were observed for isolates originating from Linda.

Similarly, A2 isolates from Agria had the highest AULEC, followed by the A1 isolates originating from Linda while AULEC was lowest for the two other host of origin by mating type combinations (Figure 1b). However, these interactions between host and mating type were not significant in test 2 (Figure 1b). The reversal of the order for the isolates originating from Linda between test 1 and 2 is the reason why the main effect mating type was only significant in test 2 for IE and AULEC (Table 3).

There were no significant interactions between host of origin and SC of the mating types, however, the effect of storage on the SC of A2 isolates can be clearly seen (Figure 1c).

Correlation of fitness parameters

The separate correlation analyses for test 1 and test 2 revealed significant correlations (except for SC) among all fitness parameters IE, AULEC and MGR (data not shown). Therefore, the joint analysis of both datasets (n=208) is presented (Table 4). Sporulation capacity was the only parameter that did not correlate well with any of the others. Not surprisingly, MGR was highly correlated to AULEC and IE (R^2=0.83 and 0.46, respectively). There was no significant correlation between the number of virulences of the tested isolates and any of the fitness parameters, however (data not shown).

Discussion

Cultivar of origin and mating type and their interaction appeared to be very important factors in this study, influencing fitness parameters of *P. infestans* isolates. Overall, the Area under the lesion expansion curve (AULEC) appeared to be the best parameter to identify cultivar specific adaptation in this study.
An important factor affecting the outcome of the tests was the storage time as evident from test 2. Loss of fitness during storage is a commonly observed phenomenon with *P. infestans*. Tooley et al. (1986) found significant negative reductions of the infection efficiency and fitness index after increasing storage of isolates (R^2=-0.65 and R^2=-0.42, respectively). Also, the storage of *P. infestans* isolates in liquid nitrogen (Day & Shattock, 1997) as well as continuous inoculation on plants or plant parts in the laboratory or greenhouse resulted in fitness losses of the isolates (Spielman et al., 1992; Sujkowski, 1986). Even experiments within two months revealed significant interactions of storage time with cultivar and isolate (Peters et al., 1999). Storage not only affected fitness in general but interacted with mating type. Thus, a simple repetition of aggressivity tests for *P. infestans* appears not to be possible.

The lack of significant effects of sampling time is in contrast to our preliminary results and results from others. Pilet (2003) reported a significant increase of aggressiveness of isolates during late blight epidemics, however, this was cultivar dependent. As adaptation within season was not evident in this study, the cultivar-specific adaptation must have happened before or right at the beginning of the epidemic. The initial differences in adaptation could either be due to selection during seed potato storage or at the site of production in the previous year. On the other hand, if the epidemic was established by incoming inoculum the initial selection must have happened during establishment of the *P. infestans* populations.

Differences in fitness could also arise by chance if the pathogen population is largely clonal and different clones were established on the cultivars in the beginning. The isolates used were not clonal, however. This was shown in a larger study on the population structure of *P. infestans* on the experimental farm and in the surroundings (Bouws-Beuermann and Finckh, submitted). In that study, 18 of the 90 isolates that were used in this study were included. Among these 18 isolates, 16 multilocus haplotypes and eight pathotypes were identified. The study by Bouws-Beuermann & Finckh (submitted) also showed that seed-borne inoculum may play an important role in shaping the pathogen population structure. It could not be established, however, if the populations on Agria and Linda originated from seed borne infections or from incoming inoculum. Thus, with our data it is not possible to decide if selection acted during storage or on incoming external inoculum.

The fact that A2 isolates originating from the more resistant host cultivar Agria were more aggressive is in line with results by Day & Shattock (1997) reporting A1 isolates to be more aggressive on susceptible Maris Piper, while A2 isolates were better adapted towards moderately resistant Cara. More evidence for better adaptation of A2 isolates to resistant cultivars comes from field collections. Isolate collections of *P. infestans* in the Republic of Ireland in the years 1988 to 1994 yielded 46 % of the A2 isolates originating from the cultivar Cara indicating an association of A2 mating type and this variety (O'Sullivan et al., 1995). Similarly, out of more than 1,000 isolates collected from more than thirty cultivars in Canada, A2 isolates were recovered from a wider range of cultivars and they infected moderately to highly resistant varieties to a greater extent and with faster growth (*P*=0.02) than the A1 isolates (Chycoski & Punja, 1996). If A2 isolates are more associated to moderately and highly resistant potato varieties which stay longer green during an epidemic this increases the probability of sexual reproduction on the plant**,** i. e. in-the-green-crop sex (Zwankhuizen et al., 2000). The results from Bouws-Beuermann and Finckh (submitted) are suggestive of sexual and asexual reproduction both occurring in the area studied.

In order to make use of cultivar specific adaptive processes within a farming system, several conditions have to be met. Different cultivars have to be present and population subdivision during the cropping season has to be favoured. This is probably not the case in typical conventional potato growing areas such as e.g. several US regions or European countries as e.g. France, the Netherlands and Belgium, where only a few (often susceptible) potato varieties are cultivated in most of the potential potato growing areas (Inglis et al., 1996; Erbe & Lüthke, 2004;). In contrast, in organic farming systems usually more different crops and several cultivars are grown in generally smaller fields. In such a more diversified system overall inoculum pressure should be reduced as has been reported for other diversified cropping systems such as barley (Wolfe, 1992) or rice (Zhu et al., 2000). Additional diversification strategies within such a generally more diverse system might then be of interest.

Various diversification strategies have been tested for their effects on late blight epidemic development such as cultivar mixtures (Finckh et al., 2003; Garrett & Mundt, 2000; Philipps, 2004; Pilet, 2003), alternating rows or strips of different varieties (Andrivon & Lucas, 1998; Andrivon et al., 2003; Beuermann et al., 2000) and intercropping of potatoes (Bouws-Beuermann & Finckh, 2002; Finckh et al., 2004). In pure stands compared with mixed stands of potato cultivars the selective pressure on *P. infestans* populations is expected to be higher (Garrett & Mundt, 2000; Philipps, 2004). In mixtures and alternating rows inoculum interchange between cultivars appears to result in disease reductions on the susceptible host while the severity on the resistant hosts may even be higher (Andrivon & Lucas, 1998; Andrivon et al., 2003; Conolly et al., 1995; Finckh et al., 2003; Garrett & Mundt, 2000; Philipps, 2004; Pilet, 2003). This inoculum interchange in mixed stands may lead to a homogenisation of *P. infestans* populations leading to less cultivar-specific adaptation processes. In contrast, intercropping of potatoes with strips of different potato cultivars interspersed with other crops might result in population subdivision and thus allow for disruptive selection among *P. infestans* populations.

The results of this study are suggestive of cultivar specific adaptation towards the non-race-specifically moderately resistant cultivar Agria. This might be of interest especially in diversified farming systems that rely on the use of different varieties and species. In order to verify these results it will not be possible to repeat the same experiment with the same isolates due to the large effects of storage time on the fitness of *P. infestans* isolates. Instead, different *P. infestans* populations originating from different varieties in different years should be studied in order to determine under which conditions cultivar specific adaptation might play a role in the field.

Acknowledgements

Part of this study was financially supported by the ZFF fund of the University of Kassel. We thank H.-P. Piepho (University of Hohenheim, Germany) for statistical support of data analysis, J. Kossmann and E. Geithe for technical assistance in our laboratory.

Table 1. Host of origin, epidemic stage and mating type of *P. infestans* isolates collected from experimental potato plots in 2002 that were used in two laboratory tests of aggressiveness.

factor 1 **host cultivar**	factor 2 **mating type**	sampling date	test 1 1.-9.9.02	test 2 1.-9.3.02
Agria		early	15	(5) [1]
		late	15	(22)
Linda		early	15	(7)
		late	15	(26)
Agria	**A1**		(20)	16
	A2		(10)	11
Linda	A1		(18)	15
	A2		(12)	18
		isolates tested in total	60	30+30 [2]
		test cultivar (factor 3)	**Agria** and **Linda**	
		number of replications per isolate each test cultivar	8	12
		total inoculated leaves	960	1440

[1] numbers in parentheses indicate the number of isolates possessing a trait not considered when originally selecting isolates for test 1 (in that case mating type). Due to unequal sample size sampling date was not a factor in test 2.

[2] 30 isolates from test 1 were repeated in test 2 plus 30 other isolates of the collection 2002

Table 2. Mean fitness parameters [1] for isolates with potato host of origin Agria and Linda inoculated on leaflets of these two test cultivars in two laboratory tests [2].

	test 1 (1.-9.9.02)					test 2 (1.-9.3.03)				
Test cultivar	Agria	Linda	Agria	Linda		Agria	Linda	Agria	Linda	
Host of origin	**Agria**		**Linda**		*P*-value [3]	**Agria**		**Linda**		*P*-value
IE	0.77	0.79	0.72	0.63		0.50	0.49	0.52	0.51	
IE mean	**0.78**		0.68		**0.04**	0.49		0.51		0.75
AULEC	96.8	85.9	77.8	76.2		45.3	34.6	32.6	32.3	
AULEC mean	**91**		77		**0.07**	**40.0**		32.4		**0.15**
MGR	0.051	0.049	0.047	0.046		0.035	0.029	0.032	0.031	
MGR mean	0.050		0.046		**0.19**	0.032		0.031		0.87
SC *1000	20.3	23.5	18.9	22.4		13.0	11.1	12.9	15.1	
SC mean	21.9		20.6		0.52	12.1		**14.0**		0.52

[1] IE = infection efficiency, i.e. frequency of sporulating lesions; AULEC = Area under lesion expansion curve, i.e. accumulated % diseased leaf area from day 4 to day 6 after inoculation.; MGR = maximum growth rate, calculated with Gompertz growth model; SC = sporulation capacity per cm^2 lesion at day 7. The parameters were compared with t-tests (GLM), LS-Means are shown

[2] see Table 1 for test details

[3] parameters were compared with t-tests (GLM), LS-Means are shown

Table 3. Mean fitness parameters for isolates belonging to mating type A1 or A2 in two different laboratory tests [1].

	test 1 (1.-9.9.02)			test 2 (1.-9.3.03)		
parameter [2]	A1 (36) [3]	A2 (18)	*P*-value [4]	A1 (27)	A2 (23)	*P*-value
IE	0.74	0.72	0.67	0.43	**0.58**	**0.01**
AULEC	77	**91**	**0.07**	27	**45**	**<0.01**
MGR	0.045	**0.052**	**0.01**	0.030	**0.033**	0.16
SC * 1,000	22.2	20.2	0.32	**16.0**	10.0	**0.05**

[1] see Table 1 for test details
[2] for explanation of abbreviations see Table 2
[3] number of successfully tested isolates per mating type, i.e. at least 2 sporulation spots on both test cultivars
[4] parameters were compared with t-tests (GLM), LS-Means are shown

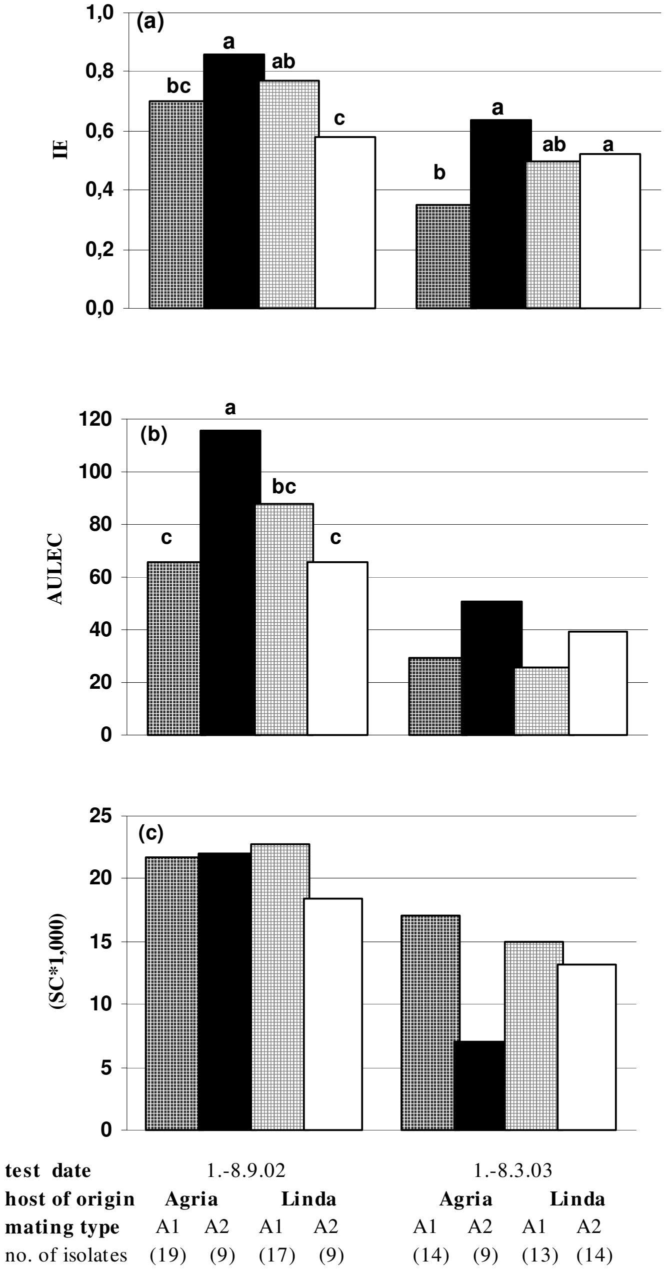
Table 4. Pearson correlation between fitness parameters for both datasets with n=208 isolates by test cultivar in two tests.

	IE	**MGR**	**SC**
AULEC	0.52 ***	0.83 ***	0.27 ***
IE		0.46 ***	0.11 n.s.
MGR			0.25 ***

*** correlations significant at *P*<0.001. n.s.= not significant

Figures

Figure 1. Effects of potato host of origin Agria (black) and Linda (white) and mating type A1 (caro) and A2 (solid bars), respectively, on (a) infection efficieny IE (b) Area under lesion expansion curve AULEC and (c) sporulation capacity per cm^2 lesion (SC*1,000) of *P. infestans* isolates in two laboratory test. Columnes marked with different letters within test differ significantly (LSMeans, GLM, t-tests, $P<0.05$).



Section 4: Consolidated report on Diversification strategies

Introduction:

Diversification can make an important contribution to plant protection and is a potential component of an integrated systems approach to the control of late blight caused by *Phytophthora infestans* in potatoes grown according to organic standards. As well mechanistic interactions such as distance, barrier and microclimatological effects, complex ecophysiological interactions such as competition, induced resistance and allelopathy may act in diversified systems to reduce disease pressure. Traditionally, diversity is implemented through rotations and growing a number of different varieties of the same crop species on an individual farm. A different approach is to grow mixtures of varieties and species within the same field (and grow them in smaller fields). Previous experience has shown that late blight development in susceptible varieties can be reduced by adopting diversification strategies, including growing varieties with different types and levels of resistance in alternating rows or in random mixtures or intercropped with different species that are unaffected by late blight.

Alternating rows: Late blight infection in susceptible varieties can be reduced and their yields increased significantly by growing these varieties in rows alternating with other varieties displaying a partial (non-race specific) resistance to the pathogen. However, the performance of such varietal associations has not been assessed with different densities of partially resistant and susceptible varieties or with mixtures of partial and complete resistance (mediated by undefeated, race specific R-genes). Greater knowledge and understanding of the underlying mechanisms for the improved performance of such "associations" and influence of environmental conditions is needed to improve their reliability. Potential mechanisms include: physical barrier to inoculum dispersal caused by rows of resistant plants; progressive adaptation (during the course of the epidemic) of the pathogen genotypes to one or the other variety used in the association, thus restricting pathogen movement between varieties; compensatory growth effects between varieties. Field experiments that test the efficacy of alternating rows of different varieties with different types and levels of resistance can be used to investigate these 3 potential mechanisms by i) modelling the spread of late blight epidemics in pure and associated plots. ii) measuring variety-specific adaptation of *P. infestans* genotypes to determine to what extent any observed temporal adaptation of the pathogen population structure contributes to the performance of associations in controlling late blight. iii) quantifying competition between varieties in associated crops which may account for yield benefits that are independent of the effect of associations on disease control.

Variety mixtures: There is now substantial evidence that the development of air-borne pathogen epidemics can be significantly restricted by the use of variety mixtures, even if the mixtures contain only three or four different forms of resistance. The most important mechanisms which are thought to restrict epidemic development in mixtures are spatial separation of plants carrying the same resistance genes, barrier effects and induced resistance (i.e. plant A induces resistance in its neighbour B by plant A allowing the persistence of a pathogen race which is virulent to A, avirulent to but resistance inducing in B). Barrier effects and induced resistance should play a greater role when plants are mixed within rows than when planted in alternating rows. However, improvements in disease control in varietal mixtures must be weighed against additional costs for separating the varieties prior to sale and only certain types of mixtures may be of practical value depending on the target market (fresh, processing, industrial).

Intercropping: Once primary infection has taken place, the relatively heavy spores of *P. infestans* are mainly distributed within the field by rain splash and through direct contact of leaves. Separation of single or multiple rows/beds of potato by suitable barrier crops of a different species should restrict the development of blight epidemics by restricting row-to-row dispersal. However, the microclimatic conditions within the crop will be affected by the type of intercrop and the width of the potato beds, which may influence the efficacy of intercropping. Effectiveness of restriction of dispersal is expected to be greater in potato varieties with some resistance than in fully susceptible crops. Intercropping with relatively tall crops such as wheat should significantly delay the spread of blight and increase yields compared with shorter crops such as clover. Furthermore, the protective, barrier effect should increase as the strips of wheat get wider.

Within Blight-MOP the potential of various diversification strategies alone or in combination with other management options for reducing late blight was studied. The aims were to determine the effects on late blight epidemics of variety mixtures, alternating rows of different varieties and strip cropping (intercropping) with different species.

Hypotheses under test were:

(i) Alternating rows and variety mixtures and intercropping reduce the amount of susceptible plant tissue per unit area and so will reduce general disease pressure.

(ii) Interactions between neighbouring varieties and species influence disease development and yield through dilution, barrier, microclimatic and competitive effects.

(iii) Resistance type (race-specific or race-non-specific) influences the epidemiological interactions in diversified stands.

Experiments were conducted in the UK, Denmark (DK), France (F) and Germany (D) in 2001, 2002. Alternating rows and different types of resistance (F, DK), variety mixtures (UK, D), plot size (UK, D) and intercropping (DK, D) were tested.

Alternating rows:

Experiments were made in France and Denmark in both 2001 and 2002 to test the efficacy of alternating rows of different varieties with different types and levels of resistance on infection with late blight and crop yield. In France, the field experiments were also used to analyse epidemiological, ecophysiological and evolutionary mechanisms implicated and identify and understand underlying mechanisms for the improved performance of such "associations" which may include: physical barrier to inoculum dispersal provided by rows of resistant plants; progressive adaptation (during the course of the epidemic) of the pathogen genotypes to one or the other variety used in the association, thus restricting pathogen movement between varieties; compensatory growth effects between varieties. Approaches included modelling the spread of late blight epidemics in pure and associated plots; measuring variety-specific adaptation of *P. infestans* genotypes to determine the extent to which any observed temporal adaptation of the pathogen population structure contributes to the performance of associations in controlling late blight; quantifying yield effects which may be due to competition and better resource use rather than to better disease control.

During two consecutive years in France in Brittany the varieties Bintje, Desiree and Naturella showed no significant disease reduction in mixed (alternating rows of contrasting varieties) vs pure (single variety) plots irrespective of the planting pattern (with single or twin rows of varieties alternating with other varieties) and of the type of resistance used. As in the variety mixtures, disease on the highly susceptible variety Bintje was reduced in alternating rows while the moderately susceptible variety Desiree suffered more disease in combination with Bintje. Disease on the highly resistant variety Naturella was not different in alternating rows. However, the spatial distribution of disease was clearly different in pure and in mixed plots, although the heterogeneity of infection was very high within plots. There were no yield effects among the varieties. This indicates that either the three varieties were very similar in competitive ability or the distance between rows of 75cm was large enough to reduce competitive interactions.
Similar results were obtained in Denmark with the varieties Kuras (highly resistant), Danva (moderately resistant) and Oleva (susceptible). These three cultivars showed neither competitive interactions when mixed nor significant reductions of potato late blight in **susceptible** varieties grown in alternate rows with varieties possessing either a partial (non-race specific) or race specific resistance in 2001 and 2002. However, in 2001, late blight infection was significantly delayed in the **resistant** variety Kuras (with high race-specific

resistance) when alternated with the susceptible variety Oleva. The delay, of approximately one week occurred after the adjacent rows of Oleva were defoliated by leaf blight.

In Kuras, disease started three to five weeks later and it was delayed by another week in alternating rows and yields were increased. This could have been due to the competitive advantage of the resistant variety following death of the foliage of the susceptible variety and/or changes in microclimate that benefited the resistant variety. Such an effect was absent in 2002 however.

In the experiments in France, populations of the pathogen differed according to host genotypes. Isolates virulent to Naturella (possessing the R2 resistance gene) were recovered almost exclusively from this cultivar, and proved weakly aggressive on Bintje (susceptible) or Désirée (partially resistant). Isolates collected on Bintje were most aggressive on both Bintje and Désirée. There was no clear indication of a strong correlation between pathogenicity characteristics and AFLP fingerprints. These patterns were similar in collections made over the two successive years. It was concluded that alternating rows of susceptible and resistant potato cultivars have a limited potential to control late blight in environments highly conducive to the disease, but may help manage the evolution of pathogen populations for virulence and aggressiveness.

Variety mixtures:

Experiments were made in the United Kingdom (UK) and Germany (D) between 2001 and 2003 to investigate growing mixtures of varieties of potatoes (with varieties mixed within the row, rather than different varieties being grown in alternate rows as described in Section 1 of this Chapter) as a diversification strategy for the management of late blight. In the UK, there were 4 experiments testing effects of variety mixtures and pure stands on blight development and severity. Five varieties with differing resistances were grown in all possible mixtures and in two differently sized plots. Susceptible or moderately resistant varieties suitable for commercial cropping were infected to similar extents in mixtures and pure stands. In two of three years, infection in variety Sante (moderately susceptible variety) was slightly less when mixed with Cara (moderately resistant), but Cara was unaffected. Mixing a late blight immune/near immune partner (Tominia) with either Cara or Sante reduced disease in these latter two varieties. However, it seemed that mixtures of varieties did not suppress the disease to an extent which exceeded that provided by the average of the resistance of individual components/varieties within the mixture. Larger plots were more infected than small plots in terms of AUDPC, but the disease progressed more rapidly in small plots. Planting density had no effect on rate or extent of infection.

Variety Cara consistently yielded better in mixtures with different varieties than expected, demonstrating positive combining ability: Appell performed worse than expected, showing negative combining ability. The combining ability of other varieties depended on their companion varieties. Overall, mixtures improved yields by about 5% compared with the yields of individual components (indicating better resource use), but whilst certain varieties showed an improvement, other varieties' yields were depressed. The advantages shown by Cara improved as the number of varieties in the mixture increased whereas Sante yielded progressively less (other varieties showed no response) and for Cara the relative benefits of mixtures over varieties was affected by plant density. It is possible that plant-pathogen interactions in some varieties in some conditions may further improve the performance of variety mixtures above that provided by favourable plant-plant interactions.

Different varieties were used in Germany (D) in 2002 and 2003 – Agria and Simone (white skinned) and Laura and Rosella (red skinned). They were grown in pure stands and four mixtures of a red and white skinned variety either following grass/clover or wheat to study effects of nutrient availability on late blight and plant-plant interactions. Yields were about 18.5t/ha in 2002 and 37.8t/ha in 2003 and in both years, tuber bulking was slower and yields less after wheat, for both pure stands and mixtures, although not significantly so. Blight was severe in 2002 but not in 2003 because of very dry conditions but severity and rate of infection was less (but not significantly) following wheat than grass/clover. Effects of mixtures on disease were small compared with pure stands. The Laura-Simone mixture was most effective (these two varieties represented the upper and lower ends of the susceptibility spectrum), especially under low disease pressure after wheat than grass clover in both years. Whilst mixing varieties did not affect overall yield, the least competitive variety in the mixture produced fewer oversized and more undersized tubers than in pure stand. Competitive interactions between varieties were similar in both years and were unaffected by nutrient availability.

Strip- or Intercropping:
The effects of strip or inter- cropping of potatoes with either cereals or grass-clover on late blight severity and yield of two potato varieties were studied from 2000 to 2002 in large-scale field experiments in Germany. In addition, effects of the orientation of the plots to prevailing winds and effects of plot size were included. Plots of different size (3 x 10 m in 2000 and 6 x 18 and 6 x 36 m in 2001 and 2002) were arranged either parallel with, or perpendicular to the main wind direction.
There were no effects of inter cropping on late blight disease in the potatoes in 2000. However, there were strong spatial patterns within and between plots indicating interference between plots. When plot size and distance were increased, area under the disease progress curve (AUDPC) was significantly reduced by 9-20 % in 2001 and 4-12 % in 2002. Reductions were highest in plots planted perpendicular to the wind grown next to grass-clover. The most important mechanism leading to disease reduction is loss of inoculum outside of the plots due to the distances between strips and the barrier function of neighbouring non-potato hosts. The effects of the cropping strategy did not interact with variety. However, yields in the (edge) potato rows directly neighboured by cereals were significantly reduced in all three years.A maximum of 20 % of the variation in yield could be explained by differences in AUDPC depending on variety and year but there were no effects of competition with cereals on the yield loss relationship. In 2000, there was a three-fold effect on the yield of the more resistant but late bulking variety compared with the yield of the susceptible variety indicating that early bulking might be more important than resistance. The lack of correlation between AUDPC and yield in 2002 can be explained by the very dry spring which impeded the mineralization of organic matter in the soil resulting in much reduced nutrient supply to the crop. Thus, nutrient limitation rather than disease were responsible for the reduced yields.

Conclusion: The epidemiological effects of diversification strategies are extremely dependent on the variety (level and type of resistance) used and the spatial variation in disease pressure. Inter cropping experiments indicated that potatoes are highly susceptible to competition from different varieties as well as from different species. In order to make use of diversification strategies the reactions of different varieties to competition from other varieties and species have to be determined to identify varieties suitable for this approach. It could become an aim of breeding for organic farming to improve the competitive ability of varieties. This might also be of interest with respect to weed suppression and making the best use of limited resources for growth. Diversification strategies may contribute to a reduction in disease pressure but the extent to which this is achieved in a potato cropping system may be dependent upon the size of the plots or beds of the different varieties and species. The most effective sizes may be much larger than experimental plot sizes.