

## Heidi Bouws-Beuermann (Autor) Effects of strip intercropping on late blight severity, yields of potatoes (Solanum tuberosum Lindl.) and on population structure of P. infestans



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## **Chapter 1 – General introduction**

#### Potato late blight and its causal agent Phytophthora infestans

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary is considered to be the most devastating pathogen of both, conventionally and organically grown potatoes (*Solanum tuberosum* Lindl.) worldwide. When it first appeared in the Netherlands in the summer of 1845, within a few months potatoes as far away as Ireland, Poland, Switzerland and France were severely infested (Bourke, 1964; Fry et al., 1993), resulting in wide spread starvation especially the so-called Irish potato famine (Woodham-Smith, 1962).

The fungus not only attacks potatoes but also several other solanaceous species such as tomatoes (*Lycopersicon spec.*) eggplants (*Solanum melongena*) (Adler et al., 2002) and weeds, e.g. hairy nightshade or bittersweet *S. nigrum* and *S. dulcamara*, respectively (Dorrance et al., 1999; Goodwin et al., 1998; Peters et al., 1999). On potatoes and tomatoes all plant parts except the roots are affected.

*P. infestans* can reproduce asexually and sexually. During the **asexual cycle**, numerous zoosporangia are produced within lesions on leaves and stems and in storage on tubers (Agrios, 1997). Those sporangia are dispersed by **wind and rainsplash** (Harrison and Lowe, 1989; Johannes, 1953) and zoospores released from sporangia germinate on new host tissue (Schöber and Ullrich, 1985). At around 15 °C and a relative humidity >90 % (Croxall and Smith, 1976; Harrison, 1992; Harrison et al., 1994; Minogue and Fry, 1981; Mizubuti and Fry, 1998) *P. infestans* can produce up to two generations within one week (Schöber, 1981). Forecasting systems as e.g. the negative prognose of Ullrich and Schrödter (1966) and SIMPHYT among several newly developed modelling systems (Kluge and Gutsche, 1990) make use of the fungus' climatic preferences for the prediction of the optimal start of fungicide application. When zoospores are washed into the soil they may also infect the tubers and usually the fungus survives in **latent infected tubers** (as hibernating mycelium) in storage or in the field until the next growing season.

As *P. infestans* is heterothallic, **sexual reproduction** can only take place if the two opposite mating types A1 and A2 occur together within host tissue (Gallegly and Galindo, 1958). The resulting oospores are thick-walled, persistent propagules which can survive in

the ground for more than three years (Drenth et al., 1995) and thus present an often unpredictable inoculum source.

#### The "new" P. infestans populations

Prior to the 1980s, only the A1 mating type occurred throughout the world, whereas both mating types co-existed in central Mexico, the centre of origin of the fungus (Niederhauser, 1956). In the early 1980s, however, a **world-wide migration** of *P. infestans* populations consisting of both mating types occurred replacing the old relatively homogeneous population (Fry and Goodwin, 1997). Sexual reproduction in the new *P. infestans* populations is considered the main reason for high phenotypic and genotypic diversity of the pathogen in the USA and Canada (Chycoski and Punja, 1996; Dorrance et al., 1999; Fraser et al., 1999; Gavino et al., 2000; Goodwin et al., 1998) and several European countries.

Depending on the region **in Europe**, sexual reproduction may or may not play a role. Ooospores have been found in Scandinavia (Andersson et al., 1998; Brurberg et al., 1999), the Netherlands (Drenth et al., 1993; Fry et al, 1991; Turkensteen et al., 2002; Zwankhuizen et al., 1998), former East Germany (Daggett et al., 1993), Poland (Therrien et al., 1993; Sujkowski et al., 1994) and Russia (Elansky et al., 2001). In contrast, asexual reproduction appears to predominate in France (Andrivon et al., 1993; Lebreton and Andrivon, 1998), Switzerland (Knapova and Gisi, 2002), the United Kingdom (Carlisle et al., 2001; Day and Shattock, 1997; Day et al., 2004) and likely also in western Germany, (Rullich and Schöber-Butin, 2000; Rullich et al., 2002; Schöber, 1983).

In contrast to the old A1 populations, the new populations consist of more and complexer pathotypes (Drenth et al., 1994; Elansky et al, 2001; Goodwin et al., 1995; Peters et al., 1999; Rullich et al., 2002) and the overall resistance level against the systemic fungicide Metalaxyl has increased (Cooke et al., 2003; Daayf et al., 2000; Davidse et al., 1989; Fry et al., 1991; Holmes and Shannon, 1984; Jaime-Garcia et al., 2000; Marshall-Farrar et al., 1998).

The newly introduced *P. infestans* populations have also been reported to be **fitter** than the old pre-1980 clonal lineage (Tooley et al., 1986) with higher growth rates and lesion expansion, and shorter latent periods compared to isolates belonging to the old US-1 lineage (Medina et al., 1999; Miller et al., 1998; Peters et al., 1999). As a result, late blight epidemics caused by the new populations are increasingly difficult to control (Goodwin et al., 1995; Turkensteen et al, 2002; Sujkowski et al., 1996).

Economic efficiency of conventional potato production is decisively affected by specific on-time applications of fungicides (Bradshaw and Vaughas, 1996; Kellermann, 1998; Maykuss et al., 1998) with yield losses of 30-50 % if left unsprayed and tuber blight in storage (Fry, 1978; Guenther et al., 2001; Johnson et al., 2000; Marshall-Farrar et al., 1998).

For the comparison of P. infestans populations of different origin several molecular methods have been developed such as isoenzymes (Tooley et al., 1985), restriction fragment length polymorphsims (RFLPs) (Forbes et al., 1998; Goodwin et al., 1992), amplified fragment length polymorphisms (AFLPs) (Vos et al., 1995), randomly amplified polymorphic DNA (RAPD) (Carlisle et al., 2001; Mahuku et al., 2000; Maufrand et al., 1995; Peters et al., 1999) or microsatellites (Knapova and Gisi, 2002). The allozymes Gpi and Pep are used to define and compare different clonal (US)-lineages (Forbes et al., 1998; Goodwin et al., 1995). In addition, RFLP fingerprinting with probe RG-57 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 and the determination of migrations among populations (Drenth et al., 1993; Goodwin et al., 1992; Sujkowski et al., 1994). While RAPD-PCR (polymerase chain reaction) technology resulted in 10 to 30 polymorphic DNA loci (Carlisle et al., 2001; Ghimire et al., 2002; Mahuku et al., 2000; Punja et al., 1998), with AFLPs a higher number of identifiable polymorphisms, i.e. 50 to more than 100 loci were detected (Abu-El Samen et al., 2003; van der Lee, 1997; Zwankhuizen et al., 1998) leading to even more detailed differentiation among isolates. However, depending on the markers used, isolates of e.g. different clonal lineages may group together in different ways (Forbes et al., 1998; Ghimire et al., 2002; Knapova and Gisi, 2002).

**Migrations** of *P. infestans* populations within continents or transcontinentally have most likely taken place **via infected seed potatoes** (Fry et al., 1993; Goodwin, 1997; Hermansen et al., 2000; Jaime-Garcia et al., 2000; Niederhauser, 1991; Punja et al., 1998; Wangsomboondee et al., 2002). This is underlined by the fact that even at present, regions with predominantly own seed production appear to be dominated by more homogenous pathogen populations as was shown for Ecuador (Forbes et al., 1997), China, the Philippines

and Taiwan (Koh et al., 1994), Siberia and the Far East (Elansky et al., 2001), Brazil (Reis et al., 2003), South Africa (Mc Leod et al., 2001; Ochwo, et al., 2002), and also in European countries e.g. France (Andrivon et al., 1993) or the United Kingdom (Carlisle et al., 2001).

In contrast, phenotypic and genetic diversity is high in the Netherlands (Drenth et al., 1993; Fry et al., 1991; Zwankhuizen et al., 2000) and Poland (Sujkowski et al., 1996), i.e. countries that frequently import seed potatoes from Mexico. However, similarly high genetic diversities were found among Scandinavian *P. infestans* populations (Andersson et al., 1998; Brurberg et al., 1999; Hermansen et al., 2000) and in the former German Democratic republic (Daggett et al., 1993). These countries import mainly seed potatoes from the Netherlands and Poland, respectively, suggesting an influence of seed transfer on migrations of (sexual) *P. infestans* populations.

Often, it appears that visibly infected tubers die in storage and do not cause disease in the field (Boyd, 1980; Doster et al., 1989; Hirst and Stedman, 1960b; Jenkins and Jones, 2003). On the other hand, it has been observed that apparently healthy tubers will produce infected shoots and thus provide the initial inoculum within fields (Adler, 1999; Appel et al., 2001; Zwankhuizen et al., 1998). Examination of seed lots with PCR has revealed that the **frequency of latent infections** is much higher than previously thought (Adler, 2001). Humid climatic conditions during the growing season and harvesting (Andersson et al., 1998; Croxall and Smith, 1976) the disease level in the previous year (Zwankhuizen and Zadoks, 2002) and varietal resistances to tuber blight (Berggren et al., 1988; Doster et al., 1989; Platt and Tai, 1998; Stewart et al., 1983) influence tuber infection rates. The transmission rate of the pathogen from infected seed tubers into potato sprouts apparently depends on the field conditions and growing practices e.g. texture and humidity of the soil (Andrivon, 1994; Andrivon, 1995; Bäßler et al., 2004).

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#### Late blight management in organic farming

The only effective sprays against late blight in organic potato cropping are copper based with a maximum dosage of 3 kg pure copper per ha and season allowed in Germany (Anonymous, 2005). As a contact fungicide copper would need to be applied preventively for best results. However, in order to reduce inputs in organic farming application of **copper** is only allowed once the occurrence of late blight has been confirmed in the field 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; Schüler et al., 1999). Also, copper is a heavy metal especially toxic to aquatic organisms and it remains in the environment (Brümmer et al., 1986; Filius, 1993). Only 200-300 g\*ha\*year-1 are taken up by plants as a micronutrient (Filipinsky, 1989) Therefore, it is generally agreed that there is a need for alternative strategies for the management of late blight.

Plant extracts and compost extracts have until now failed to reliably reduce the disease under conducive natural field conditions (Hein, 1998; Schmitt, 1997). However, when disease pressure was lower, these **alternatives** have been reported to stabilize potato tuber yield (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.

The most effective strategy for late blight reduction in organic farming is **resistance** resulting in later and less severe epidemics (Beuermann et al., 2000; Bouws-Beuermann et al., 2002; Meinck and Kolbe, 1999; Möller et al., 1999; Phillips, 2004; Stöppler et al., 1990). Unfortunately, resistant varieties often are late maturing (Colon et al., 1995; Thurston, 1971; Umaerus et al., 1983; Umaerus and Umaerus, 1984). In addition, the market may demand other varieties thus limiting the varietal choice (Dierauer and Speiser, 1998; Möller et al., 1996; Phillips et al., 2002; Tamm et al., 2004).

To reduce epidemic pressure in general, of course field hygiene and the removal of infected seed tubers before planting are of prime importance. Also, fields located in well aerated areas are generally less affected than fields surrounded by trees or bushes or in depressions (Hirst and Stedman, 1960a). For the same reason, it is generally recommended

to plant the crop in the main wind direction (Stevenson, 1993) to allow for better drying of plants.

Diversity of crops and varieties is known to reduce diseases and pests (Finckh and Wolfe, 1998), and cultivar mixtures are highly effective in cereals (Finckh et al., 2000) However, results with respect to late blight in potatoes are not straight forward. When growing differentially resistant potato varieties either as mixtures or in alternating rows higher disease severity on the resistant component and lower severity on the susceptible one is commonly reported (Beuermann et al., 2000; Finckh, et al., 2003; Garrett and Mundt, 2000; 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 (Waggoner, 1962). 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.

#### Possible effects of late blight management strategies on P. infestans

Any management strategy for a disease has to be concerned with the **long-term effects** of the strategy on the host-pathogen interactions. The ability of *P. infestans* to adapt to major resistance genes has been known for many years and race-specific resistance genes (vertical resistance) are usually overcome only a few years after their introduction (Malcolmson and Black, 1966; Pilet, 2003; Turkensteen, 1993; van der Plank, 1971). In contrast, there are reports of potato varieties with non-race-specific horizontal or field resistance against late blight that have maintained considerable resistance for several decades of extensive use (Colon et al., 1995; Turkensteen, 1993; van der Plank, 1971; Zwankhuizen et al., 2002).

Apart of **race-specific resistance**, many more factors such as the frequency of sex, environmental conditions, the fungicide applications, the production intensity and the cropping frequency of potato (varieties) may influence adaptation processes of the pathogen to its host. For example, resistance against the systemic fungicide Metalaxyl increased especially in the US due to regular applications (Davidse et al., 1989; Goodwin et al., 1992; Marshall-Farrar et al., 1998; Wangsomboondee et al., 2002), however, resistant strains have been reported even under unsprayed conditions (Fraser et al., 1999; Goodwin et al., 1995; 1998; Chycoski and Punja, 1996; Holmes and Shannon, 1984).

In typical conventional potato growing areas such as e.g. several US regions or European countries as e.g. France, the Netherlands and Belgium, only a few popular (often susceptible) potato varieties are cultivated (Inglis et al., 1996; Erbe and Lüthke, 2004). These agronomic practices may enable epidemic spread of *P. infestans* among susceptible potato hosts within a region.

Generally, adaptation processes of *P. infestans* populations and distributional patterns of clonal lineages are host-related. Among P. infestans genotypes (or clonal lineages), generally isolates prefer either tomatoes or potatoes (Fraser et al., 1999; Hammi et al., 2002; Knapova and Gisi, 2002; Lebreton and Andrivon, 1998; Reis et al., 2003; Suassuna et al, 2004; Wangsomboondee et al., 2002). Recent laboratory studies indicate that cultivar-specific adaptation towards potatoes may also happen (Berggren et al., 1988; Bjor and Mulelid, 1991; Caten, 1974; Carlisle et al, 2002; Day and Shattock, 1997; Jeffrey et al., 1962; Pilet, 2003) and that this might possibly be associated with the new populations. Comparison of A1 isolates belonging to the sexual (Mexico) or asexual (Great Britain) population suggested that the "new populations" were fitter than the old clonal lineage (Tooley et al., 1986). Fitness tests of Day and Shattock (1997) revealed A1 isolates to be more aggressive on susceptible Maris Piper, while A2 isolates appeared better adapted towards moderately resistant Cara. Similarly, the majority of A2 isolates originated from moderately to highly resistant potato varieties in Canada (Chycoski and Punja, 1996) and in the Republic of Ireland (O'Sullivan et al., 1995). These findings may indicate cultivar-specific adaptation of P. infestans towards potato host under natural field conditions.

#### **Objectives and thesis outline**

The main objectives of the research described in this thesis were **i**) to quantify the effects of strip intercropping on late blight epidemics and tuber yields in organic farming and **ii**) to determine the possible effects of this management strategy on adaptation processes within and among populations of *P. infestans*. In 1999, the University of Kassel rented the 300 ha farm "Domäne Frankenhausen" which had been until then under conventional production. The contiguous area of the farm with relatively large homogeneous fields allowed the arrangement of plots far enough apart to minimise interplot interference. As no potatoes had been grown for over 30 years at the farm, this offered the unique opportunity to study the establishment and evolution of *P. infestans* populations from the start as influenced by the cropping patterns and origin of seed potatoes.

In Chapter 2, field experiments on strip intercropping with different potato varieties, planting pattern, plot size and neighbour cultures that were conducted from 2000 to 2002 are described. The effects on late blight and tuber yield are analysed with special emphasis on yield loss relationships under organic conditions and competition due to the neighbouring crops. Chapter 3 presents the characterisation study of a total of 639 P. infestans isolates originating from experimental plots of 90 to 648  $m^2$  size, potato production fields (>2ha) located at the experimental farm or "old fields" in the surrounding area, that had a potato cropping history. Isolates were analysed for their mating type, pathotype and molecular fingerprints using rep-PCR (repetitive extragenic palindromic). The phenotypic diversity of P. infestans populations is discussed with respect to cropping history, origin of seed potatoes and varietal resistance of potato fields from which the isolates originated. Possible adaptation processes of the pathogen towards potato varieties of different levels of resistance were investigated in Chapter 4. A total of 90 P. infestans isolates of either A1 or A2 mating type originating from two different potato varieties (suceptible and moderately resistant) were tested for their aggressivity on these two varieties. The infection efficiency, area under lesion expansion curve, maximum growth rate and sporulation capacity of isolates were analysed. The process of cultivar-specific adaptation of P. infestans isolates and the implications for resistance gene deployment with special respect to diversification strategies are discussed.

In the final discussion (Chapter 5) the results of Chapter 2 to 4 are summarised and discussed.

# Chapter 2 – Effects of strip intercropping on late blight severity and potato tuber yields in field experiments, 2000-2002

### Abstract

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  $3 \times 10$  m in 2000 and  $6 \times 18$  and  $6 \times 36$  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.