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# INFLUENCE OF FARMING SYSTEM, SPECIFIC CULTIVATION METHODS AND SITE PARAMETERS ON POTATO QUALITY

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## Table of contents

# Zusammenfassung

Qualitätsmängel gehören zu den wichtigsten Ursachen für eine ungenügende Rentabilität im schweizerischen Kartoffelbau. Seit einigen Jahren wird die Kartoffelqualität von Seiten des Handels und der verarbeitenden Industrie zunehmend kritisiert. Heute werden über 90% der Kartoffeln gewaschen verkauft und in den Supermärkten gewinnen Selbstbedienungsangebote an Bedeutung. Als Folge davon werden auch kleine äussere Qualitätsmängel von den Konsumenten als störend empfunden. Die Qualitätskontrolle im Handel erfolgt hauptsächlich an sortierten aber ungewaschenen Kartoffelproben. Von Drahtwürmern (*Agriotes ssp.*), drycore (*R. solani*) oder Schnecken (*Deroceras reticulatum*, *Arion ssp.*) verursachte Qualitätsmängel werden nicht klar unterschieden und unter dem allgemeinen Begriff „Aeussere Mängel“ zusammengefasst. Wegen fehlender Information zur Anbautechnik und zu den Standortfaktoren der Kartoffelfelder sind keine Rückschlüsse über die Ursachen der auftretenden Mängel möglich. Das Ziel dieser Dissertation war die Erforschung der relativen Bedeutung der verschiedenen Qualitätsmängel sowie deren Beeinflussung durch das Anbausystem (Biolandbau, Integrierte Produktion, Konventioneller Anbau), die Anbautechnik und die Standortfaktoren. In der vorliegenden Arbeit werden Resultate zu den äusseren Qualitätsmängeln verursacht durch Drahtwürmer, Schnecken, drycore und Rhizoctonia-Pocken präsentiert.

Im ersten Teil (2000) der Arbeit wurde auf fünf kommerziellen Kartoffelfeldern von 0.8 ha Grösse die räumliche Verteilung verschiedener Qualitätsmängel und deren Beziehung zueinander untersucht. Alle Knollen von 400 Pflanzen pro Feld wurden für die Qualitätsbeurteilung geerntet und die Koordinaten der Pflanzen wurden festgehalten. Die Daten wurden auch zur Bestimmung eines Stichprobenplans genutzt, der sich für eine grosse Anzahl Kartoffelfelder eignet und zuverlässige Qualitätsbestimmungen (% befallene Knollen) mit einer Präzision von +/- 2%-4% befallener Knollen ermöglicht.

In einem zweiten Teil wurde die Qualität von 278 Kartoffelfeldern aus verschiedenen Anbausystemen (Bio 58, Integriert 69 and Konventionell 151) aus den wichtigsten Kartoffelanbaugebieten der Schweiz beurteilt und alle relevanten Daten zur Anbautechnik und zum Standort erhoben. Unmittelbar vor der Ernte wurden auf jedem Feld nach einem genau definierten Stichprobeplan 810 Knollen von 270 Pflanzen geerntet. Die Qualitätsbeurteilung erfolgte nach einem speziell erarbeiteten Evaluationssystem welches sowohl wissenschaftliche Kriterien als auch die Anforderungen des Handels berücksichtigte.

Im dritten Teil wurde die Anastomosegruppe von 60 Isolaten des Pilzes *Rhizoctonia solani* bestimmt, welche von Sklerotien und drycore auf Knollen aus den verschiedenen Anbausystemen gewonnen wurden. Die Analyse erfolgte mittels Pektin-Zymogram, AG 3 spezifischen Primern und der Hyphenfusion mit Tester-Isolaten. Die Bestimmung der genetischen Variabilität innerhalb der Isolate von *R. solani* erfolgte durch die Sequenzierung der ITS-Region der rDNA und mit Hilfe der ERIC und REP-PCR-Methode.

Im letzten Teil der Arbeit (2005 und 2006) wurden randomisierte Feldversuche und Topfversuche unter kontrollierten Bedingungen in der Klimakammer durchgeführt, um die Hypothese zu prüfen, dass Verletzungen an den Knollen durch Drahtwürmer oder andere Mittel das Eindringen von *R. solani* in die Knollen erleichtern und damit die Bildung von drycore-Symptomen fördern. Diese Hypothese basierte auf den Resultaten der ersten drei Teile der Arbeit. Die in den Versuchen gewonnenen Isolate von *Rhizoctonia solani* wurden mittels ISSR-PCR analysiert.

Die räumliche Verteilung der untersuchten Qualitätsmängel im Feld war meist nicht-binomial. Folglich war die Variabilität der Qualitätsmängel zwischen Knollen von verschiedenen Pflanzen oft höher als zwischen Knollen der gleichen Pflanze. Als Konsequenz wurden die Vertrauensintervalle für die geschätzten Mittel von befallenen Knollen reduziert, wenn eine definierte Anzahl Knollen pro Feld von einer zunehmenden Anzahl Pflanzen geerntet wurde. Basierend auf diesen Ergebnissen wurde für eine zuverlässige Qualitätsbestimmung im „on-farm“-Programm in Teil 2 eine optimale Stichprobe von 810 Knollen pro Feld (drei zufällig gezogene Knollen von 270 Pflanzen) bestimmt, mit welcher der Prozentsatz befallener Knollen mit einer Genauigkeit von +/- 2-4% geschätzt werden kann.

Schäden durch Drahtwürmer, Schnecken und drycore waren in allen Anbausystemen für bedeutende ökonomische Verluste verantwortlich. Als Folge erreichten 36% (Bio), 13% (Integriert) respektive 12% (Konventionell) der Kartoffelfelder den geforderten Qualitätsstandard nicht ohne Aussortieren von beschädigten Knollen (Toleranzgrenze = 18% beschädigte Knollen, bei Qualitätsbeurteilung an gewaschenen Knollen). Im biologischen Anbausystem lag der Anteil beschädigter Knollen für alle drei Qualitätsmängel am höchsten. Die Bedeutung von Schneckenschäden war höher als erwartet. Schneckenschäden allein führten zu Preisreduktionen bei 14% der untersuchten Felder. Schneckenschäden wurden vermutlich in Qualitätsbestimmungen des Handels oft mit Drahtwurmschäden verwechselt. Ein bedeutender Einfluss der Witterungsbedingungen wurde beobachtet. Im sehr heissen und trockenen Jahr 2003 (75% des Mittels der langjährigen Niederschlagsmenge) traten in allen Anbausystemen weniger Schneckenschäden auf. Im nassen Jahr 2001 (140% des Mittels der langjährigen Niederschlagsmenge) wurden die Schneckenschäden signifikant durch die Fruchtfolge beeinflusst. Die Schneckenschäden wurden mit zunehmender Bodenbedeckung (Kleegras, Gründüngung) und einem erhöhten Anteil an günstigen Kulturen für Schnecken (z.B. Gemüse, Raps, Sonnenblumen) in den drei Jahren vor Kartoffeln verstärkt. Bemerkenswert sind die deutlich erhöhten Schneckenschäden auf Feldern mit regelmässigem Gemüseanbau (z.B. Spinat, Salat, Karotten). Bedeutende Schneckenschäden wurden auf allen Bodentypen unabhängig vom Ton- und Humusgehalt beobachtet, auch auf Böden mit weniger als 15% Ton.

Die Tatsache, dass Drahtwurmschäden allein bei 12% aller Felder zu Preisreduktionen führten unterstreicht die Bedeutung dieses Schädlings. Im biologischen Anbausystem war der Anteil Felder mit bedeutenden Schäden höher als in den anderen Anbausystemen, aber nur 2001 und nur im Vergleich zum integrierten Anbausystem war der Unterschied signifikant ($p=0.05$). 2001 waren die Drahtwurmschäden in allen Anbausystemen leicht höher als in den Jahren 2002 und 2003. Mit zunehmender Anzahl Jahre Anbaupause nach Kleegras und mit abnehmendem Anteil Kleegras in der Fruchtfolge wurde das Risiko für Drahtwurmschäden deutlich verringert. Während bei circa 50% der Kartoffelfelder im ersten Jahr nach Kleegras deutliche Drahtwurmschäden auftraten, lag dieser Anteil signifikant tiefer, wenn die Kartoffeln erst im vierten oder fünften Jahr nach Kleegras angebaut wurden (weniger als 10% der Felder mit deutlichen Schäden).

Der Befall durch Rhizoctonia-Pocken war im biologischen Anbausystem am höchsten, aber der Unterschied zu den anderen Anbausystemen war nicht signifikant. Bei 50% der biologischen Felder lag der Anteil befallener Knollen über 20%, im Vergleich zu 29% bei integrierten und konventionellen Feldern ohne chemische Pflanzgutbehandlung und 11% mit chemischer Pflanzgutbehandlung. Die Varianzanalyse mit den Daten der integrierten und konventionellen Felder ergab einen signifikanten Einfluss der Pflanzgutqualität (% befallene Knollen) und des Erntejahres sowie einen signifikanten Einfluss einer chemischen Pflanzgutbehandlung bei einem höheren Pflanzgutbefall (mehr als 20% der Knollen mit Befall). Nicht signifikant war der Einfluss der Sorte, des Anbausystems, des Misteinsatzes und der Vorkultur.

Drycore war der wichtigste äussere Mangel im biologischen Anbausystem und trat in allen drei Jahren signifikant häufiger und stärker auf als in den übrigen Anbausystemen. Auf 29% der biologischen Felder führte drycore allein zu Preisreduktionen, im Vergleich zu 3% in integrierten und konventionellen Feldern. Der drycore-Befall wurde signifikant beeinflusst durch Drahtwurmschäden, den Pflanzgutbefall mit Rhizoctonia-Pocken und Kleegras als

Vorkultur. Im Gegensatz zum Schneckenbefall wurde das Auftreten von drycore durch die Jahreswitterung nicht beeinflusst und lag in allen drei Jahren auf dem gleich hohen Niveau. Starke drycore-Schäden wurden sowohl auf leichten als auch schweren Böden gefunden, unabhängig vom pH, Ton-, und Humusgehalt. In allen drei Anbausystemen wurde nie bedeutender drycore-Befall beobachtet, wenn nur ein geringer Drahtwurmbefall auftrat und Pflanzgut mit geringem Pockenbefall gepflanzt wurde. Zudem trat drycore auch bei sehr starkem Befall der Ernteknollen mit Rhizoctonia-Pocken nur sehr selten auf, wenn die Knollen keine Drahtwurmschäden aufwiesen. Diese Resultate wurden durch die Berechnung des relativen Befallsrisikos basierend auf den Daten von 32'000 kontrollierten Knollen von 40 Feldern mit einem erhöhten Drycorebefall bestätigt. Das Befallsrisiko für drycore war signifikant erhöht, wenn Rhizoctonia-Pocken oder Drahtwurmschäden auf der gleichen Knolle auftraten. Im Gegensatz dazu erhöhte sich das Risiko für drycore nicht, wenn Schneckenschäden auf der gleichen Knolle auftraten.

AG 3 ist die häufigste Anastomosegruppe von *R. solani* im schweizerischen Kartoffelbau unabhängig vom Anbausystem. Neunundfünfzig von 60 Isolaten von Rhizoctonia-Pocken und drycore gehörten zur AG 3 und nur eine einzige zur AG 5. Die Untersuchungen mit ERIC und REP-PCR zeigten einen hohen Grad an Heterogenität innerhalb der AG 3 Isolate mit einer genetischen Ähnlichkeit zwischen 20% und 87%. Keine höhere genetische Übereinstimmung konnte bei den Isolaten aus der gleichen Region oder der gleichen Kartoffelsorte beobachtet werden. Die genetischen Unterschiede zwischen den Isolaten des biologischen Anbausystems waren tendenziell geringer als zu den Isolaten der anderen Anbausysteme.

Die Resultate der Feldversuche und Topfversuche unter kontrollierten Bedingungen mit Isolaten der AG 3 bestätigen die Hypothese aus dem „On-farm“-Forschungsprogramm auf 278 Kartoffelfeldern. Drycore Symptome auf den Ernteknollen wurden nur in den Verfahren mit *R. solani* beobachtet und in den meisten Fällen nur, wenn die Knollen entweder natürlich durch Drahtwürmer oder künstlich mit Nadeln verletzt waren. Unverletzte Knollen wiesen nur sehr selten drycore auf, auch wenn die Knollen von Rhizoctonia-Pocken befallen waren. In den Verfahren ohne Infektion mit *R. solani* wurden nie Pocken oder drycore gefunden. Alle Isolate von *R. solani* von Pocken und drycore in den Feld- und Topfexperimenten gehörten zur AG 3. Zudem wiesen alle in den Topfexperimenten gewonnenen Isolate einen identischen ISSR-PCR Fingerprint auf wie das Isolat, welches für die Inokulation der sterilisierten Erde verwendet wurde. Damit wurde bewiesen, dass drycore durch *R. solani* AG 3 verursacht wird und dass natürliche (Drahtwürmer) oder künstliche (Nadeln) Verletzungen der Knollen die Bildung von drycore fördern. Demnach kann das im „On-farm“-Programm beobachtete, deutlich stärkere Auftreten von drycore im biologischen Anbausystem durch den häufigen Anbau von Kartoffeln nach mehrjährigem Kleegras erklärt werden, da dadurch das Risiko für Drahtwurmschäden deutlich erhöht wird. Weitere fördernde Faktoren sind der höhere Anteil von Pflanzgutposten, welche die Bekämpfungsschwelle für Rhizoctonia-Pocken überschreiten und das Fehlen einer wirksamen Pflanzgutbehandlung im biologischen Anbausystem.

Die Fruchtfolge hat einen grossen Einfluss auf das Auftreten von Qualitätsmängeln verursacht von drycore, Schnecken und Drahtwürmern. Zur Verbesserung der Kartoffelqualität müssen die Fruchtfolgen optimiert werden. Dies gilt im speziellen für das biologische Anbausystem, wo Kleegras als Vorkultur zu Kartoffeln als wichtige Stickstoffquelle betrachtet wird. Die Zunahme von Schneckenschäden in Fruchtfolgen mit erhöhter Bodenbedeckung im Winterhalbjahr (Kleegras, Zwischenkulturen) zeigt, dass Zielkonflikte zwischen den Qualitätsanforderungen des Handels und den ökologischen Zielen einer umweltschonenden Produktion bestehen. Forschung ist nötig, um Pflanzen mit einer geringen Akzeptanz durch Schnecken zu finden, die sich als Zwischenfutter oder Gründüngung eignen. Der hohe Anteil an Pflanzgutposten, welche die Bekämpfungsschwelle für *Rhizoctonia solani* überschreiten und damit das Risiko für Pocken und drycore erhöhen, unterstreicht die Wichtigkeit einer Verbesserung der Pflanzgutqualität. Zielsetzungen für zukünftige Forschung könnte die Entwicklung von Methoden zur Produktion von befallsfreiem Pflanzgut und zur biologischen Bekämpfung von Drahtwürmern sein.

## Summary

Quality deficiencies are one of the main reasons for insufficient economy in Swiss potato production. Potato quality has been increasingly criticized by traders and the potato industry in recent years. Today over 90% of the sold potatoes are washed and self pick service increases in the supermarkets. As a result consumers notice even minor outer quality deficiencies. The quality assessment in the potato industry is mainly conducted with graded but unwashed samples. Quality deficiencies caused by wireworms (*Agriotes ssp.*), drycore (*R.. solani*) or slugs (*Deroceras reticulatum*, *Arion ssp.*) are not distinguished in detail but subsumed under the generic term "outer quality deficiencies". Because information on management (e.g. crop rotation) and site parameters (e.g. soil characteristics) are largly unknown a conclusive appraisal of the factors responsible for the quality deficiencies is not possible. The aim of this thesis was to investigate the relative importance of quality deficiencies and the influence of farming systems (organic, integrated, and conventional), cultivation methods and site parameters. Data is presented on quality deficiencies caused by wireworm (*Agriotes ssp.*), slugs (*Deroceras reticulatum, Arion ssp.*), drycore and black scurf (*Rhizoctonia solani).*

In the first part (2000) the spatial distribution of the different quality deficiencies and possible relations between them were studied on five commercial potato fields of 0.8 ha. All tubers of 400 plants per field were sampled for quality assessment and the coordinates of the plants were recorded. The resulting data was also used to determine the sampling plan for reliable quality assessments of potatoes with a precision of +/- 2% to 4% for the estimated mean of damaged tubers on a large number of potato fields.

In a second part potato quality of 278 potato fields of different farming systems (organic 58, integrated 69 and conventional 151) across the major potato growing regions of Switzerland was assessed and all relevant data concerning cultivation methods and site parameters were recorded. Immediately before harvest a sample consisting of 810 tubers from 270 plants was collected on each field according to a specified sampling plan. The quality assessment was done with a specifically designed evaluation system which contained both scientific criteria and requirements set by the trade.

In a third part the anastomosis groups of 60 isolates of *Rhizoctonia solani* obtained from black scurf and drycore on potatoes of different farming systems in Switzerland were analysed with Pectic zymograms, AG 3 specific primers and anastomosis typing. ITS sequencing, ERIC and

REP-PCR was used to determine the degree of genetic variability between Swiss isolates of *R. solani*.

In the last part randomized open-air experiments and pot experiments under controlled conditions in the climate chamber were conducted to check the hypothesis that injuries on the tubers caused by wireworms or other means facilitate the penetration of *Rhizoctonia solani* into the tuber and favour the formation of drycore. This hypothesis was based on the results of the first three parts of the thesis. The isolates obtained in these experiments were analysed with ISSR-PCR.

The spatial distribution of quality deficiencies in the field was mainly non-binomial and therefore the variability of quality deficiencies was often higher between tubers of different plants than between tubers of the same plant. As a consequence the confidence intervals for the estimated means of damaged tubers were reduced when a defined number of tubers per field were sampled from an increasing number of plants. In conclusion a sampling procedure of three tubers of 270 plants per fields (810 tubers) was determined for the on-farm program in the second part of this work to get reliable quality assessments with a precision of +/- 2% to 4% for the estimated mean of damaged tubers.

Wireworms, slugs and drycore were responsible for important economic losses in all farming systems. Due to these deficiencies 36% (organic), 13% (integrated) and 12% (conventional) of the fields did not meet the quality standard without sorting out damaged tubers (limit = 18% of damaged tubers for washed potatoes). In the organic farming system the quality damage was higher for all three deficiencies. The relative importance of slugs as a potato pest was higher than expected. Slug damage alone led to price reductions for 14% of the fields. Damage caused by slugs was probably often confused with wireworm damage in quality assessments of the traders. An important influence of the meteorological conditions was observed. In the very hot and dry year 2003 (75% of the precipitation of the long term annual mean) less slug damage occurred in all farming systems. In the wet year 2001 (140% of mean precipitation) slug damage was significantly influenced by the crop rotation. With increasing soil cover (grass clover ley, catch crops) and crops favourable for slugs (e.g. oilseed rape, sunflower, vegetables) in the three years preceding potatoes slug damage increased. The higher slug damage on fields with regular vegetable production (spinach, salad, carrots) was especially remarkable. Important slug damage was observed in all soil types independently from their content of clay and organic matter, even on sandy loams with less than 15% clay content.

The fact that wireworm damage alone led to price reductions for 12% of the fields underlines the importance of this pest. In the organic farming system the proportion of fields with important wireworm damage was higher than in the other farming systems, but only in 2001 the difference was significant between the integrated and organic fields. In 2001 wireworm damage was slightly higher in all three farming systems than in 2002 and 2003. The risk of wireworm damage was clearly decreased with increasing number of years between grass clover leys and potatoes and with decreasing proportion of grass clover leys in the crop rotation. About 50% of all fields in the first year after grass clover leys had important wireworm damage. Potatoes in the fourth and fifth year after grass clover leys showed significantly lower occurrence of wireworm damage than in the first year after grass clover leys (less than 10 % of all fields with important wireworme damage).

Black scurf attack was highest in the organic system, but the difference to the other farming systems was not significant. Fifty percent of the organic fields showed black scurf levels of harvested tubers of more than 20% infested tubers, compared to 29% of the conventional and integrated fields without seed treatment and 11% of the conventional and integrated fields with fungicide treatment. Analysis of variance for black scurf with the data of integrated and conventional fields showed a significant influence of seed quality (% seed tubers with black scurf) and the year as well as a significant influence of a fungicide seed treatment at higher seed infestation level (more than 20% tubers with black scurf). No significant influence was found for the factors variety, farming system, manure application and preceding crop.

Drycore was the most important outer quality deficiency in the organic farming system and the occurrence was significantly more frequent and higher on organic fields than in the other farming systems in all three years. On 29% of the organic fields drycore damage alone led to price reduction, compared to 3% in the integrated and conventional farming system. The infestation level of drycore was significantly influenced by wireworm damage, black scurf level on seed tubers and grass clover ley as preceding crop. In contrast to slug damage occurrence of drycore was not influenced by the meteorological conditions and was on a similar level in all three years. Heavy drycore damage was found on fields with light and heavy soils independently from pH and clay and organic matter content. In all three farming systems important drycore damage was never observed on fields with both a low occurrence of black scurf on the seed tubers and a low occurrence of wireworm damage. Even on fields with heavy black scurf infestation on progeny tubers, drycore occurred very rarely without injuries by wireworms. These results were confirmed by the relative risk for drycore calculated with the data of 32’000 assessed tubers from 40 fields with important drycore damage. Progeny tubers had a higher risk for drycore if black scurf or wireworm damage were on the same tuber. In contrast, no higher risk for drycore was observed on tubers with slug damage.

AG 3 is the most frequent anastomosis group of *R. solani* in Swiss potato production independently from the farming systems. Fifty-nine of sixty isolates of *Rhizoctonia solani* obtained form black scurf and drycore belonged to AG 3 and only one to AG 5. Analysis with ERIC and REP-PCR showed a high level of heterogeneity among the isolates of AG 3 with a genetic similarity ranging from 20% to 87%. No higher genetic similarity could be observed among isolates of the same region or the same variety, but genetic differences between isolates of the organic system tended to be fewer than between isolates of different farming systems.

The results of the additional open-air experiments and pot experiments under controlled conditions with isolates of AG 3 strongly support the hypothesis of the on-farm program. Drycore symptoms on harvested tubers were only observed in treatments where *R. solani* was present and potato tubers were injured either naturally by wireworm or artificially with needles. Non-wounded tubers very rarely had drycore symptoms even when tubers were infested with black scurf. In the treatments without *R. solani* infection black scurf or drycore was never observed. All isolates of *R. solani* obtained from black scurf or drycore symptoms belonged to AG 3. Furthermore, all isolates obtained from black scurf and drycore in the experiments under controlled conditions presented an identical ISSR-PCR fingerprinting genotype to the isolate used to inoculate the sterilized soil. Thus, it was proven that *R. solani* AG 3 is the causal agent of drycore and that injury on tubers caused by wireworm or artificially by needles favour the formation of drycore. Therefore the higher occurrence of drycore in the organic farming system found in the on-farm program can be explained by the fact that potatoes are often grown after several years of grass clover leys, which enhances the risk for wireworm damage. Other favouring factors are the higher proportion of seed lots which exceeded the threshold for black scurf and the lack of an effective seed treatment in the organic farming system.

Crop rotation has a large influence on the occurrence of drycore, slugs and wireworm. To improve potato quality the crop rotation has to be optimized especially in the organic farming system, where grass-clover leys preceding the potato crop are regarded as an important source of nitrogen. The increasing risk for slug damage with increasing soil cover over winter (catch crops, grass-clover leys) indicates that conflicting aims exist between the quality standards set by the trade and ecological aims of an environmentally friendly production. Research is needed to find plants with a low acceptability for slugs which are suitable for catch crops or green manure. Seed tuber infestation with black scurf often exceeds the threshold and increases the risk for black scurf and drycore especially in organic farming system. This underlines the importance to improve seed quality. Our results suggest that a promising issue for future research might be to develop methods to produce seed free of black scurf in organic production and to find biological control methods against wireworm.

# 1. General introduction

## Global importance of potatoes

In 2003 – 2005 the global potato surface averaged 19 million hectares with an average yield of about 17 tons per hectare. Yields in the industrialized countries exceeded 40 t per hectare, compared to less than 10 t per hectare in developing countries. With a global production of 320 million metric tons potato is the fourth important field crop world wide behind maize, rice and wheat. With 70 million metric tons China is by far the most important potato growing nation, followed by Russia, India, USA and the Ukraine. These five nations together produced over 50% of the word's potato crop (FAO, FAOSTAT Data, 2006). Potatoes are used primarily for food and industrial products such as starch or alcohol. By-products of the industry and potato lots which do not reach the quality standards for human consumption, are used for animal feeding.

Potato production in Switzerland is in a period of rapid change. In 1980 980'000 t of potatoes were produced on a surface of about 24`000 ha. Between 1980 and 2005 this surface decreased rapidly to about 13'500 ha with a total yield of 500-600`000 t (swisspatat, 2006). The number of potato growers decreased in the same period from 44'000 to about 9'000 (swisspatat, 2006). With a per head consumption of 42.3 kg potatoes are still ahead of pastry (39.8 kg) and rice (21.6 kg). The potato industry in Switzerland has a demand of about 300'000 t per year (57% fresh consumption, 43% processing industry), 27'000 t are used as seed potatoes. Potatoes which do not reach the Swiss quality standards are used fresh or dried for animal feed. Because the price for animal feed is rather low and production costs are very high compared to other crops, quality deficiencies are a high economical risk for potato growers.

## Farming systems of potato growing farms in Switzerland

Since the introduction of minimum ecological requirements as a condition for direct payments by the agricultural policies in the early 90ties crop production in Switzerland changed considerably. The input per hectare of nitrogen, phosphor and potassium and of pesticides has been clearly reduced. The percentage of fields with green manure or catch crop over winter increased rapidly. Today 87% of the total potato surface are produced on conventional farms, which have to fulfil minimum ecological requirements concerning e.g. crop rotation, fertilization, crop protection and soil protection. Nine percent are produced according to the guidelines of integrated production for the label IP-Suisse. In addition to the minimum requirements of conventional farms chemical weed control and chemical haulm destruction is forbidden. Four percent of the potato surface is on organic farms, which have to fulfill the high standard of the official Swiss guidelines for organic production on the total surface of the farm. No synthetic fertilizers or pesticides are allowed. Weed control is done mechanically and haulm destruction mechanically or with gas. The use of copper to control late blight (*Phytophtora infestans*) is limited to 4 kg/ha.

### Potato quality

Quality assessment in the Swiss potato industry is done according to the Swiss potato quality standard (Schweizerische Kartoffelkommission, 1989). Samples of 10 kg for potato lots up to 5 tons, 2 times 10 kg for lots between 5 and 12t and 3 times 10 kg for lots over 12 tons are controlled. The maximum tolerance for the sum of outer and inner quality deficiencies (e.g. wireworm, drycore, hollow heart) is 12% and 18% of weight for unwashed and washed potatoes respectively. Over 4% or 10% deficiencies respectively lead to price reductions. Potato lots with more than 2% of tubers with bacterial soft rot and dry rot (*Phytophtora infestans*, *Fusarium ssp*. and *Phoma ssp.*) or more than 30% of tubers with heavy attack (more than 25% of tuber surface) of netted scab (Streptomyces ssp.) are refused. “Qualiservice” is the controlling body to check if potato traders and retailers comply with the Swiss quality standard. In 2002-2005 76-81% of 800 assessed samples of packed potatoes for the fresh market had good quality, 15-17% were sufficient and 4-7% insufficient (Swisspatat, 2006). Before these potato lots were controlled about 30% (about15% each on the farm and in the trading company) of the tubers were sorted out (F. Stucki, Fenaco, Switzerland, pers. com., 2004). Quality deficiencies are a major reason for insufficient economy in potato production. There are several possible reasons for the fact that potato quality has been increasingly criticized by traders and the potato industry in recent years. In 1980 around 70% the potatoes were sold unwashed, where as today over 90% are washed (Swisspatat, 2004). As a result consumers notice even minor quality deficiencies like e.g. black scurf. This is especially the case for self pick selling in supermarkets. Thus, outer quality deficiencies have become more important. Furthermore because of the decreasing potato surface in Switzerland, the potato industry has to choose their demand from a smaller total yield than some years ago, which leads to a higher risk for an insufficient availability of high quality. There is no difference in the quality standards for the different farming systems. Around 75% of all organic potatoes are sold by the biggest retailers in Switzerland, Migros and Coop. Being in the highest price segment and in competition with other less expensive labels (e.g. IP Suisse), only top quality can be marketed as organic potatoes.

The quality assessment in the potato industry is mainly conducted with graded but unwashed samples. Quality deficiencies like e.g. wireworm, drycore or slug damage are not distinguished in detail and the management and site parameters of the potato fields are unknown. Therefore, the actual cause for the claimed quality problems and the importance of different quality deficiencies are largely unknown.

In a three year research program (2001-2003) in the framework of which this thesis was carried out the relative importance and the causes of quality deficiencies of potatoes were studied on the main varieties Agria, Charlotte, Bintje and Eba in different farming systems (organic, integrated and conventional) across the major potato growing regions of Switzerland. Although some important damage by powdery scab as well as by *Streptomyces* ssp. were found in some situations and on certain genotypes, the most important causes of quality deficiencies over all three years were wireworms (*Agriotes* ssp.), slugs (*Deroceras reticulatum, Arion* ssp.) and *Rhizoctonia solani* (drycore and black scurf). The occurrence of drycore, black scurf, wireworms and slugs was highest in the organic farming system. No difference between farming systems could be observed for powdery scab, common scab and all other assessed quality deficiencies. On average over the three years 2001-2003 the Swiss potato quality standard was achieved for 30% of the fields without sorting out deficient tubers. For a further 48% the requirements could be reached if it is assumed that halve the deficient tubers are sorted out. For 22% of the fields the quality was insufficient to meet the standard. Looking at the results of the varieties Agria and Charlotte only, the quality was comparable for the integrated and conventional system (15% and 18% insufficient quality respectively). In contrast, the proportion of fields with insufficient quality was 40% for organic production (Fig.1.1). The higher percentage of fields with insufficient quality in organic production can be explained by the higher damages caused by drycore, wireworm and slugs.

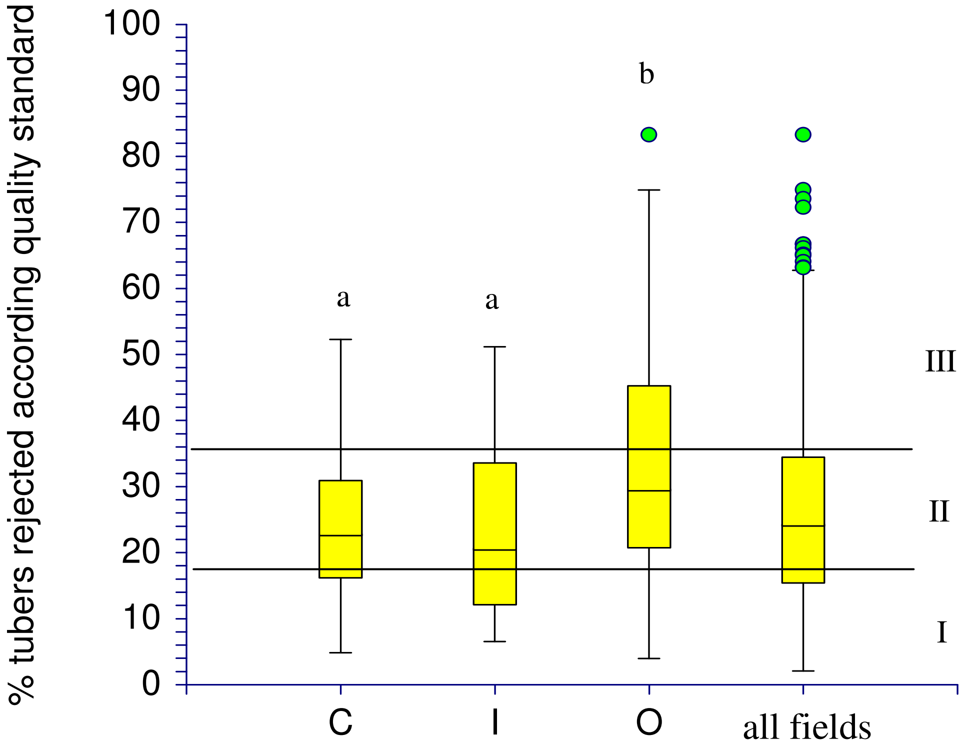


Fig. 1.1 Percentage of tubers rejected 2001-2003 according to the Swiss quality standards due to all quality deficiencies except netted scab, dry- and soft rot. For the comparison of the farming systems only the varieties Agria and Charlotte are considered. C = conventional, I = integrated, O = organic. N: all fields = 278, C = 82, I=39, O=47. The box plot shows the 50% inter quartile range of the values (50% of the plots are in the box) as well as the median (middle line in the box). The points represent the outliers. In order to have a global α-level of 0.05 the Bonferroni-Holm procedure was applied (Holm 1979). Results with different denominator are statistically different. I = accepted without sorting out damaged tubers, II = accepted after sorting out, III = insufficient quality.

### Quality deficiencies caused by black scurf, drycore, wireworms and slugs

#### *Rhizoctonia solani* (*dry core and black scurf*)

*Rhizoctonia solani Kühn* (telemorph*: Thanatephorus cucumeris* (Frank) Donk) is a soil borne plant pathogen affecting many agricultural crops worldwide (Ogoshi, 1987; Sneh et al., 1991). On potatoes the disease causes delayed emergence, lesions on stems (stem canker) and stolons and sclerotial formation on tubers (black scurf) (Baker, 1970; Anderson, 1982). Attacks on stolons may induce the development of malformed tubers and tubers with growth cracks (Weinhold et al., 1978; Jeger et al., 1996). Besides stem canker and black scurf, other symptoms have also been attributed to *R. solani* most notably drycore, restricted brown cavities up to several millimetres deep with a diameter of 3 to 6 mm (Schwinn, 1961; Grütte, 1940; Ramsey, 1917; Müller, 1925). Schwinn (1961) presented evidence that *Rhizoctonia solani* is the causal agent of the drycore disease of potato tubers. Infections with the mycilium of *R. solani* were successful through mechanically wounded normal lenticels as well as naturally grown lenticels excrescences with the mycilium of *R. solani* were successful. He postulated that the development of drycore is stimulated under wet soil conditions when lenticels tend to form excrescences. Other symptoms are scabby lesions on tuber skin which closely resemble netted scab symptoms caused by *Streptomyces reticuliscabiei* and some strains of *Streptomyces europaeiscabiei* (Boucheck-Mechiche et al., 2000).

The most useful system for the classification of *R. solani* is based largely on hyphal fusion with anastomosis tester isolates. To date, fourteen anastomosis groups (AG 1 to AG 13 and AG BI) have been identified, seven of them (AG 1, AG 2, AG 3, AG 4, AG 6, AG 8, AG 9) being divided into sub-groups that differ for pathology, biochemical or genetic characteristics (Anderson, 1982; Cubeta and Vilgalys, 1997; Gonzalez et al. 2001, Carling et al., 2002). AG 3, 4, 5 and 8 are capable of doing moderate to extensive damage to potato plants. AG 3 has been known as the most host-specific type of *R. solani* causing disease of potato. It is by far the most important cause of black scurf because most of the sclerotia isolated from potato tubers belong to AG3 (Carling and Leiner, 1986; Chand and Logan, 1983; Campion et al., 2003).

In contrast to many *Rhizoctonia* diseases of other crops *Rhizoctonia* disease of potato (Solanum tuberosum L.) has an important seed borne (tuber borne) phase that provides a mechanism for long distance dispersal of the pathogen (Ceresini, 2002). Once established in the soil, the mycelium and sclerotia of the pathogen may provide an additional source of primary inoculum. Soil borne inoculum mainly contributes to stolon and root damage and black scurf on progeny tubers, while tuber-borne inoculum affects sprout emergence, causes stolon damage and represents the stem canker phase of the disease (Adams et al., 1980; Frank and Leach, 1980). Tuber-borne inoculum is nearest to the developing shoots and is of greater importance in causing stem canker than soil-borne inoculum, which may reach the shoots only when they have become more resistant to infections after emergence (Van Emden, 1965). Mature tissues express greater resistance to infection than tissues in a formative stage (Hide et al., 1985). Therefore all practices which favour rapid emerge will generally aid in decreasing stem canker infections. These practices include: Seed pre-germination, planting in warm soil (15°C), shallow planting (Sneh et al., 1996).

The key for the control of this disease lies in the use of scurf-free seed tubers because of their importance of disseminating the disease and adding to the pool of soil borne inoculum. The planting of scurf-free seed tubers alone will not guarantee freedom from disease. The soil borne inoculum needs to be kept minimal by the use of crop rotations. Rotations of at least 3-5 years should be used to reduce losses caused by *R. solani* (Scholte, 1992). Carling et al. in Alaska (1986) recovered isolates of AG 3 from soils free of potato for two years but not from soils free of potato for five years and more. For chemical control a vide range of fungicides are used, from the broad-spectrum dithiocarbamate metham sodium to the phenylurea pencycuron, which is extremely selective against *R. solani* and *Rhizoctonia*-like binucleate fungi (Jeger et al., 1996). The most common application method in Switzerland is seed treatment during storage or at planting. No soil treatments are allowed. Various antagonistic or hyper parasitic fungi and bacteria are partly effective in the suppression of the pathogen. Some of these show promise, but as yet, no satisfactory biological control agents is commercially available (Sneh et al., 1996). Promising is the application of *V. biguttatum* conidia in green-crop-lifting or green-crop-harvesting, which has lead to predictable and relevant control of black scurf and an appreciable reduction of the viability of tuber-borne sclerotia (Mulder et al.,1992; Jeger et al., 1996).

The technique for haulm destruction has an important influence on the black scurf infestation level. Mechanical haulm destruction by haulm pulling stimulates black scurf formation less than chemical destruction or cutting of the shoots. This is probably because it immediately stops sap flow from roots to tubers. After haulm destruction, black scurf formation is stimulated by changes at the tuber surface due to accelerated tuber maturation. These changes probably start within three to six days. The final mass of the sclerotia, however, is probably influenced most by stimulatory and inhibitory volatile tuber exudates. Inhibition dominates during tuber growth, decreases gradually during tuber maturation and disappears rapidly after haulm killing (Djist, 1989).

Differences in susceptibility to *Rhizoctonia solani* can be seen between cultivars (Scholte, 1989), but no resistant cultivars have been identified so far. Buhr (1989) showed that reactions to tuber deformations varied significantly and reproducible between cultivars. For all other symptoms the influence of environmental conditions was bigger than the influence of cultivars.

### *Wireworm (Agriotes ssp.)*

Wireworms are the larvae of click beetles (*Elateridae*). There are about 150 species of click beetles recorded in middle Europe, but only few of them can be classified as pests. By far the most important pest species are those belonging to the genus *Agriotes*. Three species, *Agriotes* *linneatus* (L.), *Agriotes obscurus* (L.) and *Agriotes sputator* (L.) are responsible for the vast majority of attacks in Britain (Parker et al. 2001). In Switzerland *Agriotes obscurus* (L.) and *Agriotes lineatus* (L.) are the most important species in the north of the Alps, whereas *Agriotes littigiosus* and *Agriotes obscurus* are dominant in the south of the Alps (Jossi et al., 1997). Other wireworm genera can sometimes be found in both grassland and arable fields, but these rarely exceed 10% of the total population. Jossi et al (1997) found that 10.6 % of the total wireworm population in the north of the Alps belong to *Athous ssp*. and recent surveys in western France (Blot et al. 1999) indicated that *Athous ssp*. wireworms were present in 19% of insecticide-treated fields of maize-wheat rotations. In general non-*Agriotes* species tend to be more common in marginal areas such as rough grazing land or in higher altitudes (Erichson Jones 1944). High wireworm populations have traditionally been associated with fields in long-term grassland (Miles, 1942; Anonymus, 1948), as this undisturbed habitat is generally favourable for wireworm survival. Crops susceptible to injury are cereals, maize, potatoes, sugar beet and vegetables. Legumes are less likely to be injured (Glogoza, 2001). Wireworms damage crops by feeding on the germinating seed or the young seedling. Damaged plants can wilt and die, resulting in thin stands and bare spots may appear. In heavy infestation reseeding may be necessary. In potatoes the new tubers can be severely damaged. Infested tubers have narrow tunnels resulting in a lower market value or even total refusal of the potato lot by the retailer. Wireworm damage may be confused with slug damage. However, slug often hallow out large cavities within the tuber flesh, whereas wireworms do not (Gratwick, 1989). Wireworms usually take three to five years to develop from the egg to an adult beetle. Most of the time is spent as a larva. There are two main periods of wireworm activity, one in March to May and a second one in September to October. The latter period of activity is when most damage to potatoes occurs (Gratwick, 1989). The time taken to complete larval development may vary considerably between genera and individual sites (Miles, 1942). Wireworms usually reach maturity in July to September. Pupation takes place 5-30 cm below the soil surface. After 3 to 4 weeks they become adults but usually remain in the soil for hibernation (Gratwick, 1989). Generations overlap, so larvae of all ages may be in the soil at the same time. Female click beetles lay there eggs in May or June just below the soil surface either single or in clusters and usually within the protection of grassy or weedy spots which minimizes the risk of desiccation. There is evidence that oviposition is reduced on arable land compared with grassland (Gough and Evans, 1942), possibly because of lack of cover at key times. In potatoes wireworms were until recently regarded as minor but locally important pest in mixed arable and livestock farming areas. However in the last couple of years wireworm damage has become an increasing problem including the occurrence of damage in fields in all-arable rotations even without long-term grassland history (Parker, 2001; Demmler, 1999). The reasons for this apparent change in pest status of wireworm are not clear. A contributory factor could be the increasingly stringent quality demands from retailers, because an increasing part of washed potatoes is sold and even smallest injuries are recognized by the consumer. There may also be biological reasons for the growing pest status of wireworm. There is some evidence that crops following long-term set-aside (1-5 years follow) provide a suitable habit for wireworm and this may be contributing to the observed problems in arable rotations (Hancock et al., 1992; Parker, 1999). Strickland *et al.* (1962) suggested that the apparent decline in wireworm damage in the period of 1944-54 could be at least in part be explained by an increasing use of persistent soil insecticides such as Aldrin. Since the beginning of the 1990ies the use of insecticides against wireworms is limited to the seed treatments of cereals, maize and sugar beet in Switzerland. Soil treatment with insecticide granulates is restricted to control the nematode *Ditylenchus dispaci* in infested fields. The apparent increase of wireworm problems in Switzerland could at least in part be attributed to the strong decrease of the treatments with persistent insecticide caused by the new agricultural policies that favours sustainable production systems. As well in the Netherlands the application of soil pesticides has been curtailed for environmental reasons (Ester et al., 2004). The implementation of the Assured Produce Protocols requires growers to justify their pesticide inputs. A good estimation of the wireworm infestation level of a field and the prediction of the likely level of damage is essential for an effective risk assessment method which must be part of a wireworm management strategy. A difficulty is that insecticides against wireworms have to be applied before or at planting. Therefore the assessment of the likely damage risk has to be done well in advance of damage actually occurring in the crop.

Unless a clear relationship between wireworm catches with bait traps and subsequent damage to potatoes can be demonstrated, baiting can only be used as a presence/absence indicator of wireworm infestation. The damage assessment work carried out by different authors (Chabert and Blot, 1992; Parker, 1996) indicated that such a relationship is unlikely to be found for both soil sampling with laboratory extraction and bait traps. It is therefore unlikely that the treatment threshold for potatoes can be raised above the level of presence/absence (Parker, 1996), especially because a relatively low percentage of wireworm damaged tubers already leads to price reductions for the grower. Important damage on potatoes can occur even in fields where very few wireworms are detected. On the other hand low wireworm damage was recorded on fields with a high wireworm infestation level (Parker, 1996). These results indicate both the patchy distribution of wireworms within a field and the sampling errors involved in trying to estimate the population size accurately. Furthermore the wireworm damage level must be influenced by other factors like climate, chemical and mechanical characteristics of the soil, cropping and cultivation history (Parker, 1996). The best indicator of wireworm presence or absence is the duration of grassland in the cropping history of individual fields. Parker and Seeney (1997) found that the proportion of grass fields infested was relatively constant at 60-70% on fields used as grassland for 10-15 years. Over 3 years they collected data on 62 grassland fields in England and Wales on site specific variables including soil physical characteristics, grass duration, grass genera diversity and abiotic factors such as field aspects, altitude and meteorological parameters. Only grass duration and soil bulk density showed any association with wireworm infestation. Trapping of adult click beetles with species specific sex pheromones is used in recent work to record the different species and their flight peaks in different host plants. Between 1999 and 2003 Ester et al. (2004) captured large numbers of *A. lineatus* and *A. obscurus* in fields with seed grass, grass clover ley, barley, lucerne, peas, and roadside verges. *A. sputator* was manly found in the verges. Other species seem to be of minor importance in the Netherlands. Adult Elateridae do not migrate and any movement is likely to be limited to local dispersal only, probably largely by walking because some species (e.g. *Agriotes obscurus*) do not seem to fly readily (Parker et al., 2001). Measuring beetle activity in a given locality should therefore indicate the presence of an established local population, thus contributing to an assessment of an overall risk of wireworm infestation (Parker et al., 2001).

### *Slugs (Deroceras ssp., Arion ssp)*

Most slug damage in arable crops in Europe is caused by two types of slug, the grey field slug *Deroceras reticulatum* (Müller) and the round back slug *Arion* ssp. In England, Belgium and locally in France a third type, the keeled slug *Tandonia* ssp is responsible for important slug damage in arable crops (Hommay, 1995). In Switzerland the most frequent slug species in arable crops are *Deroceras reticulatum* (Müller) and *Arion* ssp.. *Tandonia* ssp are mainly soil-dwelling although they will also feed on plants above ground. In England they are a particular problem in potatoes where they bore into tubers (Hommay, 1995). Like *Tandonia* ssp. *Arion hortensis* is attracted by underground organs of the plant (e.g. carrots, potatoes, seed grains). *Deroceras reticulatum* (Müller) are the most important slug pest of arable crops in temperate climate (Godan, 1983; Port and Port, 1986; South, 1992). Reproduction can take place all year round apart from periods with temperatures below 5° C or very dry periods. Nevertheless periods with highest reproduction activities are in springtime and autumn. *D. reticulatum* has two generations per year. The duration of one generation varies in function of climatic conditions (Hommey, 1995). The lifespan is 9 to 12 months. Roundback slugs (*Arion spp.*) are commonly found, represented by a range of species varying in size and local importance. The lifecycle of *Arion spp.* is generally annual. The reproduction period is autumn to spring. The highest percentage of sexually major slugs is reached in springtime and most of the adults die in June or July with an age of 12 to 18 month (Phillipson, 1983). *Tandonia* spp. has two generations per year with spring and autumn egg hatch.

Although slugs are regarded as generalist feeders, they are capable of a high degree of selectivity and exhibit a distinct preference between different plants. Physical barriers such as the silicon content in the plant can keep slugs from feeding (Wadham and Wynn Parry, 1981). The choice of food depends generally on the presence of antiappetentes or phagostimulants. *Arion rufus* generally doesn’t feed on plants containing alkaloids (Wink, 1984). In contrast plants with higher sugar content stimulate the consumption of *D. reticulatum* (Henderson et al., 1992). The palatability of legumes to *Deroceras reticulatum* was tested in controlled-environment experiments. A clear hierarchy of acceptability was shown, with red clover, lucerne, lupine and white clover showing significantly higher acceptability indices than the other legumes tested. Red clover was consumed in significantly greater quantities than any other legumes (Brooks et al., 2003).

Differences in resistance of crop varieties are well known. Port and Port (1986) reported that the palatability of different wheat species (Triticum ssp.) correlated with the total nitrogen content of the seeds. Newer varieties of oilseed rape (00-type) with low Glucosinolate content are more susceptible to slug damage than conventional varieties. Several authors observed differences in susceptibility of different potato varieties (Gould, 1965; Winfield, 1967; Warley, 1970; Pinder, 1974; Airey, 1987 and Johnston, 1994). Pinder (1974) observed that the initiation of slug damage coincided with the completion of tuber growth and suggested that biochemical changes in tubers at this time render them more susceptible to slug damage. On resistant varieties slugs didn’t eat the skin, but the tuber flesh where it was exposed (Pinder, 1974). High phenolase activity was identified as the chief resistance factor by Johnston (1994). High phenolase activity is located in the surface layer of the tuber and differs between cultivars in parallel with resistance. In bioassays slugs avoided feeding on the skin if other tissues were available and they grew less well on the skin plus cortex than on other parts of the tuber (Johnston, 1993). However, slugs can be secondary feeders when other organisms such as wireworm or millipedes have already broken through the skin (Cooper et al., 1989). In this case important slug damage can even occur on varieties with a good slug resistance. In the British variety list slug resistance is indicated with a score from 1 to 9 (1 = resistant, 9 = very susceptible). For most of the varieties grown in Switzerland slug resistance is unknown. In a laboratory box test, the slug *Deroceras reticulatum* affected only wounded tubers and, although slime was present on the unwounded potatoes, they were not damaged by slugs (Ester and Trul, 2000).

Changes in the agricultural practice due to economical pressure and increasing importance of ecological criteria (e.g. catch crops to reduce nitrate leaching, reduced soil tillage) seem to increase slug problems. There is higher risk for slug damage in reduced and especially zero tillage systems, where activity and abundance of slug species is increased (Voss et al., 1998; Glen et al, 2002). Taupin et al. (1997) reported that reduced soil tillage in the intercrop period and the sowing of a catch crop increased the slug population in the main crop in springtime. Slugs cause problems mainly in crops grown on clay soils in which they shelter in cracks and between clods (Rayner, 1975). Glen et al. (2002) also found that the risk for slug damage is higher on heavier soils especially in reduced tillage systems with a high proportion of inter crops or green manure. Chabert (2003) concludes that under present large-scale arable farming systems, it is proving very difficult to keep slugs under control. Slug baits based on Metaldehyde or Methiocarb are the main measure against slugs. These chemicals together account for more than 90% of the European molluscicide market. Methaldehyde principally acts on slugs through excessive secretion of mucous leading to subsequent dehydration and death (Triebskorn et al., 1998). Methiocarb has a totally different mode of action, acting on nerve tissue and inhibition of acetyl cholinesterase (Kelly and Martin, 1989). During years of especially heavy slug attack, as many as 5 million of the 12 million hectares of arable crops in France may be under molluscicide treatment (Chabert, 2003). As an alternative the parasitic nematode *Phasmarabditis hermaphrodita* is commercially available to control slugs. Results with nematodes have been promising particularly for horticulture (Speiser et al., 2001; Ester et al., 2003). The higher cost compared to chemical treatments hinder the widespread use, especially in arable crops.

## Outline and objectives

High quality is a key factor for successful potato production. Outer quality deficiencies caused by wireworms, slugs and *Rhizoctonia solani* (drycore and black scurf) are a major problem in Swiss potato production and are responsible for important economic losses. This thesis aimed at identifying the influence of farming systems, specific cultivation methods and site parameters on the occurrence of outer quality deficiencies in an on-farm program between 2000 and 2003 and in open-air experiments and pot experiments under controlled conditions in 2005 and 2006. These results were the basis for differentiated recommendations to potato growers how to improve potato quality. To guarantee a fast and wide conversion of the results into practise the on-farm program was conducted in close collaboration with extension services and potato growers, traders as well as the processing industry. The results were discussed in regular regional workshops diffuse the experience and know-how of all partners involved.

The thesis is divided into four main parts:

1. Spatial distribution of the different quality deficiencies within fields and analysis of the relationship between them. The resulting data was also used to determine the sampling plan for quality assessments of potatoes with a precision of +/– 2% to 4% for the estimated mean of damaged tubers in the on farm program between 2001 and 2003 (Chapter 2).
2. Influencing factors for outer quality deficiencies and the relative importance of wireworms, drycore, black scurf and slugs. For this purpose data of 278 potato fields of different farming systems was collected and analyzed in an on-farm program between 2001 – 2003 (Chapter 3 and 4).
3. Genetic characterization with classic (anastomosis grouping with tester strains) and molecular tools (pectic zymogram, AG-specific primers, ITS sequencing, ERIC and REP-PCR) of isolates of *Rhizoctonia solani* from black scurf and drycore symptoms of potato fields (Chapter 5).
4. Check of the hypothesis derived from the results of chapters 3 and 4 that injuries of wireworms on the tubers facilitate the penetration of *Rhizoctonia solani* into the tuber and favour the formation of drycore. For this purpose randomized field trials and pot trials under controlled conditions in the climate chamber were conducted in 2005 and 2006 (Chapter 6).

# 2. Influence of sampling procedure and spatial distribution of quality deficiencies caused by drycore, black scurf, wireworms and slugs on the precision of the potato quality assessment.

## ABSTRACT

Across the major potato growing regions in Switzerland the most important causes of quality deficiencies were wireworms (*Agriotes* ssp.), slugs (*Deroceras reticulatum, Arion* ssp.) and *Rhizoctonia solani* (drycore and black scurf). For a better understanding of the spatial distribution of their occurrence and to analyse possible relations between quality deficiencies all tubers of 400 plants were sampled and assessed on five potato fields of 0.8 hectares. Data was also used to optimize sampling procedure for a reliable assessment of potato quality for large numbers of fields.

Distribution of damaged tubers in the field was in most cases non-binomial because of a heterogeneous spatial distribution of quality deficiencies. Therefore the probability that a quality deficiency occurred on two tubers of the same plant was higher than for tubers of different plants. Consequently, for a defined total number of tubers more sampled plants improve the precision of the assessment. For the quality assessment at Swiss conditions, a sample size of 800 tubers collected from 270 plants guaranteed a sufficient precision of approximately +/- 2 to 4% for the mean of damaged tubers. The relative risk for drycore on a progeny tuber was higher if wireworm damage or black scurf was present on the same tuber.

## INTRODUCTION

Quality assessment in the Swiss potato industry is done according to the Swiss potato quality standard (Swiss potato commission, 1989). Samples of 10 kg for potato lots up to 5 tons, 2 times 10 kg for lots between 5 and 12 tons and 3 times 10 kg for lots over 12 tons are controlled. The quality assessment is mainly conducted with unwashed samples and quality deficiencies caused by wireworms (*Agriotes ssp.*), drycore (*R. solani*) or slugs (*Deroceras reticulatum*, *Arion ssp.*) are not distinguished in detail but subsumed under the generic term “outer quality deficiencies”. Therefore the relative importance of these deficiencies is unknown. For a quality improvement a clear distinction of these deficiencies is of great importance.

Wireworms are the larvae of click beetles (*Elateridae*). There are about 150 species of click beetles recorded in middle Europe, but only few of them can be classified as pests. By fare the most important pest species are those belonging to the genus *Agriotes*. In potatoes the new tubers can be severely damaged. Infested tubers have narrow tunnels resulting in a lower market value or even total refusal of the potato lot by the retailer (Appendix, Fig. A5). Wireworm damage may be confused with slug damage (Appendix, Fig. A6). However, slugs often bore small entrance hauls into the tubers to reach the flesh and hollow out large cavities, whereas wireworms do not (Gratwick, 1989).

Most slug damage in arable crops in Europe is caused by two types of slug, the grey field slug *Deroceras reticulatum* (Müller) and the round back slug *Arion* ssp. (Hommay, 1995). Potato tubers are mainly attacked in mild and wet climates (Johnston et al, 1994).

Restricted brown cavities up to several milimetre deep with a diameter of 3 to 6 mm, denominated as drycore, have been attributed to *Rhizoctonia solani* (Grütte, 1940; Ramsey, 1917; Müller, 1925). Schwinn (1961) presented evidence that *Rhizoctonia solani* is the causal agent of the drycore disease of potato tubers. *Rhizoctonia solani Kühn* (telemorph*:* *Thanatephorus cucumeris* (Frank) Donk) is a soil borne plant pathogen affecting many agricultural crops worldwide (Ogoshi, 1987; Sneh et al., 1991). On potatoes the disease causes delayed emergence, lesions on stems (stem canker) and stolons and sclerotial formation on tubers (black scurf) (Baker, 1970; Anderson, 1982). Attacks on stolons may induce the development of malformed tubers and tubers with growth cracks (Weinhold et al., 1978; Jeger et al., 1996) (Appendix, Fig. A4).

In the year 2000 five potato fields of 0.8 ha each were sampled for quality assessment. For each field the sample consisted of the tubers of 400 plants. To get an optimal sample of the observed potato fields and to avoid the influence of different harvest techniques used in potato production, tubers were manually collected in the field before harvest. Coordinates of all plants were noted to create maps of the spatial distribution of quality deficiencies caused by wireworms, slugs, drycore and black scurf. The data was used to analyse the relationship between different deficiencies. The infestation levels in percent damaged tubers were studied in relation to sample size to determine the optimal sample size to investigate the influence of farming systems, specific cultivation methods and site parameters on the potato quality on a large number of fields.

## MATERIAL AND METHODS

### Description of the experimental sites and experimental design

In collaboration with Fenaco, the biggest wholesale trader for potatoes in Switzerland, five potato fields with a high probability for the occurrence of quality deficiencies were chosen for the quality assessment (Table 2.1). All fields were within a radius of 30 km around Bern, 435m to 620m above sea level. Soil types were sandy loam and clay loam. Soil characteristics of the fields were homogenous with a small spatial variation of clay content, organic matter and pH (Table 2.1). The management of the fields followed the usual practice of the respective farms. The long term annual mean temperature (1961 to 1990) in the area was between 8.0° C and 9.8° C. 2000 was a warm year with mean temperatures 2° C higher than the long term mean. The annual precipitation in 2000 at the different sites (1000 – 1100 mm) corresponded with the long term annual mean (Swiss Meteorological Institute Zurich).

### Sampling plan

For the definition of the sampling plan the inhomogeneous distribution of quality deficiencies within a field had to be taken into consideration. To get an exact map of the distribution of different quality deficiencies in the field all tubers of 400 plants per 0.8 hectare were harvested and the coordinates of each plant was noted. The distance between two sampled ridges was three or six meters respectively according to a specially designed sampling plan. Within these ridges the distance between two sampled plants was 5 meters. The total number of sampled ridges varied in dependence of the length of the field. Tubers of each plant were packed separately in bags and stored in a potato storeroom until quality assessment.

### Quality assessment

All tubers were washed before quality assessment to facilitate a clear differentiation of the different quality deficiencies. The number of lesions per tuber caused by wireworm, drycore and slugs were counted. To control the depth of the quality deficiencies all tubers were peeled with a special knife to a depth of 4 mm. Tubers with lesions deeper than 4 mm or more than two lesions are not accepted according to the official quality standard (Swiss potato commission, 1989). Black scurf was assessed using grades from 1 (no black scurf) to 7 (75% to 100% of the tuber surface with black scurf).

Table 2.1 Description of the experimental sites

| field | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Altitude (m.a.sl) | 450 | 435 | 545 | 450 | 620 |
| Soil type | sandy loam | sandy loam | loam | clay loam | sandy loam |
| pH | 5.5 | 7.5 | 8 | 7.8 | 6.3 |
| organic matter (%) | 3.4 | 1.8 | 5.2 | 4.2 | 2.3 |
| clay content (%) | 14.8 | 13.3 (12-13.7) | 28 | 33.5 (30-34) | 14.5 |
| silt content (%) | 33 | 35 | 45.2 | 47.4 | 31.2 |
| Variety | Agria | Bintje | Bintje | Agria | Agria |
| Preceding crops | | | | | |
| 1989 | maize | carrots | maize/ catch crop | grass clover ley | marley |
| 1990 | barley | oats | wheat/ catch crop | grass clover ley | maize/ catch crop |
| Fungicide seed treatment on potatoes[1)] | yes | yes | no | no | yes |
| Insecticide seed treatment in the preceding crop[2)] | no | no | yes | no | yes |

1) against *R. solani*, 2) against wireworm (*Agriotes ssp.*)

### Soil analyses

Thirty to fifty soil samples per field were analysed to check the variability of the soil characteristics within the fields according to the reference method of the Swiss federal research stations (2004): clay, silt and sand contents (analytic), soil type (finger test), pH ($H_20$) and organic matter (analytic).

### Spatial distribution of quality deficiencies within a field and within the plants

For the quality deficiencies of each field a map of their spatial distribution was drawn. To describe the variability within and between plants all assessed plants per field were grouped according to their number of tubers. Within each group, it was tested whether the infected tubers were binomially distributed using a Chi-square test. For deficiencies with a non-binomial distribution the variability between tubers within a plant is smaller than between tubers of different plants. To check the within field distribution of drycore and possible relations between an influencing factor and drycore the relative risk for drycore damage on a tuber could be calculated for the influencing factors black scurf (field 4) and wireworm damage (fields 1 and 4). The relative risk is the ratio of the event rate between comparative groups and is calculated with the following equation: Relative risk (RR) = ((a/a+b)/(c/c+d)), a = tubers with drycore and influencing factor present, b = tubers without drycore, but influencing factor present, c = tubers with drycore but influencing factor not present, d = tubers without drycore und no influencing factor. A relative risk > 1 means that drycore symptoms are more likely to occur if on the same tuber black scurf or wireworm, respectively, are present.

### Determination of the optimal sample size

For the determination of the optimal sample size the lower (p=2.5%) and upper confidence level (p=97.5%) for the mean of infested tubers was determined for sample sizes of 200 to 1400 tubers. Per field and per quality deficiency 1000 random samples for each sample size were simulated. To consider the heterogeneous distribution of certain deficiencies in the field confidence levels were determined for different combinations of harvested numbers of plants and tubers per plant for a defined sample size (number of tubers per field). Based on these results the optimal sampling plan (number of plants per field and tubers per plant) for a precision of approximately +/- 2-4% for the estimated mean of damaged tubers was determined. The simulations were calculated with R, a language and environment for statistical computing (R Foundation for statistical computing, 2006, Vienna, Austria).

## RESULTS

### Spatial distribution of quality deficiencies within a fields and within the plants

*Wireworm damage*

On three fields moderate (3.5% and 3.6%) to heavy wireworm damage was observed (48.1%). No wireworm damage was found on the two fields where the seed of the preceding crop had been treated with an insecticide against wireworms. The infestation level of un-graded (all tubers) and graded tubers (42.5-70 mm) was comparable (Table 2.2). Plants with damaged tubers were distributed over the whole field and no higher infestation was observed along the borders of the plots. A Chi square test showed a non-binomial distribution of wireworm damage on Field 1. Therefore the variability within the tubers of one plant was smaller than between plants. The possibility that two tubers are damaged by wireworm was higher for tubers of the same plant than for tubers of different plants. On Fields 2 and 4 wireworm damage was lower (3.5 and 3.6% respectively) and the distributions within the fields was binomial (Appendix, Table A1).

*Slug damage*

Four of five fields had important slug damage (4.8-37.2% damaged tubers). Infestation level was comparable for un-graded (all tubers) and graded tubers (42.5-70mm) (Table 2.2). The patchy distribution of slug damage over the whole field in Field 2 shown in Figure 2.2 was representative for all fields. Distinct differences in the infestation level occurred between the subplots. No concentration of slug damage along the borders of the fields was observed. The Chi square test suggested a non binomial distribution for damaged tubers in all fields (Appendix, Table A1).

*Drycore and black scurf (Rhizoctonia solani)*

A high proportion of tubers with black scurf was found in two fields with 81% and 22% (Table 2.2). For both fields Chi square tests suggested a very strong non-binomial distribution of tubers with black scurf within the field (Appendix, Table A1). Therefore the variability observed for the tubers of the same plant was much smaller than between plants. Important drycore damage was found in two fields with 3.8 and 12.4% infested tubers, respectively. These fields had highest clay content (28% and 33.5%, respectively) of all five fields. The soil of the fields was homogenous and the clay content varied only few (Table 2.1). In both fields plants with infested tuber were spread over the whole field and the Chi square tests suggested a non-binomial distribution of damaged tubers (Appendix, Table A1). In Field 4 (Fig.2.1) the infestation level of drycore was much higher in the subplots I to IV on the left side of the field and decreased towards the right side. On Fields 1 and 4 the relative risks (RR) for drycore damage with respect to influencing factors were the following: a. factor wireworm damage, RR=5.14 (LCL=3.06; UCL=8.68; p=0.0001; n=2462) and RR=2.88 (LCL=2.07; UCL=3.88; p=0.0001; n= 3320), respectively; b. factor black scurf on Field 4, RR=1.77 (LCL=1.43; UCL=2.17; p=0.0001; n=3320). This indicates that the risk for drycore damage on a tuber was significantly higher if wireworm damage or black scurf was found on the same tuber.

### Determination of optimal sampling procedures

On the basis of the upper (p=97.5%) and lower (p=2.5%) confidence level for different series of simulations the precision of the quality assessment of the potato lots was determined (Fig. 2.3, Appendix Table A2). The precision of the quality assessment was distinctly improved with an increasing sample size between 200 and 800 tubers. With sample sizes of 1000 and more tubers the confidence interval (difference between lower and upper confidence level) was only marginally reduced. For sample sizes of 200 and 400 tubers the confidence interval was larger than the assessed mean for deficiency levels between 3 to 4% damaged tubers and about 50% of the assessed mean for deficiency levels of 12-14%. A precision of +/- 2 to 4 % was reached for all levels of assessed means with a sample size of 800 tubers collected from 270 plants (Fig. 2.3).

For quality deficiencies with a binomial distribution in the field the confidence interval for an assessed mean was not influenced by the number of plants from which a defined number of tubers was sampled (Fig. 2.4a). In contrast, the precision of the assessment of a deficiency with a non-binomial distribution in the field improved with an increasing number of sampled plants (Fig. 2.4b).

Table 2.2 Quality deficiencies caused by slugs, wireworm, black scurf and drycore in % infested tubers for all tubers and for graded tubers (size 42.5.70mm). Number of assessed tubers from 400 harvested plants: n = 2462 (Field 1), 6909 (Field 2), 4454 (Field 3), 3317 (Field 4) and 4790 (Field 5).

| | Tuber size | Field 1 | Field 2 | Field 3 | Field 4 | Field 5 |
|---|---|---|---|---|---|---|
| Slug | all tubers | 40.5 % | 10.4% | 8.4 % | 3.8 % | 0.2% |
| | 42.5-70mm | 37.2% | 14.0% | 9.5% | 4.8% | 0.3 % |
| Wireworm | all tubers | 51.1 % | 2.3 % | 0.4% | 3.3 % | 0.0% |
| | 42.5-70mm | 48.1% | 3.5% | 0.6% | 3.6% | 0.0 % |
| Black scurf | all tubers | 2.2% | 0.04 % | 75.0% | 21.3 % | 1.1% |
| | 42.5-70mm | 3.2 % | 0.1% | 81.2 % | 22.3% | 1.0% |
| Drycore | all tubers | 4.1% | 0.2 % | 0.2 % | 10.4 % | 0.1 % |
| | 42.5-70mm | 3.8 % | 0.3% | 0.2% | 12.4% | 0.1% |

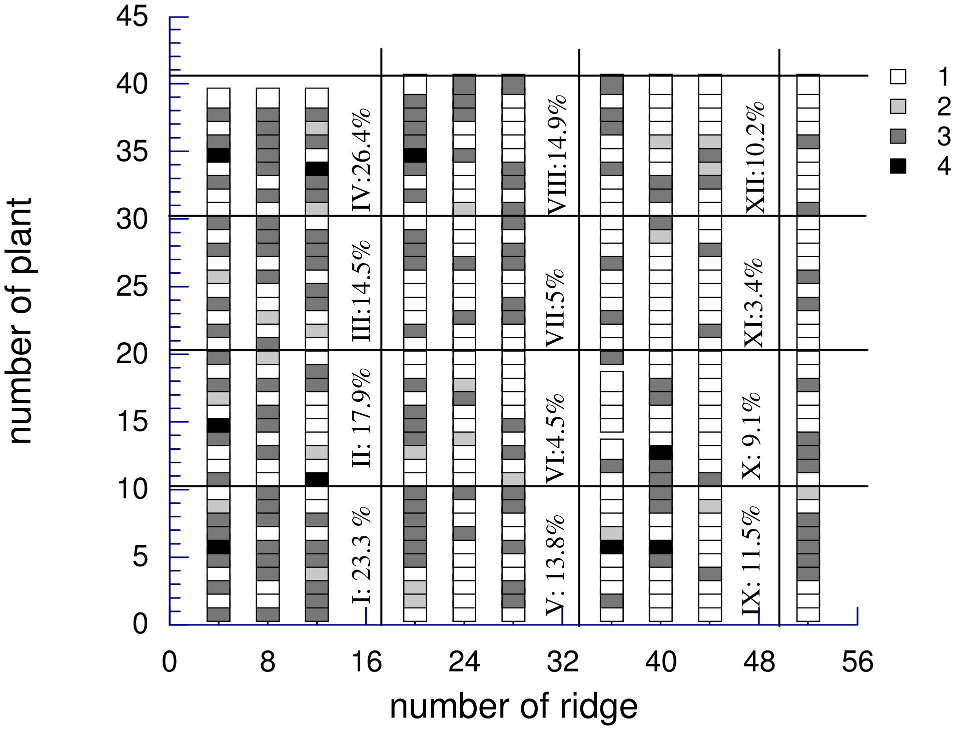


Fig. 2.1 Spatial distribution of drycore damage in field 4. Squares indicate % tubers per plant with drycore symptoms. 1 = 0%, 2 = 0.1-10%, 3 =10.1-50%, 4 > 50%. Distance between two ridges is 0.75 meters and between two sampled plants 5 meters. I-XII: Slug damage in % infested tubers in subplots.

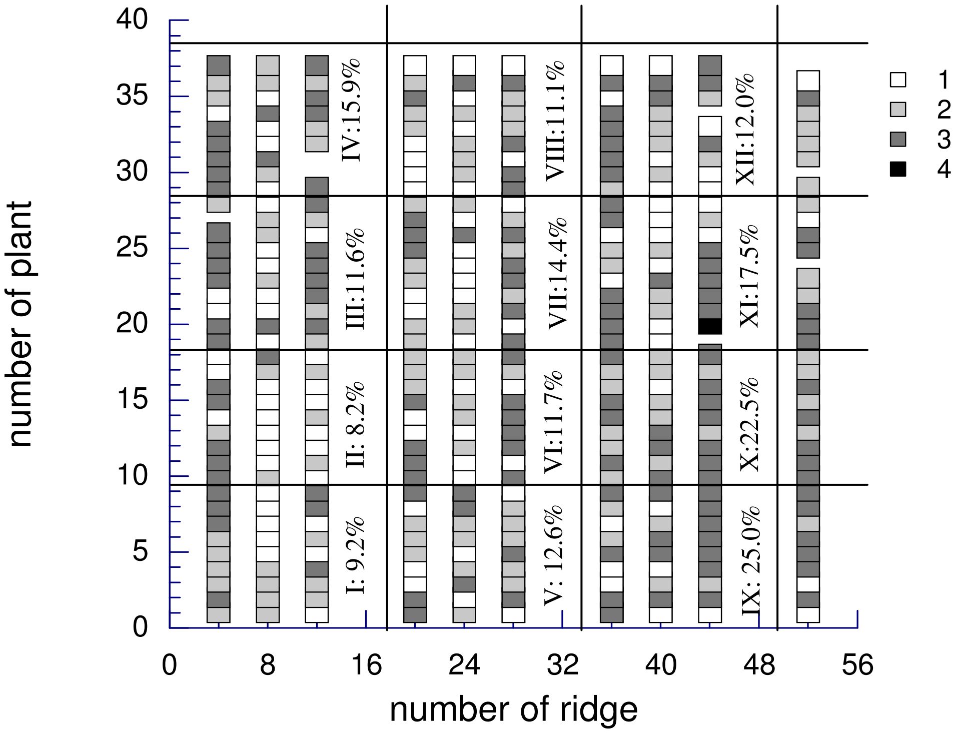


Fig. 2.2 Spatial distribution of slug damage in field 2. Squares indicate % tubers per plant with slug damage. 1 = 0%, 2 = 0.1-10%, 3 =10.1-50%, 4 > 50%. Distance between two ridges is 0.75 meters and between two sampled plants 5 meters. I-XII: Slug damage in % infested tubers in subplots.

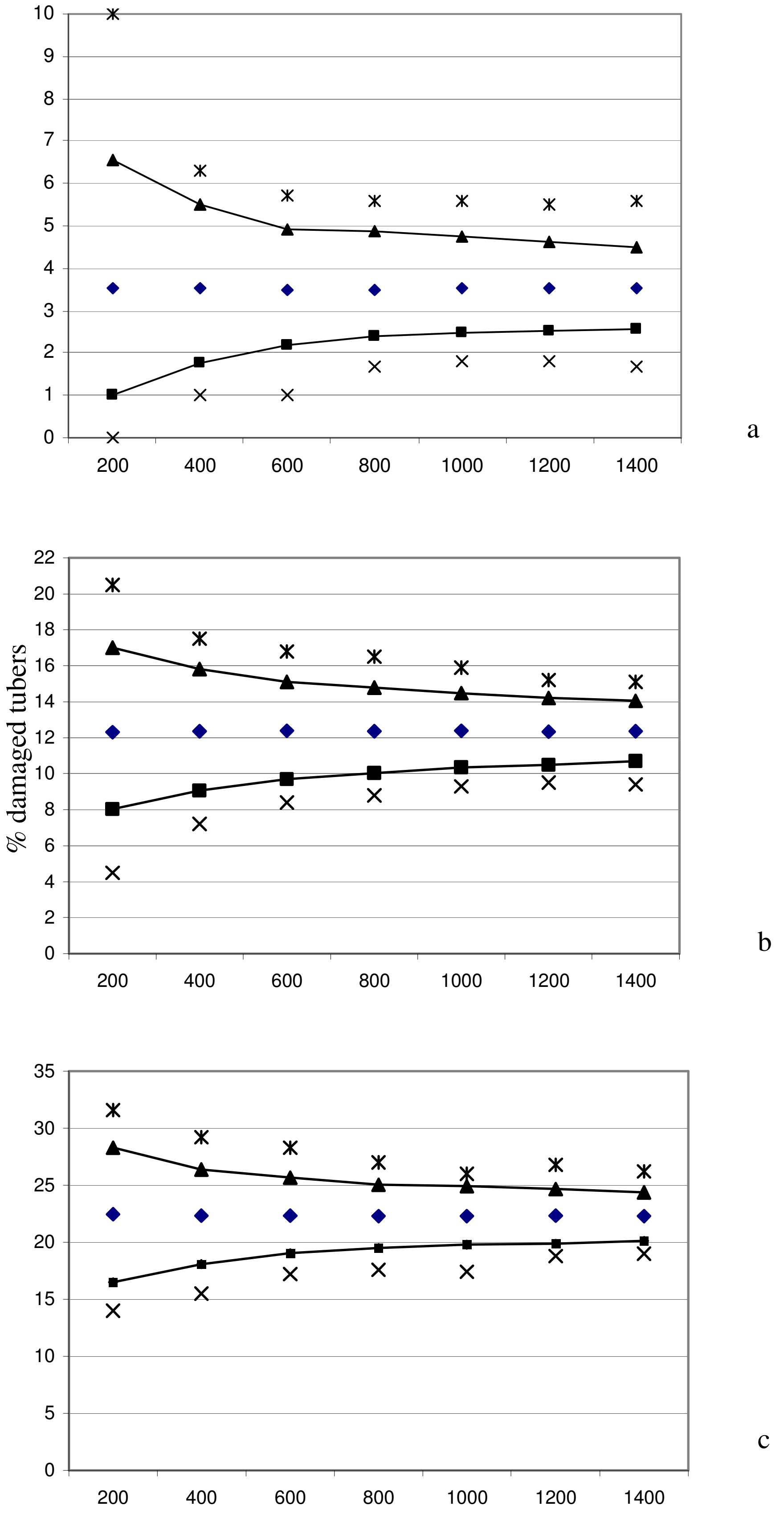


Fig. 2.3 Mean (◆), upper (▲)and lower (■)confidence level (p=2.5 and 97.5%) and minimal (×) and maximal (⚹)value of simulations of 1000 random samples for sample sizes of 200 to 1400 tubers per field for a) wireworm, b) drycore and c) black scurf.

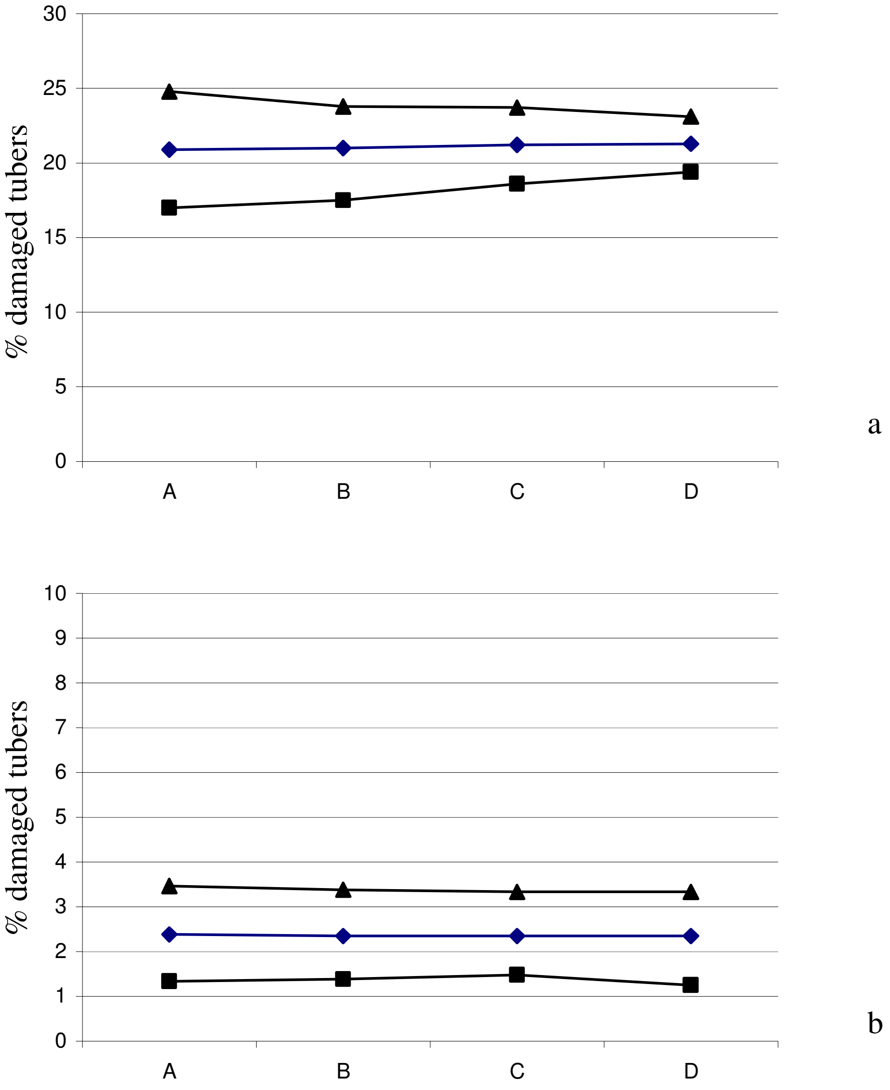


Fig. 2.4 Mean (◆), upper (▲) and lower (■) confidence level for a sample size of 800 tubers in dependence of the number of sampled plants for deficiencies with non-binomial (a; e.g. black scurf) and binomial distribution (b; e.g. wireworm) within the field.: A= 160 plants, B= 200 plants, C=270 plants, D=400 plants.

## DISCUSSION

The distribution of tubers with quality deficiencies was non-binomial in all fields for black scurf, drycore and slugs. For wireworm distribution was non-binomial in one field and binomial in two fields. A non-binomial distribution of a quality deficiency could be expected, when a disease was transmitted by infested seed tubers (e.g. *Rhizoctonia solani*) or if spatial distribution in the field is patchy. Female click beetles (Agriotes ssp.) do not migrate and any movement is likely to be limited local dispersal only, probably largely by walking because some species (e.g. *Agriotes obscurus*) do not seem to fly readily (Parker, 2001). They lay there eggs in May or June just below the soil surface either single or in clusters and usually within the protection of grassy or weedy spots which minimizes the risk of desiccation (Gough and Evans, 1942). This behaviour suggests a patchy distribution of wireworms in the field and therefore a non-binomial distribution of damaged tubers could be expected. However, only on field 1 (48% damaged tubers) a clear non-binomial distribution was observed, maybe because the infestation level on the other plots was rather low.

The distribution of slug damage indicates that the slug population was already established in the field and slugs did not immigrate after the potato crop was planted as no higher infestation level was observed along the field borders. Slugs move only locally over short distances as long as they are sheltered by vegetation that serves them for nutrition (Ricou et al., 1977). Higher slug damage along grass fields or wild flower stripes are often observed, but Frank (1998) found significantly higher slug damage in rape and wheat only up to one meter from grass fields and wildflower stripes. The patchy distribution of slug damage with important differences in the infestation level within the field (Fig. 2.2) could not be explained by changes of soil characteristics as the soil was very homogenous.

The very strong non-binomial distribution of black scurf indicates that variability of black scurf infestation was much smaller for tubers of the same plant than between tubers of different plants. This can be explained by the fact that infested seed tubers are an important factor in transmission of *Rhizoctonia solani*. Thus, the probability for progeny tubers from an infested plant to be affected by black scurf or drycore is higher than for progeny tubers from healthy plants. Higher infestation levels with black scurf occurred only on fields where seed tubers were not treated with a fungicide before planting. Both fields with important drycore damage had higher clay content (28% and 33.5%, respectively) than the other fields. This is consistent with the postulate of Schwinn (1961) that the development of drycore is stimulated especially in soils with higher clay content especially under wet soil conditions when lenticels tend to form excrescences. But the patchy distribution of drycore on Field 4 (Fig.2.2) can not be explained by soil characteristics. The field was homogenous and the clay content varied between 30% and 34%. The fact that progeny tubers with heavy black scurf infestation can be free of drycore (Field 3, Table 2.2) indicates that beside the infection of the plant with *Rhizoctonia solani* further conditions have to be fulfilled for the formation of drycore symptoms on tubers. Schwinn (1961) presented evidence that infection with the mycelium of *Rhizoctonia solani* through mechanically wounded normal lenticels as well as naturally grown lenticels excrescences causes drycore on tubers. This might explain why drycore occurred only on Fields 1 and 4, where *Rhizoctonia* infections and wireworm damage were present, but not on Field 3, where no wireworm damage was observed. Also the calculated relative risk (RR) for drycore for the influencing factors black scurf and wireworm indicated that the risk for drycore was higher if black scurf or wireworm damage were present on the same tuber.

A sample size of 200 tubers corresponds approximately to that used for quality assessment in trade according to the Swiss quality standard; but this does not meet the demanded precision for scientific investigations. With the determined confidence intervals (difference of upper and lower confidence level) it is probable that potato lots that meet the quality standard are mistakenly refused or vice versa. This well corresponds with recent experiences that sample sizes of 100 to 300 tubers lead to confidence intervals that are too wide in Elisa tests for a reliable classification of potato seed lots with a low infestation level of virus (Pre-basic and basic seed) ( J. Derron, Agroscope Changins, Switzerland, unpublished). The labour-intensive quality assessment often prevents an adequate sample size. Assessments with a sample size of 800 tubers met the demanded precision of +/– 2 to 4% for the estimated mean of damaged tubers. With sample sizes of 1000 and more tubers the marginal gain of precision cannot be justified by the time needed for the assessment of such big samples. As the distribution of the assessed quality deficiencies was often non-binomial, the probability that two tubers have the same deficiency was higher for tubers of the same plant than for tubers of different plants. For a quality assessment before harvest, the precision of the assessment is influenced by the number of plants from which a defined number of tubers is sampled. For black scurf which showed a clear non-binomial distribution in the field, the confidence interval was reduced with an increasing number of sampled plants (Fig.2.4a). The optimal combination of the number of sampled plants and tubers per plant must be derived from a compromise between precision demands and the available work capacity. For large surveys we propose a sample size of three tubers of 270 plants for fields of 0.8 ha each.

## CONCLUSIONS

The distribution of quality deficiencies is mainly non-binomial due to a patchy distribution in the field (slugs, wireworm, drycore) or because of the transmission of a disease through infected seed tubers (black scurf, drycore). Therefore the variability of quality deficiencies is often higher between tubers of different plants than between tubers of the same plant. As a consequence the number of plants from which a defined total number of tubers is sampled, has an influence on the precision of the quality assessment. For a reliable quality assessment tuber samples have to be harvested from a sufficient number of plants. With a sample size of 800 tubers harvested from 270 plants per field the precision of the assessed means is +/– 2 to 4%. A relationship between drycore and wireworm damage in a field is strongly indicated.

# 3. Quality deficiencies caused by *Rhizoctonia solani*, wireworms (*Agriotes* ssp.) and slugs (*Deroceras reticulatum, Arion hortensis*) in different farming systems.

## ABSTRACT

Today over 90% of the potatoes for the fresh market are washed; therefore consumers already object to minor outer quality deficiencies. The quality assessment performed by potato traders does not distinguish in detail between quality deficiencies and potential links to the farming systems and site parameters are unknown. Thus, the cause for the observed increase of outer quality problems is unknown. From 2001 to 2003 the extent and the potential causes of quality deficiencies were studied in a standardized sampling pattern of 810 tubers each on totally 278 fields on conventional, integrated and organic farms across the major potato growing regions of Switzerland. Wireworms, slugs and drycore were responsible for important economic losses in all farming systems. In the organic farming system the quality damage was higher for all three deficiencies. While wireworm and slug damage were of general importance, drycore was most severe in the organic farming system, with the difference being statistically significant in all three years. Grass clover ley in the years preceding potatoes increased the risk for all three quality deficiencies. Slug damage increased with soil cover (catch crops) and with the percentage of crops favourable to slugs in the crop rotation (e.g. vegetables). Seed tubers without black scurf infestation reduced the occurrence of black scurf and drycore on harvested tubers. Fungicide seed treatment reduced black scurf significantly if seed tubers were infested. Insecticide seed treatment for cereals preceding potatoes as well as molluscicide treatments in the potato crop had a beneficial but not always sufficient effect.

## INTRODUCTION

According to the Swiss quality standard the maximum tolerance for the sum of quality deficiencies (e.g. wireworms, drycore, and slugs) is 12% and 18% of weight for unwashed and washed potatoes respectively. Quality deficiencies are one of the main reasons for insufficient economy in Swiss potato production. Potato quality has been increasingly criticized by traders and the potato industry in recent years. One reason for the increasing quality deficiencies could be the increasing importance of ecological production, another one the increasing quality standards set by the market: in 1980 around 70% the potatoes were sold unwashed, today over 90% are washed. Minor quality deficiencies like e.g. black scurf (*Rhizoctonia solani*) that were not subject to the Swiss potato quality standard previously have become more important than ten years ago (Schweizerische Kartoffelkommission, 1989). The quality assessment in the potato industry is mainly conducted with graded but unwashed samples. Quality deficiencies caused by wireworms (*Agriotes ssp.*), drycore (*R. solani*) or slugs (*Deroceras reticulatum*, *Arion ssp.*) are not distinguished in detail but subsumed under the generic term "outer quality deficiencies". Management (e.g. crop rotation) and site parameters (e.g. soil characteristics) are unknown and consequently not taken into consideration. Therefore the relative importance of the different quality deficiencies and their influencing factors are unknown.

Drycore with its restricted brown cavities up to several mm deep with a diameter of 3 to 6 millimetres, has early been attributed to *Rhizoctonia solani* (Grütte, 1940; Ramsey 1917; Müller, 1925). Schwinn (1961) presented evidence that *Rhizoctonia solani* is the causal agent of the drycore disease of potato tubers (Appendix, Fig. A4 c-g). Infections with the mycelium of *R. solani* through wounded normal lenticels and lenticels excrescences were successful. A stimulation of drycore through the infection with *R. solani* was therefore postulated for wet soil conditions as this furthers the formation of lenticels excrescences. *Rhizoctonia solani Kühn* (telemorph*: Thanatephorus cucumeris* (Frank) Donk) is a soil borne plant pathogen affecting many agricultural crops worldwide (Ogoshi, 1987; Sneh et al., 1991). On potatoes the disease causes delayed emergence, lesions on stems (stem canker) and stolons and sclerotial formation on tubers (black scurf) (Baker, 1970; Anderson, 1982). Attacks on stolons may induce the development of malformed tubers and tubers with growth cracks (Weinhold et al., 1978; Jeger et al., 1996) (Appendix, Fig. A4). To date, fourteen anastomosis groups (AG 1 to AG 13 and AG BI) have been identified and seven of them (AG 1, AG 2, AG 3, AG 4, AG 6, AG 8, AG 9) are divided into sub-groups that differ in pathology, biochemical or genetic characteristics (Anderson, 1982; Cubeta and Vilgalys, 1997; Gonzalez et al., 2001, Carling et al., 2002). AG 2-1, 3, 4, 5, 8, and 9 are capable of causing moderate to extensive damage to potato plants. AG 3 has been known as the most host-specific type of *R. solani* causing disease of potato. It is by far the most important cause of black scurf because most of the sclerotia isolated from potato tubers belong to AG 3 (Carling and Leiner, 1986; Chand and Logan, 1983; Campion et al., 2003).

Wireworms are the larvae of click beetles (*Elateridae*). About 150 species of click beetles are recorded in middle Europe, but only few of them can be classified as pests. By fare the most important pest species are those belonging to the genus *Agriotes*. Three species, *Agriotes linneatus* (L.), *Agriotes obscurus* (L.) and *Agriotes sputator* (L.) are responsible for the vast majority of attacks in Britain (Parker et al., 2001). In Switzerland, *Agriotes obscurus* (L.) and *Agriotes lineatus* (L.) are the most important species in the north of the Alps, where as *Agriotes littigiosus* and *Agriotes obscurus* are dominant in the south of the Alps (Jossi et al., 1997). In potatoes the new tubers can be severely damaged by narrow tunnels resulting in a lower market value (Appendix, Fig. 5A). Wireworm damage may be confused with slug damage (Appendix, Fig. A6). However, slugs often hallow out large cavities within the tuber flesh, whereas wireworms do not (Gratwick, 1989). There are two main periods of wireworm activity, one in March to May and a second in September to October. The latter period of activity is when most damage to potatoes occurs (Gratwick, 1989). In potatoes wireworms were until recently regarded as generally minor but locally important pest in mixed arable and livestock farming areas. However in the last few years wireworm damage has become an increasing problem including the occurrence of damage in fields in all-arable rotations even without long-term grassland history (Parker, 2001; Demmler, 1999). The reasons for this apparent change in pest status of wireworms are not clear.

Most slug damage in arable crops in Europe is caused by two types of slugs, the grey field slug *Deroceras reticulatum* (Müller) and the round back slug *Arion* ssp. (Hommay, 1995). These two species are also regarded as the most frequent slug species in arable crops in Switzerland. *Arion hortensis* is attracted by underground organs of the plant (e.g. carrots, potatoes, seed grains). *Deroceras reticulatum* (Müller) is the most important slug pest of arable crops in temperate climate (Godan, 1983; Port and Port, 1986; South, 1992). Potato tubers are mainly attacked in a mild and wet climate (Johnston et al, 1994). Slugs often bore small entrance hauls into the tuber to reach the tuber flesh and hallow out large cavities. Pinder (1974) observed that the initiation of slug damage coincided with the completion of tuber growth and suggested that biochemical changes in tubers at this time render them more susceptible to slug damage. On resistant varieties, slugs did not eat the skin, but the tuber flesh where it was exposed (Pinder, 1974). Slugs can be secondary feeders when other organisms such as wireworm or millipedes have already broken through the skin (Cooper et al., 1989).

In Switzerland 87% of the potatoes (Swisspatat, 2006) are produced on conventional farms, 9% and 4% according to the guidelines of integrated or organic production, respectively. There is no difference in the quality standard for the different farming systems.

In a three year on-farm project (2001 to 2003) the relative importance of different quality deficiencies and the influence of farming systems (organic, integrated, conventional), cultivation methods and site parameters were studied on totally 278 potato fields across the major potato growing regions of Switzerland. Data is presented on quality deficiencies caused by wireworm (*Agriotes ssp.*), slugs (*Deroceras reticulatum, Arion ssp.*), drycore and black scurf (*Rhizoctonia solani).*

## MATERIAL AND METHODS

### Description of the experimental sites and experimental design

Over the years 2001-2003 278 potato fields (91 to 94 per year) with a surface of 0.8 ha distributed across the major potato growing regions in Switzerland were included in the survey. The selection and grouping of fields was done according to the following criteria (Tab.3.1): farming system, practised for at least 8 years (organic (O), integrated production (I) and conventional (O)); varieties (Agria, Charlotte, Bintje, Eba), crop rotation (with or without grass clover leys in the 3 years preceding the potato crop); farm manure application to the potato crop (yes or no) and crop protection (seed tuber treatment against *Rhizoctonia solani* Kühn., Insecticide application against wireworms (*Ag*riotes ssp.) and application of molluscicide pellets against slugs (*Deroceras reticulatum*, *Arion ssp*.)).

Soil types were mostly sandy loam (61%) and clay loam (22%). Their pH varied widely between 5.4 and 8.2. Organic matter contents between 1.7 and 6.8% were observed in 80% of the fields, only 5% had more the 10% (Table 3.2). The analytical results on soil nutrient status (P, K and Mg) ranged between “adequate” and “enriched” for about 75% of the fields and was “slightly deficient” for about 24%.

The long term annual mean temperature (1961 to 1990) in the main potato growing area is between 8.0° C and 9.8° C. However, 2002 and especially 2003 belonged to the hottest years ever with mean temperatures 1 to 2.5° C higher than the long term mean. The annual precipitation varied strongly among different years and regions. The annual precipitation was 140% (1150 – 1450 mm) and 125% (1000 – 1300 mm) of the long term annual mean in 2001 and 2002 respectively. In contrast, 2003 was extraordinary dry and hot in all regions with a precipitation of 600 to 750 mm (75% of the long term mean) (Bundesamt für Meteorologie und Klimatologie, Zurich). In 2001 potatoes were planted late in April and in Mai due to a wet spring. In 2002 and 2003 fields were mostly planted before the end of April.

*Varieties*

Four main varieties from the Swiss variety list were selected for the project. Charlotte (maturity: early) and Agria (early to intermediate) are the most important varieties in all three farming systems. Charlotte is the most popular variety for the fresh market, whereas Agria is produced for both the fresh market and the processing industry (French fries). Bintje (early to intermediate) is still one of the most important varieties for the fresh market in the integrated and conventional farming systems. Eba (intermediate to late) is used for French fries production in the processing industry.

*Crop rotation*

Intervals between two potato crops were between 3 and 5 years for 25% of the fields and more than 5 years for the other 75%. In the organic production system the most frequent preceding crops were grass clover leys (43%), followed by cereals (24%) and vegetables (22%). On integrated and conventional fields grass clover leys were much less frequent (23 and 10% respectively), while cereals, maize and sugar beet where most frequent (51 and 46 %).

*Plant protection*

The average number of fungicide treatments against late blight (*Phytophtera infestans*) was 7.6 for conventional and 7.4 for integrated fields. On organic fields the average number of treatments was 3.6 mainly with copper. In the integrated system on 36% of the fields potato seed tubers were treated with a fungicide against *Rhizoctonia solani*, compared to 59% in the conventional system. 75% of all treatments were done at planting, 25% during storage of the seed potatoes. The most frequent product used was Monceren with the active substance Pencycuron. No treatments with synthetic fungicides are allowed in organic agriculture. 30% of the conventional and integrated fields were treated once and around 10% twice with molluscicide pellets against slugs. In the integrated and conventional system an insecticide seed treatment was used at least once in the 5 preceding years to potatoes on 19% and 21% respectively of the fields cropped with cereals, sugar beet or corn. Insecticide treatments of seed potatoes or soil applications are not allowed in Switzerland.

*Fertilization*

Manure was applied on 36% (organic and integrated) and 28% of the fields (conventional). An application of liquid manure was much more frequent in the organic system (34%) than in the integrated and conventional system with 13% and 17% respectively. On 14% (organic), 3% (Integrated) and 5% (conventional) of the fields both liquid manure and manure was applied. Manure was most frequently applied in spring before ploughing. On 48% (integrated) and 50% (conventional) of the fields no manure was applied compared to 16% in the organic system. On organic fields over 90% of the total amount of phosphor, potassium and magnesium per hectare and 70% of nitrogen was applied in the form of manure. In the integrated and conventional farming system about 50% of phosphor, 66% of potassium and magnesium and 80% of nitrogen fertilization were applied with mineral fertilizers. The average amount of N, P, K and Mg in kg/ha in dependence of the farming system is shown in Table 3.3.

Tab. 3.1 Number of potato fields included in the on-farm experimental program grouped according to selection criteria.

| | | | | | |
|---|---|---|---|---|---|
| Fields/Year | 2001: 93 | 2002:94 | 2003:91 | | |
| Fields/Farming system | Organic:58 | Integrated:69 | Conv.:151 | | |
| Varieties | Agria: 109 | Charlotte: 59 | Bintje:47 | Eba:39 | Other:24 |
| Crop rotation with grass clover leys | No: 164 | Yes: 114 | | | |
| Application of manure | No:172 | Yes:106 | | | |
| Only system C and I: | | | | | |
| Fungicide seed treatment | No: 108 | Yes: 112 | | | |
| Insecticide against wireworm in preceding crop | No: 103 | Yes: 117 | | | |
| Application of molluscicide pellets | No: 123 | Yes: 97 | | | |

Tab. 3.2 Soil characteristics of the fields included in the project

| Soil type | % of fields | % clay | % silt | pH | organic matter |
|---|---|---|---|---|---|
| Sandy loam | 61% | 11-16 | 11-31 | 5.4-8.1 | 0.4-20.7 |
| Clay loam | 22% | 21-31 | 31-41 | 5.9-8.2 | 0.8-8 |
| Sandy silt loam | 8% | 6-16 | 41-51 | 5.9-7.9 | 1.3-35.7 |
| Loamy sand | 6% | 6-11 | 11-21 | 6.1-8.0 | 1.3-4.6 |
| Clay | 2% | 41-46 | > 40 | 7.2-7.7 | 2.4-7.7 |
| Sandy clay loam | 1% | 21-26 | 21 | 7.6-8.1 | 1.6-2.2 |

Table 3.3 Average fertilization of N, $P_2O_5$, $K_2O$ and Mg in kg per hectare on project fields in dependence of the farming system.

| System | N | $P_2O_5$ | $K_2O$ | Mg |
|---|---|---|---|---|
| Organic | 90 | 65 | 171 | 17 |
| Integrated | 131 | 71 | 245 | 25 |
| Conventional | 130 | 82 | 270 | 27 |

**Data collection and quality assessment**

Potato growers filled out a questionnaire with all relevant data concerning crop rotation and management for each field included in the project. In 2002 and 2003 one hundred tubers were randomly sampled from the seed tuber lots of each field. Tubers were washed and controlled for black scurf (*Rhizoctonia solani* Kühn*)* according to the guidelines of Swisssem, the organisation of the Swiss seed multiplier companies (SSPV, 2002). Just before harvest samples consisting of 810 tubers were collected on each field. On every fourth ridge of each 0.8 ha field a total of 270 plants was harvested, three tubers of each plant were randomly sampled. This scheme was based on preliminary studies in 2000. All tubers were washed before assessment of quality deficiencies. The number of lesions per tuber caused by wireworm, drycore and slugs were counted. To control the depth of the quality deficiencies all tubers were peeled with a special knife to a depth of 4 mm, the maximum depth for acceptance (Swiss potato commission, 1989). Black scurf on seed tubers and harvested tubers was assessed using grades from 1 (no black scurf) to 7 (75% to 100% of the tuber surface with black scurf).

Soil samples of all 278 fields in the project were analysed according to the reference method of the Swiss federal research stations (2004) for clay, silt and sand content (finger test), soil type, pH ($H_20$), organic matter (analytic), and ammoniumacetat-EDTA extractible P, K and Mg contents.

### Influence of crop rotation and soil cover on slug damage

To assess the influence of crop rotation and soil cover on slug damage of potatoes, all fields were grouped into four risk groups according to the soil cover during previous years (Table 3.4). Each main crop and each catch crop was assigned a specific number of risk points and the sum of risk points was determined for each plot for a period of three years preceding the potatoes. Based on the sum of risk points each plot was assigned to one of the four risk groups with 1= low risk and 4 = high risk for slug damage).

Table 3.4 Risk groups according to the sum of risk points: 1= less than 12 points (low risk), 2= 13-15 points, 3= 1-18 points and 4= more than 18 points (high risk).

| Risk points | Main crop | Catch crops, green manure or second crops |
|---|---|---|
| 0 | | Black fallow |
| 1 | Sugar beet, maize | Autumn sown main crops |
| 2 | Spring cereals | Grass clover mixtures for animal feeding |
| 3 | Winter cereals, oil seed rape, sunflower, grain legumes, tobacco, | Phacelia, mustard, sunflower, turnip |
| 4 | Grass clover leys | Spinach, salad |
| 5 | Vegetables | |

### Statistical Analysis

To determine the influence of various factors on different quality parameters, the project fields were grouped according to management practice (e.g. farming system, crop rotation) and site parameters (e.g. clay content, pH). Measurement results were statistically checked for normal distribution and comparable variability. Depending on the result, averages or medians were compared with the following methods:

- Two - and higher way analyses of variance. In certain cases arcsin $\sqrt{p}$ transformation was done before analyses of variance. From the full model all interactions with a probability level $> 0.15$ were removed stepwise and residuals of the final model were checked for normal distribution. Main factors from the final model were removed to test stability of the result.
- t-test for two independent samples of normally distributed but heterogeneous data.
- Wilcoxon rank-sum test for not normally distributed data.

In order to have a global $\alpha$-level of 0.05 for the family of the tests performed, the tukey-Kramer Test respectively the Bonferroni-Holm procedure was applied (Holm, 1979). All statistical analysis was done with NCSS 2004 (Number Cruncher Statistical Software, Kaysville, Utah 84037 Utah, USA).

*Relative risk for drycore*

To check the within field distribution of drycore and black scurf and the relations between these deficiencies, the relative risk for drycore damage on a tuber was calculated for the influencing factor black scurf for 40 fields (totally 32'000 tubers) with an important drycore level of more than 5% damaged tubers. The relative risk is the ratio of the event rate between comparative groups and is calculated with the following equation: relative risk = ((a/a+b)/(c/c+d)), where a = tubers with drycore and influencing factor present, b = tubers without drycore, but influencing factor present, c = tubers with drycore but influencing factor not present, d = tubers without drycore und no influencing factor. A relative risk > 1 means drycore symptoms are more likely to occur if black scurf is present on the same tuber.

# RESULTS

## Quality deficiencies caused by wireworms, drycore and slugs

Quality deficiencies caused by wireworms, slugs and drycore belonged to the most important quality problems in all three years and their occurrence was highest in the organic farming system (Fig 3.1). For all other quality deficiencies assessed in this project (e.g. powdery scab, *Spongospora subterranea*) no influence of the farming system was observed (data not shown). While 36% of the organic fields did not meet the standard due to wireworm, drycore and slug damage it was only 13% and 12% for integrated and conventional fields respectively. For a further 20% (organic), 12% (integrated) and 11% (conventional) of the fields the price was reduced, if the proportion of damaged tubers was not below 10% after sorting out.

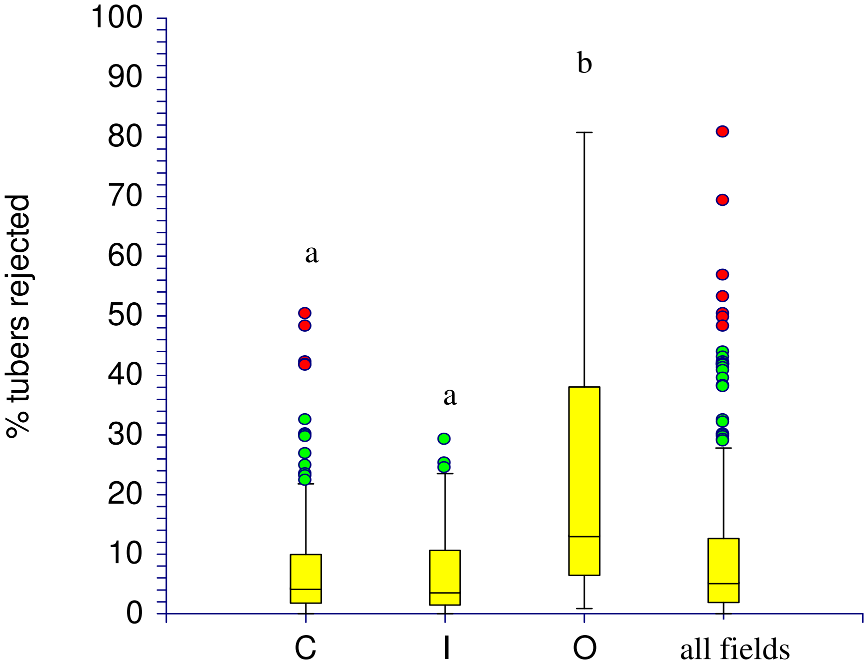


Fig. 3.1 Percentage of tubers rejected during 2001-2003 according to the Swiss quality standard due to wireworm, drycore or slug damage. C = conventional, I = integrated, O = organic. N: all fields = 278, C = 151, I=69, O=58. The box plot shows the 50% inter quartile range of the values (50% of the fields are in the box) as well as the median (middle line in the box). The points represent the outliers, red = extreme outliers. In order to have a global α-level of 0.05 the Bonferroni-Holm procedure was applied (Holm, 1979). Results with different denominator are statistically different.

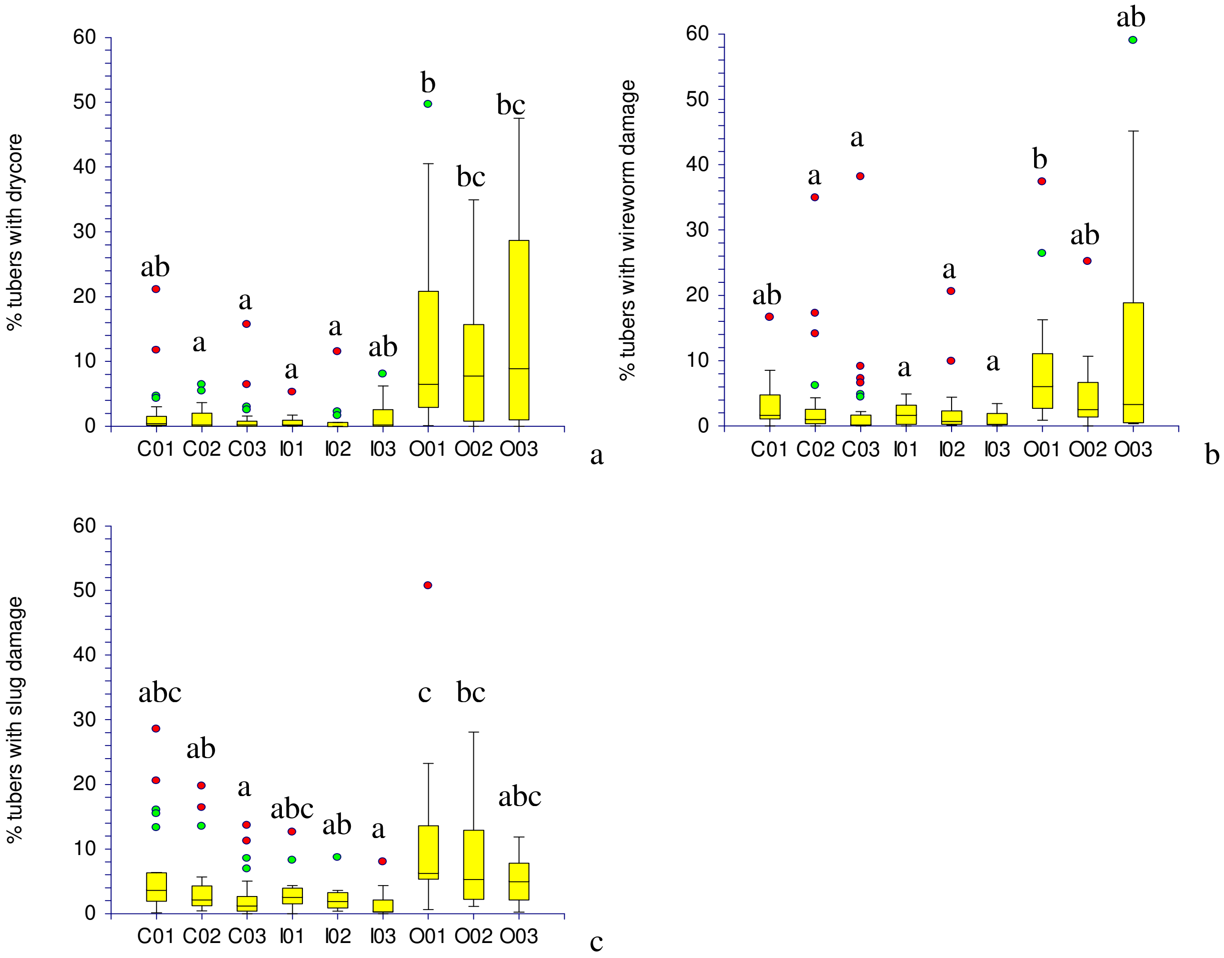


Fig.3.2 Percentage of tubers with drycore (a), wireworm (b) and slug damage (c) in dependence of the year and the farming system for the varieties Charlotte and Agria. C= conventional, I= integrated, O= organic, 01=2001, 02=2002, 03=2003. The box plot shows the 50% inter quartile range of the values (50% of the fields are in the box) as well as the median (middle line in the box). The points represent the outliers, red = extreme outliers. In order to have a global α-level of 0.05 the Bonferroni-Holm procedure was applied (Holm 1979). Results with different denominator are statistically different.

**Rhizoctonia solani (black scurf, drycore and malformed tubers)**

*Seed quality*

In 2002 and 2003 29% (integrated and conventional) and 40% (organic) of the seed lots exceeded the quality standard for certified seed of 20% tubers with black scurf (SSPV, 2002). For such lots a seed treatment with a fungicide is recommended. In the integrated and conventional fields a seed treatment was applied by just in 51% the cases with an exceeded threshold. However, 56% of the seed lots were treated even when the threshold was not exceeded (Appendix Fig. A1).

*Quality of harvested tubers*

The most frequently observed infestation level for black scurf of individual tubers was score 2 (0.1-5% of tuber surface with black scurf) followed by score 3 (5.1-10%). Black scurf on more than 10% of the tuber surface was only observed on 0.4% (C 2003) to 5.6% (O 2002) of the tubers. Black scurf attack was highest in the organic system, but the difference to the other farming systems was not significant. Fifty percent of the organic fields showed black scurf levels of harvested tubers of more than 20% infested tubers, compared to 29% in conventional and integrated fields without seed treatment and 11% with fungicide treatment. Analysis of variance for black scurf with the data of integrated and conventional fields showed a significant influence of seed quality (% seed tubers with black scurf) and the year. The influence of a fungicide seed treatment was also significant at a higher seed infestation level (more than 20% tubers with black scurf). No significant influence was found for the factors variety, farming system, manure application and preceding crop (Tab.3.5). There was no clear relationship between black scurf infestation of seed tubers and black scurf level of harvested tubers (r = 0.43). However, infestation levels of more than 20% of harvested tubers were rarely observed, if less than 10% of the seed tubers were infested.

In all three years the occurrence of drycore was significantly more frequent and higher on organic fields than for the other farming systems (Fig. 3.2a). On 29% of the organic fields drycore damage alone led to price reduction (>10% of damaged tubers when quality assessed on washed potatoes), compared to 3% in the integrated and conventional farming system. Seventeen percent of the organic plots did not reach the official quality standard because of drycore damage alone (>18% tubers with drycore for quality assessment on washed tubers). Thus drycore was the most important outer quality deficiency in the organic farming system. In the excessively dry year 2003 drycore damage was at a similar level as in 2001 and 2002. Important damage was found on all soil types independently from clay and organic matter content. In the organic farming system drycore damage was significantly highest (p=0.05) when more than 20% of seed tubers were infested with black scurf and with grass-clover leys in the two preceding years to potatoes. When clean seed was planted on fields without grass clover in the two preceding years important drycore damage never occurred (Fig. 3.3). The correlation between black scurf level on harvested tubers and the appearance of drycore was just r=0.38. Nevertheless calculations of the risk level for 40 fields with important drycore damage showed that the occurrence of drycore was significantly increased on tubers with black scurf (risk level = 2.23 +/- 0.23). Fields with heavy seed infection and *Rhizoctonia* symptoms on plants could be absolutely free of drycore, but drycore symptoms on tubers were never observed on plants without any symptoms of *Rhizoctonia solani*.

Important levels of malformed tubers caused by *Rhizoctonia solani* were very rarely observed, even on fields with a high level of seed tuber infestation (data not shown).

Table 3.5 Analysis of variance of black scurf on tubers for integrated and conventional fields. Year (2002, 2003), farming system (integrated, conventional), variety (Agria, Charlotte, Bintje), seed treatment (yes/no), manure (yes/no), preceding crop (grass-clover ley, cereals, other).

| Source of variation | DF | SQ | MQ | F-Ratio | Probability level |
|---|---|---|---|---|---|
| Year | 1 | 0.4497 | 0.4497 | 12.24 | 0.0007 |
| System | 1 | 3.6036E-02 | 3.6036E-02 | 0.98 | 0.3247 |
| Variety | 2 | 0.1520 | 7.6002E-02 | 2.07 | 0.1325 |
| Seed quality | 2 | 0.3666 | 0.1833 | 4.99 | 0.0088 |
| Seed treatment | 1 | 4.7139E-03 | 4.7139E-03 | 0.13 | 0.7211 |
| Seed quality * Seed treatment | 2 | 0.2731 | 0.1365 | 3.72 | 0.0282 |
| System * variety | 2 | 0.4190 | 0.2095 | 5.70 | 0.0046 |
| Seed treatment * Variety | 2 | 0.1443 | 7.2136E-02 | 1.96 | 0.1464 |
| Manure | 1 | 1.4551E-02 | 1.4551E-02 | 0.40 | 0.5307 |
| Preceding crop | 2 | 0.1492 | 7.4586E-02 | 2.03 | 0.1374 |
| S | 89 | 3.2707 | 3.6749E-02 | | |
| Total adjusted | 105 | 5.5433 | | | |
| Total | 106 | | | | |

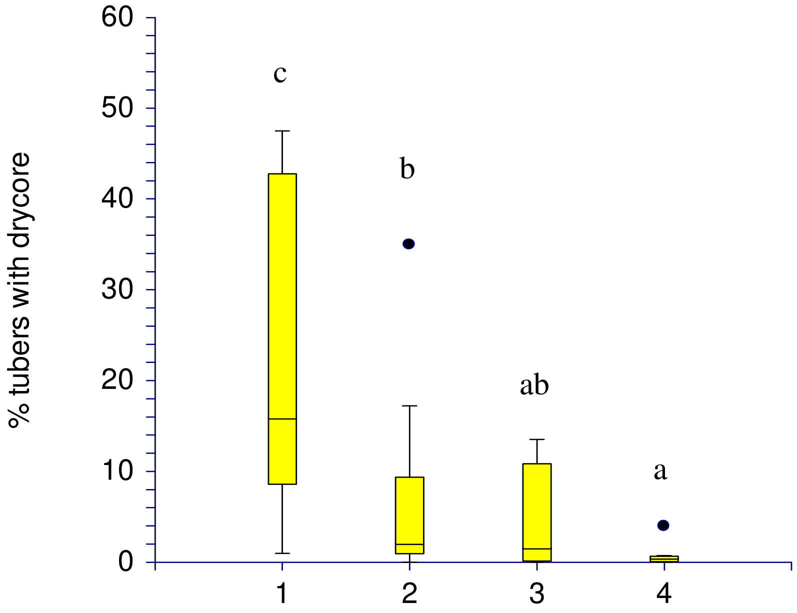


Fig. 3.3 Percent of tubers per field with drycore in the organic farming system in dependence of black scurf infestation on seed tubers and preceding crop. 1 = more than 20% of seed tubers with black scurf, grass-clover ley in the two preceding years, 2= less than 20% seed tubers with black scurf, grass-clover ley in the two preceding years, 3 = more than 20% of seed tubers with black scurf, no grass-clover in the two preceding years,4 = less than 20% of seed tubers infested with black scurf, no grass-clover in the two preceding years. The box plot shows the 50% inter quartile range of the values as well as the median (middle line in the box). The points represent the outliers. N = 11 (1), 14 (2), 4 (3) and 9 (4). In order to have a global α-level of 0.05 the Bonferroni-Holm procedure was applied (Holm 1979). Results of groups with different denominator are statistically different.

### *Agriotes ssp. (wireworm)*

The fact that wireworm damage alone led to price reductions for twelve percent of the 278 fields (>10% of damaged tubers on washed potatoes) underlines the importance of this pest. Soil characteristics, crop management and crop rotation could not sufficiently explain the wireworm damage of all 278 fields. No higher way analysis of variance could be performed because the data was heterogeneous and not normally distributed even after transformation. In the organic farming system the proportion of fields with important wireworm damage was higher than in the other farming systems, but only in 2001 the difference between the integrated and organic fields was significant. In all three farming systems wireworm damage was slightly higher in 2001 than in 2002 and 2003 (Fig. 3.2b). The risk of wireworm damage was clearly decreased with increasing length of time between grass clover leys and potatoes (Fig.3.4) and with decreasing proportion of grass clover leys in the crop rotation. In the first year after grass clover leys about 50% of all fields had important wireworm damage. Potatoes in the fourth and fifth year after grass clover leys showed a significantly lower occurrence of wireworm damage than in the first two years after grass clover leys. Nevertheless, in some cases important wireworm damage was also observed four or more years after grass clover leys or even in crop rotation without grass clover leys. However, no or only very low wireworm damage in spite of grass clover leys as preceding crop occurred as well. Potatoes grown after winter wheat with insecticide seed treatment had a significantly reduced occurrence of wireworm damage. Nevertheless, the effect was dependant of the position in the crop rotation. If the insecticide seed treatment was for wheat following grass-clover leys its effect was sometimes not sufficient.

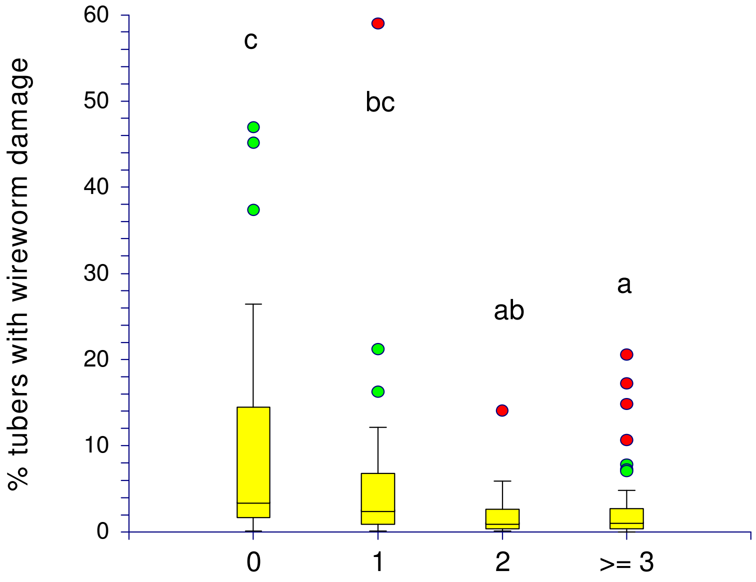


Fig. 3.4 Percent of tubers per field with wireworm damage in dependence of the crop rotation on fields without insecticide treatments. Crop rotation groups: 0= potatoes grown after grass clover leys. 1= one year break between grass clover leys and potatoes, 2= 2 years break, 3= 3 or more years break. The box plot shows the 50% inter quartile range of the values as well as the median (middle line in the box). The points represent the outliers, red = extreme outliers. N= 39 (0), 26 (1), 12 (2) and 74 (3). In order to have a global α-level of 0.05 the Bonferroni-Holm procedure was applied (Holm 1979). Results of rotation groups with different denominator are statistically different.

***Slugs***

Slug damage alone led to price reductions for fourteen percent of the 278 fields (>10% of damaged tubers in washed potatoes). Small entrance holes and more or less deep cavities were typical for slug damage because slugs apparently avoided the tuber skin. Often, the slug holes were already overgrown by a corky layer at harvest. Important slug damage was observed on all soil types independently from their clay and organic matter content, even on sandy loams with less than 15% clay content. No difference between the different varieties was observed in the occurrence of slug damage. For the organic system the proportion of fields with serious slug damage was non-significantly higher than in the other farming systems (Fig.3.2c). The most serious slug damage was observed in 2001 after a mild winter and a wet spring whereas in the exceptionally dry and hot year 2003 less damage occurred. Because of unequal variance of the data, each year had to be analysed separately. In 2001 slug damage was significantly influenced by the crop rotation (Table 3.6). With increasing soil cover (grass clover ley, catch crops) and occurrence of crops favourable for slugs (e.g. oilseed rape, sunflower, vegetables) in the three years preceding potatoes slug damage increased (Fig. 3.5). The clearly higher slug damage on fields with regular vegetable production (spinach, salad, carrots) was especially remarkable. If the fields were used for grass clover leys or vegetable in the three years preceding potatoes, slug damage was significantly higher. If potatoes were preceded by cereals, important slug damage was only observed if a catch crop was grown after cereals. On fields with crop rotation with higher slug risk, slug damage was reduced in the year 2001 when molluscicide pellets were used (Table 3.7). No influence of crop rotation and molluscicide treatment was observed in 2002 und 2003.

To determine the slug species causing the damage, traps baited with chicken layers’ mash in upturned flower pots saucers were placed on 15 fields with important slug damage (Kottmann, 2005). Over 95% of the caught slugs belonged to the species *Deroceras reticulatum*, while *Arion ssp.* was found only relatively infrequently.

Table 3.6 Analysis of variance of slug damage on potatoes for fields without molluscicide treatment in 2001. Farming systems (organic, integrated and conventional), crop rotation (risk groups 1-4: 1= low risk, low percentage of crops favourable for slugs, 2 and 3= increasing risk because of higher percentage of crops favourable for slugs, 4= highest risk, high percentage of grass clover leys and vegetables.).

| Source of variation | DF | SQ | MQ | F-Ratio | Probability level |
|---|---|---|---|---|---|
| Farming system | 2 | 5.7544E-03 | 2.8772E-03 | 0.25 | 0.7762 |
| Crop rotation | 3 | 0.3409 | 0.1136 | 10.06 | <0.0001 |
| S | 46 | 0.5198 | 1.1300E-02 | | |
| Total (adjusted) | 51 | | | | |
| Total | 52 | | | | |

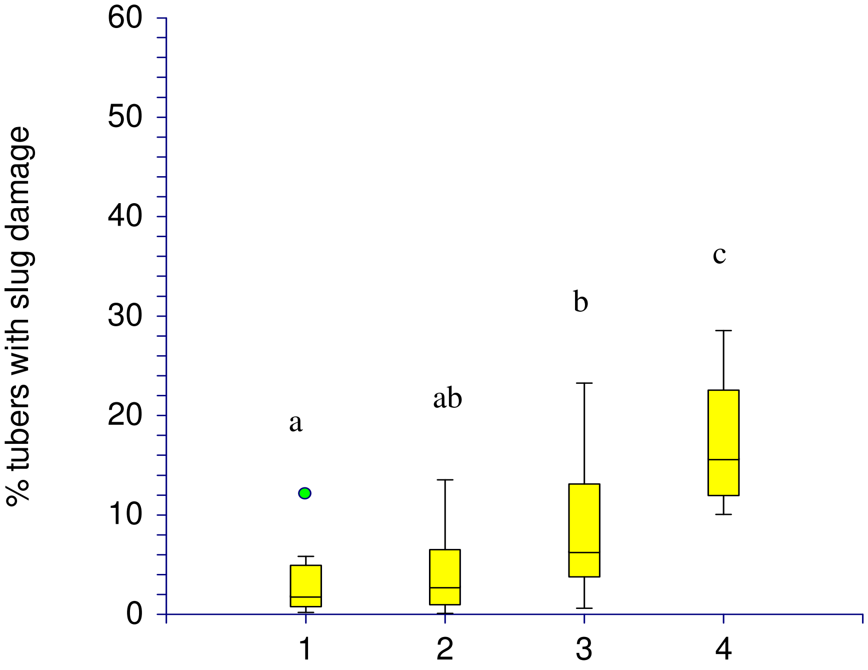


Fig. 3.5 Percent of tubers per field with slug damage in 2001in dependence of the crop rotation and soil cover in the three years preceding potatoes for fields without molluscicide treatments. Risk group: 1= low risk, low percentage of crops favourable for slugs, 2 and 3= increasing risk because of higher percentage of crops favourable for slugs, 4= highest risk, high percentage of grass clover leys and vegetables. The box plot shows the 50% inter quartile range of the values as well as the median (middle line in the box). The points represent the outliers. N= 13 (1), 13 (2), 20 (3) and 6 (4). In order to have a global α-level of 0.05 the Bonferroni-Holm procedure was applied (Holm 1979). Results of risk groups with different denominator are statistically different..

Table 3.7 Analysis of variance of slug damage on potatoes for conventional and integrated farming systems in 2001. Influencing factors, crop rotation (grass clover ley and/ or vegetables in the three years preceding potatoes), molluscicide treatment.

| Source of variation | DF | SQ | MQ | F-Ratio | Probability level |
|---|---|---|---|---|---|
| Crop rotation | 1 | 6.8895E-02 | 6.8895E-02 | 7.52 | 0.0080 |
| Molluscicide | 1 | 6.9286E-02 | 6.9286E-02 | 7.56 | 0.0078 |
| Crop rotation * Molluscicide | 1 | 0.1727 | 0.1727 | 18.85 | <0.0001 |
| S | 62 | 0.5679 | 9.1602E-03 | | |
| Total (adjusted) | 65 | 0.9161 | | | |
| Total | 66 | | | | |

## DISCUSSION

### *Rhizoctonia solani* (black scurf, drycore and malformed tubers)

The threshold for black scurf on seed potatoes is 20% infested tubers (Häni et al., 1976). This limit also corresponds to the quality standard for certified seed in Switzerland (SSPV, 2002). The high percentage of seed lots in our survey which exceeded the threshold (Appendix, Fig. A1) indicates that with regard to black scurf seed quality was often insufficient. Further more the fact that only 51% of the seed lots which exceeded the threshold were treated and 56% of the seed lots were treated even when the threshold was not exceeded, indicates that fungicide treatments applied by the farmers were not well focused. The results of our survey showed that seed quality and seed treatments are the main factors influenceable by farmer that have a potential to reduce black scurf. No significant influence was found for the factors variety, manure application and preceding crop (Table 3.5). Buhr (1989) also found no difference in susceptibility between varieties. Grasses or cereals are often mentioned as good preceding crops and caution is recommended against sugar beet and solanaceous crops (Evans and Howard, 1994; Powelson et al., 1993), which is in contrast to the results of our work. In contrast to these observations our results suggest no influence of the preceding crop on black scurf. Dijst et al. (1989) showed that black scurf on harvested tubers is strongly influenced by meteorological conditions after planting, seed tuber conditioning or time between haulm destruction and harvest. This might explain the rather low relationship between black scurf infestation on seed tubers and black scurf level on harvested tubers (r=0.43) in our survey. The fact that seed quality had a significant influence on black scurf infestation of harvested tubers (Table 3.5) indicates that soil infestation with *R. solani* was of minor importance, probably due to sufficiently long intervals between two potato crops. For more than 75% of the fields included in the survey intervals between potatoes were more than five years and at least three years for the remaining 25%. Carling et al. (1986) in Alaska could not recover isolates of AG 3 from soil free of potato for five years and more.

In contradiction to the generally accepted opinion that drycore symptoms are caused by *R. solani* if lenticels tend to form excrescences under wet conditions, especially in soils with higher clay content (Schwinn, 1961), drycore damage was on the same level in all three years of our survey (Fig.3.1a) and heavy drycore infestation was found independently from soil texture and organic matter content. Occurrence of drycore was not reduced in the exceptionally dry and hot year 2003 (600 to 750 mm annual precipitation in all regions, compared to 1150 – 1450 mm and 1000 – 1300 mm in 2001 and 2002 respectively (Swiss Meteorological Institute Zurich)). Although our results showed that infections of *Rhizoctonia solani* on seed tubers or harvested tubers increase the risk for drycore, potatoes lots with heavy black scurf infestation could be absolutely free of drycore. Thus the infection of a plant with *Rhizoctonia solani* seems to be a prerequisite but not the only condition for the formation of drycore symptoms on tubers. Consistent with the observation of Grütte (1940) that occurrence of drycore is higher after ploughing of grassland, we found drycore damage to be highest in the organic farming system after grass clover leys in the two years preceding potatoes and when infested seed was planted (Fig.3.3) Grass-clover leys as preceding crop seem to favour the formation of drycore. This could explain the higher occurrence of drycore in the organic farming system where grass clover leys as preceding crop have a higher importance than in the other farming systems.

Seemingly all varieties included in our survey were sufficiently resistant to malformations caused by *R. solani*. Stem canker and stolon damage caused by early infections by tuber borne inoculum may lead to tuber malformations (Adam et al., 1980; Frank and Leach, 1980). Buhr (1989) showed, that tuber deformation is the only symptom of *R. solani* which varies significantly and reproducible between cultivars. This is consistant with the results of field trials with heavy infested seed tubers (all seed tubers with black scurf) in 2005 and 2006 where Agria and Charlotte had significantly less tubers with malformations (<10%) as compared to the variety Innovator with over 30% (Keiser et al., unpublished).

### Wireworm

Of all parameters assessed in this survey only crop rotation showed an association with wireworm damage. This is consistent with results of Parker and Seeney (1997) which collected data on site specific variables including soil physical characteristics, grass duration, grass genera diversity and abiotic factors on 62 fields over 3 years in England and Wales. Parker et al. (1996) found important wireworm damage even in fields where very few wireworms were detected; on the other hand low wireworm damage was recorded on fields with a high wireworm infestation level. The wireworm damage level must therefore be influenced by other factors like e.g. dispersal of click beetle or influence of soil moisture content, which were not assessed in our survey. Wireworm damage in a field appears to be patchy (Salt and Hollick, 1946), suggesting that the distribution of wireworm and possibly adult click beetles, in the field is also patchy. Salt and Hollick (1946) found that high larval densities were consistently located in the same areas in the course of more than a year, and linked this to soil moisture differences across the field.

Although the risk for wireworm damage was clearly reduced with increasing number of years between grass clover leys and potatoes (Fig.3.4), important damage could also be observed four or more years after grass clover leys. These results are consistent with the observations of Parker et al. (2001) and Demmler (1999) that important wireworm damage can occur in fields in all-arable situation even without long-term grassland history. The reasons for this are not clear but more sustainable production systems like catch crop and reduced soil tillage may have been favourable for the development of wireworm populations in all-arable crop rotations. Furthermore set-asides (one to several years of fallow) seemingly favour wireworms (Hancock et al., 1992; Parker, 1999). The reduced occurrence of wireworm damage in potatoes grown after winter wheat with insecticide seed treatment observed in our survey suggests that the apparent increase of quality problems might also influenced by the use of persistent insecticides which strongly decrease in the past years due to environmental reasons (Ester et al., 2004). Strickland et al. (1962) suggested that the decline in wireworm damage in the period of 1944-54 could be at least in part be explained by an increasing use of persistent soil insecticides.

Nevertheless, the presented results show that the risk for wireworm damage can be kept low if the proportion of grass clover leys in the rotation of a field is not to high and if potatoes are not planted in the first two or even better three years after grass-clover leys (Fig. 3.4). The higher percentage of organic fields with important wireworm damage can partly be due to the higher proportion of grass clover leys and the lack of insecticide treatments in this farming system. To better assess the risk of wireworm damage more information about click beetle activity and dispersal is needed. Trapping of adult click beetle with species specific sex pheromones (Ester et al., 2004) might help to understand which conditions favour the oviposition and the development of wireworm populations. Adult Elateridae do not migrate and any movement is likely to be due to local dispersal only, probably largely by walking (Parker et al., 2001). Measuring beetle activity in a given locality should therefore indicate the presence of an established local population, thus contributing to an assessment of the overall risk of wireworm damage (Parker et al., 2001).

**Slugs**

Slug damage was more frequent than expected in all farming systems. Probably slug holes were often confused with wireworm damage in quality assessments by the trade, because potato quality is mostly assessed on unwashed tubers by the trade. Slugs often cause only small entrance holes and hallow out large cavities within the tuber flesh, whereas wireworm do not (Gratwick, 1889). Johnston et al. (1994) showed that slugs avoided feeding on the skin if other tissue was available. Whereas slugs are an important pest for potatoes in Britain (Hommay, 1995), they have so far been considered a minor problem on the continent. Results of slug traps suggest that the grey slug *Deroceras reticulatum* (Müller) was the main cause for slug damage in our survey (Kottmann, 2005). This species is regarded as the most important slug pest of arable crops in temperate climate (Godan, 1983; Port and Port, 1986; South, 1992). In contrast to drycore and wireworm, slug damage was highest after a mild winter and wet spring in 2001. Johnston et al. (1994) also reported that potato tubers are mainly attacked in a mild and wet climate. Nevertheless, important slug damage was found in all years especially in the organic farming system and in crop rotations with a higher percentage of soil cover over winter (catch crops, grass clover leys) and rotations with frequent vegetable production. This is consistent with Glen (2002) who found that the risk of slug damage in arable crops is higher with a high proportion of intercrops or green manure. Also Taupin et al. (1997) reported that the sowing of a catch crop increased the slug population in the main crop in springtime.

The higher slug damage in rotations with grass clover leys (Fig.3.5, Tab. 3.6) might be due to their high proportion of red or white clover in Switzerland. Henderson (1992) showed that plants with higher sugar content stimulate the consumption of *D. reticulatum*. Red clover was consumed in significantly greater quantities than any other legumes (Brooks et al., 2003). In contrast, no slug problems were observed in crop rotations with grass seed production in the two years preceding potatoes in Netherland (A Ester, AK Lelystad, The Netherlands, pers. com., 2005). In crop rotations with a higher risk for slug damage the use of mollucicide pellets can reduce slug damage (Table 3.7). In Britain at least two full rate or four half rate applications of slug pellets with a first application just before canopy closure is recommended (Evans, 2006). In comparison, in our survey slug pellets were applied once on 30% of the integrated and conventional fields and twice on 10% of the fields. The farmers applied the pellets preventively because no reliable and practicable system of forecasting slug damage exists. Insufficient effect of slug pellets in some cases could be due to a late application only few weeks before harvest.

In contrast to Rayner (1975) and Glen (2002) who found that slugs cause damage mainly on heavier soils with higher clay content, in our survey important damage was also found on soils with less than 15% of clay.

The influence of farming system and crop management (crop rotation, seed quality and pesticide treatments) on the quality deficiencies caused by wireworm, slugs and drycore was higher than the influence of site parameters. The higher occurrence of insufficient quality in the organic farming system was explained by the higher importance of grass clover leys in the crop rotation, the higher seed infestation with black scurf and the lack of effective treatments against *Rhizoctonia solani*, slug and wireworm. The high occurrence of drycore cannot be explained by infections of *R. solani* alone. As all outer quality deficiencies were increased by grass clover leys drycore damage might be favoured on wounded tubers caused by wireworm or other means. In greenhouse experiments Schwinn (1961) presented evidence that infection with the mycelium of *R. solani* through wounded lenticels caused drycore. An optimized crop rotation appears to be the main measure to reduce the economic risk of outer quality deficiencies, as there are no reliable and practicable systems of forecasting wireworm and slug damage. On typical mixed farms in Switzerland the possibility to reduce grass clover in the crop rotation is often limited because milk production is based on grass clover. Common crop rotations of several farms with special crop rotation for the potatoes are a possible solution.

## CONCLUSIONS

Quality deficiencies caused by wireworm, slugs and drycore are responsible for important economic losses in all farming systems in Switzerland. In our survey the influence of farming system and crop management (crop rotation, seed quality and pesticide treatments) was higher than of site parameters. In the organic system the quality damage was higher for all three deficiencies. For drycore this difference was significant in all three years. The relative importance of slugs as a potato pest was higher than expected. Grass clover leys in the years preceding potatoes increased the risk for quality deficiencies caused by wireworm, slugs and drycore. This could partly explain the higher occurrence of outer quality deficiencies in the organic farming system where potatoes are often grown after two or three years of grass clover leys. The risk for slug damage was increased in crop rotations with frequent catch crops and a high percentage of crops favourable for slugs (e.g. vegetables, oilseed rape and sunflowers). The use of high seed quality with low infestation level of black scurf reduces the risk for black surf and drycore on harvested tubers. Insufficient seed quality and the lack of fungicide seed treatment explain the higher occurrence of *Rhizoctonia* infections on organic fields. Seed quality has to be improved with regard to black scurf and farmers should assess their seed quality to decide if a fungicide treatment is advised. A Fungicide seed treatment is an effective measure for integrated and conventional farms to control seed infestations.

Future research should develop reliable and practicable methods to assess the risk for slug and wireworm damage in potato crops. In future experiments the role of wounds on tubers caused by wireworm or other means in the formation of drycore has to be investigated.

# 4. Drycore results from an interaction between *Rhizoctonia solani* and wireworm (Agriotes ssp.)

## ABSTRACT

Drycore is an important quality deficiency especially in organic potato production and after grass clover leys. The drycore symptom is attributed to *Rhizoctonia solani Kühn* (telemorph*: Thanatephorus cucumeris* (Frank) Donk). In the framework of a three year survey (2001-2003) data concerning potato quality, crop rotation, management and site parameters was analyzed from 185 potato fields. The hypothesis was tested that injuries on potatoes caused by wireworms facilitate the penetration of *Rhizoctonia solani* and favour the formation of drycore. Analysis of variance for drycore showed a significant influence of wireworm damage, seed quality and grass clover leys in the crop rotation on the infestation level of drycore. On fields which had both a low occurrence of black scurf on the seed tubers and a low occurrence of wireworm damage at harvest, significant drycore damage was never observed. The relative risk for drycore damage on tubers was significantly higher if black scurf or wireworm damage was on the same tuber. In contrast no higher risk for drycore was observed on tubers with slug damage. Abiotic factors like farm manure application, organic matter content, texture and pH of the soil also had no significant influence on the level of drycore. Thus the wounding of potatoes by wireworm could be confirmed as the major variable for drycore. The mode of action has to be clarified under controlled conditions.

## INTRODUCTION

Drycore is one of the main reasons that potato lots do not meet the demanded quality standard in organic potato production but is also the cause for important losses in integrated and conventional farming system (chapter 3) (Appendix, Fig.A4 c-g). The restricted brown cavities up to several millimetre deep with a diameter of 3 to 6 mm are attributed to *Rhizoctonia solani Kühn* (telemorph*: Thanatephorus cucumeris* (Frank) Donk) (Schwinn, 1961; Grütte, 1940; Ramsey 1917; Müller 1925). *R. solani* is a soil borne plant pathogen affecting many agricultural crops worldwide (Ogoshi, 1987; Sneh et al., 1991). On potatoes the disease causes delayed emergence, lesions on stems (stem canker) and stolons and sclerotial formation on tubers (black scurf) (Baker, 1970; Anderson, 1982). Attacks on stolons may induce the development of misshapen tubers and tubers with growth cracks (Weinhold et al., 1978; Jeger et al., 1996) (Appendix, Fig.A4 a-c, h). In a survey encompassing different farming systems in Switzerland organic fields showed considerably more serious drycore damage than integrated fields. Rainfall and soil type had no impact on this damage (Chapter 3). This contradicts the postulate of Schwinn (1961) that the formation of drycore symptoms is favoured under wet soil conditions. Although some relationship between drycore damage and black scurf existed, tubers from plants with heavy seed infections and *Rhizoctonia* symptoms on the shoots could be absolutely free of drycore. However drycore symptoms on tubers were never observed on plants without any symptoms of *Rhizoctonia solani*. Thus no strong correlation was observed between black scurf level on harvested tubers and the appearance of drycore on the same tuber (chapter 3). Therefore the infection of a plant with *Rhizoctonia solani* cannot be the only factor necessary for the formation of drycore. Intriguingly, wireworms and their damages seemed to be directly or indirectly linked to drycore occurrence (Chapter 3). Wireworms are the larvae of click beetles (*Elateridae*). By fare the most important pest species are those belonging to the genus *Agriotes*. Three species, *Agriotes linneatus* (L.), *Agriotes obscurus* (L.) and *Agriotes sputator* (L.) are responsible for the vast majority of wireworm attacks in Britain (Parker et al. 2001). In Switzerland *Agriotes obscurus* (L.) and *Agriotes Lineatus* (L.) are the most important species in the north of the Alps, where as *Agriotes littigiosus* and *Agriotes obscurus* are dominant in the south of the Alps (Jossi et al., 1997).

Injuries caused by wireworm (Appendix, Fig.A5) or other mean may facilitate the penetration of *R. solani* into the tuber and favour the formation of drycore symptoms. Data on crop rotation, management and site parameters of potato fields from a large survey was analysed to check this hypothesis.

## MATERIAL AND METHODS

### Description of the experimental sites and experimental design

In a three year survey (2001 to 2003) across the major potato growing regions of Switzerland 278 potato fields from different farming systems were evaluated (58 organic, 69 integrated and 151 conventional). In the years 2002 and 2003 for which results are presented here 185 plots were included in the survey (40 organic, 42 integrated and 103 conventional). All relevant data concerning crop rotation, management and site parameters was recorded and around twenty outer quality deficiencies on the harvested tubers were assessed. Soil types were mostly sandy loam (61%) and clay loam (22%). Soil pH varied widely between 5.4 and 8.2. Organic matter contents were between 1.7 and 6.8% in 80% of the fields; only 5% had more the 10%. The soil nutrient status ranged between "adequate" and "enriched" for about 75% of the P, K and Mg analytical results, and was "slightly deficient" in about 24% of the cases.

The long term annual mean temperature (1961 and 1990) in the main potato growing areas of Switzerland was between 8.0° C and 9.8° C. 2002 and especially 2003 belong to the hottest years ever with mean temperatures 1 to 2.5° C higher than the long term mean. The annual precipitation varied strongly among different years and regions. The annual precipitation was 140% (1150 – 1450 mm) and 125% (1000 – 1300 mm) of the long term annual mean in 2001 and 2002 respectively. In contrast, 2003 was extraordinary dry and hot in all regions with 600 to 750 mm (75% of the long term mean) (Bundesamt für Meteorologie und Klimatologie, Annalen 2006). In 2001 potatoes were planted late in April and in Mai due to a wet spring. In 2002 and 2003 all fields were planted before the end of April.

Intervals between two potato crops were at least 3 years for 25% of the fields and 5 or more years for the other 75%. In the organic farming system the most frequent preceding crops was grass clover ley (43%), followed by cereals (24%) and vegetables (22%). On integrated and conventional fields grass clover leys were much less frequent (23 and 10% respectively), most frequent were cereals (51 and 46 %) followed by maize and sugar beet.

The average number of fungicide treatments against late blight (*Phytophtera infestans*) was 7.6 for conventional and 7.4 for integrated fields. On organic fields the average number of treatments was 3.6, mainly with copper. In the integrated system potato seed tubers were treated with a fungicide against *Rhizoctonia solani* on 36% of the fields, compared to 59% in the conventional system. 75% of all treatments were done at planting, 25% during storage of the seed potatoes. The most frequent product used was Monceren with the active substance Pencycuron. No treatments with synthetic fungicides are allowed in organic agriculture. 30% of the conventional and integrated fields were treated once and around 10% twice with molluscicide pellets against slugs. In the integrated and conventional system an insecticide seed treatment was used at least once in the 5 preceding years to potatoes on 19% and 21% respectively of the fields cropped with cereals, sugar beet or corn. Insecticide treatments of seed potatoes or soil applications are not allowed in Switzerland.

Manure was applied on 36% (organic and integrated) and 28% of the fields (conventional). An application of liquid manure was much more frequent in the organic system (34%) than in the integrated and conventional system with 13% and 17% respectively. On 14% (organic), 3% (Integrated) and 5% (conventional) of the fields both solid and liquid manure was applied. Manure was most frequently applied in spring before ploughing. On 48% (integrated) and 50% (conventional) of the fields no manure was applied compared to 16% in the organic system. On organic fields over 90% of the total amount of phosphorus, potassium and magnesium per hectare and 70% of nitrogen was applied with manure. In the integrated and conventional farming system about 50% of phosphorus, 66% of potassium and magnesium and 80% of nitrogen fertilization were applied with mineral fertilizers.

### Data collection and quality assessment

Potato growers filled out a questionnaire with all relevant data concerning crop rotation and management for all fields included in the project. In 2002 and 2003 one hundred tubers were randomly sampled from the seed tuber lots of each field. Tubers were washed and controlled for black scurf (*Rhizoctonia solani* Kühn) according to the guidelines of the organisation of the Swiss seed multipliers (Swisssem) (SSPV, 2002). Just before harvest samples consisting of 810 tubers were collected on each field. On every fourth ridge of each 0.8 ha field a total of 270 plants was harvested. Three tubers in the demanded size were randomly sampled from each plant. This scheme was based on preliminary studies in 2000. All tubers were washed before the assessment of quality deficiencies. The number of lesions per tuber caused by wireworm, drycore and slugs were counted. To control the depth of the quality deficiencies all tubers were peeled with a special knife to a depth of 4 mm, the maximum depth for acceptance (Schweizerische Kartoffelkommission, 1989). Black scurf on seed tubers and harvested tubers was assessed using grades from 1 (no black scurf) to 7 (75% to 100% of the tuber surface with black scurf).

Soil samples of all 278 fields in the project were analysed according to the reference method of the Swiss federal research stations (2004): clay, silt and sand content (finger test), soil type, pH ($H_20$), organic matter (analytic) and ammoniumacetat-EDTA extractible P, K and Mg content.

## Statistical Analysis

To determine the influence of various factors on different quality parameters, the project fields were grouped according to management practice (e.g. farming system, crop rotation) and site parameters (e.g. clay content, pH). Measurement results were statistically checked for normal distribution and comparable variability. Depending on the result, averages or medians were compared with the following methods:

- Two - and higher way analyses of variance. In certain cases arcsin $\sqrt{p}$ transformation was done before analyses of variance. From the full model all interactions with a probability level > 0.15 were removed stepwise and residuals of the final model were tested for normal distribution. Main factors from the final model were removed to test stability of the result.
- T-test for two independent samples of normally distributed but heterogeneous data.
- Wilcoxon rank-sum test for not normally distributed data.

In order to have a global $\alpha$-level of 0.05 for the family of the tests performed, the tukey-Kramer Test respectively the Bonferroni-Holm procedure was applied (Holm, 1979). All statistical analysis was done with NCSS 2004 (Number Cruncher Statistical Software, Kaysville, Utah 84037 Utah, USA).

### *Relative risk for drycore*

To test the within field distribution and possible relations between an influencing factor and drycore the relative risk for drycore damage on a tuber was calculated for the influencing factors black scurf, wireworm damage and slug damange for 40 fields (totally 32’000 tubers) with an important drycore level of more than 5% damaged tubers. The relative risk is the ratio of the event rate between comparative groups and is calculated with the following equation: relative risk = ((a/a+b)/(c/c+d)). a = tubers with drycore and influencing factor present, b = tubers without drycore, but influencing factor present, c = tubers with drycore but influencing factor not present, d = tubers without drycore und no influencing factor. A relative risk > 1 means drycore symptoms are more likely to occur if black scurf, is present on the same tuber.

## RESULTS

On fields with both a low occurrence of black scurf on the seed tubers and a low occurrence of wireworm damage, significant drycore damage was never observed. The significantly highest damage was observed when more than 10% of the planted seed tubers had black scurf and more than 5% of the harvested tubers showed wireworm holes (Fig. 4.1). Even on fields with heavy black scurf infestation drycore occurred very rarely without wireworm injuries on the tubers. This indicates that injuries caused by wireworm might facilitate the penetration of *Rhizoctonia solani* into the tuber. The analysis of variance for drycore showed a significant influence of wireworm damage, seed quality and grass clover ley as preceding crop on the infestation level of drycore (Table 4.1). Drycore damage was significantly highest on fields with grass clover ley in the two years preceding potatoes when more than 20% of seed were infested with black scurf. Where clean seed was planted on fields without grass clover ley in the two preceding years important drycore damage never occurred. No influence was found for the application of solid manure to potatoes (Table 4.1).

For the tubers of the 40 fields with important drycore level on which the within field distribution was studied the average relative risk for drycore damage on tubers with black scurf infestation was 2.23 (SE 0.23) and 2.46 (SE 0.24) for tubers with wireworm damage. This means that the risk for drycore symptoms was 2.23 times higher for tubers with black scurf and 2.46 for tubers with wireworm damage than for clean tubers. In contrast, no higher risk for drycore was observed on tubers with slug damage. This confirms the results shown in Fig. 4.1 and Table 4.1 that infections with *Rhizoctonia solani* (black scurf) and injuries by wireworms are important factors for the formation of drycore symptoms on tubers.

Abiotic factors like pH, organic matter content and texture of soils as well as the meteorological conditions were without influence on drycore damage.

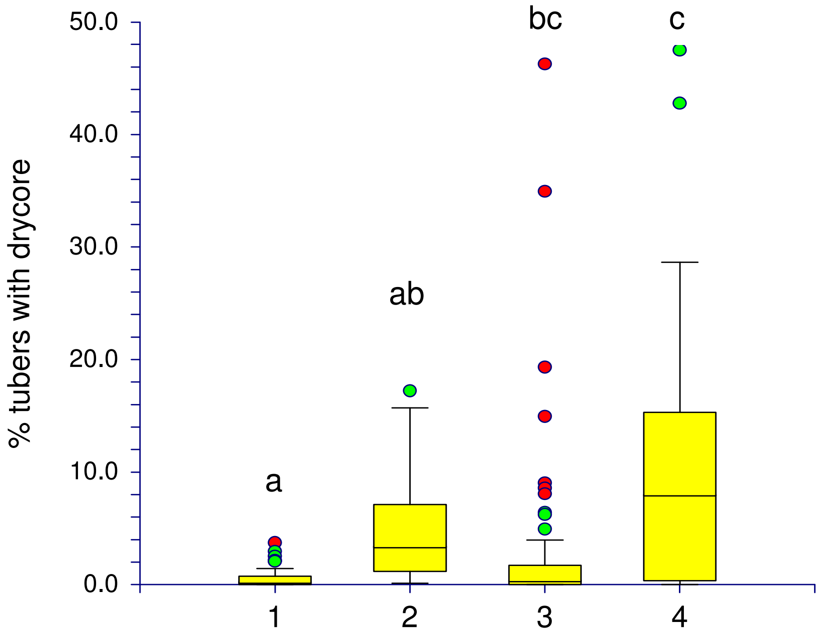


Fig. 4.1 Percentage of tubers per field with drycore damage in dependence of wireworm damage and black scurf on seed tubers 2002 and 2003. 1= less than 5% of tubers with wireworm damage and less than 10% of the seed tubers with black scurf, 2= more than 5% of tubers with wireworm damage and less than 10% of the seed tubers with black scurf; 3= more than 10% of seed tubers with black scurf, less than 5% of tubers with wireworm damage; 4= more than 5% of tubers with wireworm damage and more than 10% of seed tubers with black scurf. The box plot shows the 50% inter quartile range of the values as well as the median (middle line in the box). The points represent the outliers, red = extreme outliers. N= 73 (1), 14 (2), 74 (3) and 16 (4). In order to have a global α-level of 0.05 the Bonferroni-Holm procedure was applied (Holm 1979). Results of groups with different denominator are statistically different.

Table 4.1 Analysis of variance of drycore on potatoes for fields without fungicide seed treatment in all farming systems. Factors: Year (2002 and 2003), rotation (grass clover ley 3 year before potatoes or not), seed quality (% tubers with black scurf: 0, <20%, >20%) manure application (yes/no), covariate: Wireworm.

| Source of variation | DF | SQ | MQ | F-Ratio | Probability level |
|---|---|---|---|---|---|
| Wireworm (covariate) | 1 | 0.4543 | 0.4543 | 49.41 | <0.0001 |
| Year | 1 | 6.7938E-04 | 6.7938E-04 | 0.07 | 0.7865 |
| Crop rotation | 1 | 0.0650 | 0.0650 | 7.07 | 0.0094 |
| Seed quality | 2 | 0.1361 | 6.8041E-02 | 7.40 | 0.0011 |
| Crop rotation * Seed quality | 2 | 9.7832E-02 | 4.8916E-02 | 5.32 | 0.0068 |
| Manure application | 1 | 1.4915E-03 | 1.4915E-03 | 0.16 | 0.6882 |
| Crop rotation * Manure application | 1 | 1.9268E-02 | 1.9268E-02 | 2.10 | 0.1517 |
| S | 80 | 0.7357 | 9.1960E-03 | | |
| Total adjusted | 89 | 1.7606 | | | |
| Total | 90 | | | | |

## DISCUSSION

The presented results show that *Rhizoctonia* infections alone were rarely responsible for important drycore damage on potatoes. Drycore mainly occurred on fields where potatoes were wounded by wireworms (Table 4.1), whereas slug damage did not influence the occurrence of drycore. These results suggest that wounds on tubers by wireworm may facilitate the penetration of *R. solani* in to the tuber and favour the formation of drycore. This is consistent to Schwinn (1961) who managed to infect both mechanically wounded normal lenticels and naturally grown lenticels excrescences and reproduced typical drycore symptoms in green house experiments. He also postulated that the formation of drycore symptoms is favoured under wet soil conditions in soils with higher clay content. In contradiction to these findings no influence of the meteorological conditions were found in our survey (Chapter 3) where drycore damage was on the same level in all three years even in the very dry and hot year 2003. Furthermore, important drycore damage occurred in all soil types independently from the clay and organic matter content. Therefore seed quality and crop rotation were the most important influencing factors for drycore. As wireworms are enhanced by grass clover ley in the crop rotation (Chapter 3); this might explain in part the higher occurrence of drycore in the organic farming system as it is much more common here to grow potatoes after several years of grass clover leys. Grütte (1940) already observed that the occurrence of drycore was higher after ploughing of grassland. The fact that little research was done since 1961 indicates that drycore is only of regional importance. Drycore is regarded as a minor problem in many potato growing areas, but discussions with researchers and farmers during the last five years confirm that drycore is also an increasing problem in some regions in Germany and Austria especially on organic farms which grow potatoes in crop rotations with grass clover leys (W. Dreyer, Oekoring Niedersachsen and A. Fuchs, Bioland Bayern, pers. comm.). The higher proportion of seed lots which exceeded the threshold for black scurf in the organic farming system (Chapter 3) and the lack of an effective seed treatment could be other reasons for the severe drycore problem. The fact that seed quality had a significant influence on drycore infestation of harvested tubers (Tab.4.1) indicates that soil infestation with *R. solani* was of minor importance in our survey, probably due to sufficiently long intervals between two potato crops. For more than 75% of the fields included in the survey intervals between potatoes were more than five years and at least three years for 25%. Carling et al. (1986) in Alaska could not recover isolates of AG 3 from soil free of potato for five years and more.

Fungicide seed treatments in the integrated and conventional fields may also reduce the risk for drycore as they reduce *Rhizoctonia* infestations on plants and the infestation level of black scurf on progeny tubers (Chapter 3). The influence of fungicide seed treatments, the higher seed quality and the influence of insecticide seed treatments (Chapter 3) may explain why drycore damage occurred less in the integrated and conventional farming system even when potatoes were grown after grass clover leys.

## CONCLUSIONS

The data analyses of the 185 potato fields confirm the hypotheses that injuries of wireworm on tubers in combination with *Rhizoctonia* infestations of the potato plant increases drycore damage on potatoes. Highest drycore damage occurred when seed tubers infested with black scurf are planted and progeny tubes are wounded by wireworms. Therefore the use of clean seed without black scurf, crop rotations with low percentage of grass clover leys and intervals of at least three years between grass clover ley and the potato crop are important measures against drycore. Seed quality has to be improved especially in the organic farming system where seed infestation with black scurf often exceeds the threshold and no efficient seed treatment against *Rhizoctonia solani* is known. These results from the survey should be checked with field trials and pot experiments under controlled conditions.

# 5. Genetic variability of *Rhizoctonia solani* AG 3 isolates in Switzerland

## ABSTRACT

Black scurf and drycore attributed to *Rhizoctonia solani* are important quality deficiencies in Swiss potato production especially in organic agriculture. Up to date Swiss isolates of *R. solani* have never been characterised for anastomosis grouping. Sixty isolates obtained from black scurf and drycore on potatoes of different farming systems in Switzerland were analysed with Pectic zymograms, AG 3 specific primers and anastomosis typing. Sequence analyses of the ITS region of rDNA and ERIC and REP-PCR were used to determine the degree of genetic variability among the isolates. Fifty-nine isolates belonged to AG 3, and only one isolate to AG 5. Thus, the higher importance of drycore in the organic agriculture can not be explained by the occurrence of different anastomosis groups. Sequence similarity in the ITS region for isolates of AG 3 ranged from 98.8% to 100%, compared to 90.14% to 92.3% for isolates of different AGs. A dendrogram generated by ERIC and REP-PCR dispersed AG 3 isolates in four clusters. The genetic similarity among isolates of AG 3 ranged between 20.4 and 86.9%. The genetic similarity could not be explained by the geographical region or by the potato variety from which the isolates were obtained, but most isolates from organic fields were grouped together in the same cluster. The reason for the higher occurrence of drycore in organic agriculture has to be examined in experiments under controlled conditions.

## INTRODUCTION

*Rhizoctonia solani Kühn* (telemorph*: Thanatephorus cucumeris* (Frank) Donk) is a soil borne plant pathogen affecting many agricultural crops world wide (Ogoshi, 1987; Sneh et al., 1991). On potatoes the disease causes delayed emergence, lesions on stems (stem canker) and stolons and sclerotial formation on tubers (black scurf) (Baker, 1970; Anderson, 1982). Attacks on stolons may induce the development of malformed tubers and tubers with growth cracks (Weinhold et al., 1978; Jeger et al., 1996) (Appendix, Fig.A4 a-c, h). Besides stem canker and black scurf, other symptoms have also been attributed to *R. solani* most notably drycore (Appendix, Fig. 4A c-g), restricted brown cavities up to several mm deep with a diameter of 3 to 6 mm (Schwinn, 1961; Grütte, 1940; Ramsey 1917; Müller 1925). As shown in chapter 3, in Switzerland drycore is the most important quality deficiency in organic potato production and in other farming systems in crop rotations with grass-clover leys in the years preceding to the potato crop. Other symptoms of *R. solani* are scabby lesions on the tuber skin which closely resemble netted scab symptoms caused by *Streptomyces reticuliscabiei* and some strains of *Streptomyces europaeiscabiei* (Bocheck-Mechiche et al., 2000).

The most commonly used system for classification of *R. solani* is based largely on hyphal fusion with anastomosis tester isolates. To date, fourteen anastomosis groups (AG 1 to AG 13 and AG BI) have been identified, seven of them (AG 1, AG 2, AG 3, AG 4, AG 6, AG 8, AG 9) being divided into sub-groups that differ for pathology, biochemical or genetic characteristics (Anderson, 1982; Cubeta and Vilgalys, 1997; Gonzalez et al. 2001, Carling et al., 2002). AG 3, 4, 5 and 8 are capable of doing moderate to extensive damage to potato plants. AG 3 has been known as the most host-specific type of *R. solani* causing disease of potato. It is by far the most important cause of black scurf because most of the sclerotia isolated from potato tubers belong to AG 3 (Carling and Leiner, 1986; Chand and Logan, 1983; Campion et al., 2003). Pectic zymograms obtained by electrophoresis of pectic enzymes produced by *R. solani* are used as a routine method for rapid assignment to AG and AG subgroups. Isolates sharing a zymogram pattern constitute a zymogram group and were found to share characteristics of morphology and of pathogenicity. Based on zymogram patterns isolates can be assigned to anastomosis groups (Cruikshank, 1990). Another rapid routine method for the identification of AGs is the use of AG specific primers (Kuninaga et al., 2000). Molecular techniques enable to study genetic diversity in *R. solani*. The taxonomic significance and genetic relationship among AGs have been ascertained based on molecular techniques. Studies of the phylogenetic and taxonomic relationships of AG and AG subgroups of *R. solani* using sequence analysis of the ITS region of the nuclear encoded ribosomal DNA (rDNA) concluded that molecular relationships are largely congruent with relationships inferred with hyphal anastomosis reactions (Kuninaga et al., 1997). Repetitive DNA sequences are used to amplify inter-repeat sequences in order to assess genetic relatedness among and within different anastomosis groups by ERIC and REP-PCR (Takeshi, 1999).

The objective of this work was to identify the anastomosis groups of 60 isolates of *Rhizoctonia solani* obtained from black scurf and drycore on potatoes of different farming systems in Switzerland with Pectic zymograms, AG-3 specific primers and anastomosis typing. ITS sequencing, ERIC and REP-PCR was used to determine the degree of genetic variability between Swiss isolates of *R. solani*.

## MATERIAL AND METHODS

### Pathogen isolation

Pieces of drycore symptoms and sclerotia from potatoes from plots of all farming systems were plated on 1.5% tap water agar amended with 250 ppm chloramphenicol (WAC) and incubated for 2 to 3 days at room temperature. After a second transfer to WAC, isolates were transferred to PDA. Pure cultures of the isolates (Table 5.3) were stored at 10 °C.

### Pectic zymograms

Analysis of pectic zymograms was used as a routine method for rapid assignment of *R. solani* isolates to AG groups (Gruickshank 1979; Mc Nish et al. 1993; Schneider et al. 1997). Pure cultures were grown on pectin medium with citrus pectin (Sigma P9135, local) as a carbon source. Isolates were incubated for 10 days at 23°C in the dark. Ten µl of the culture filtrate was loaded on a native polyacrylamide gel, with 1.5 M Tris/HCL, pH 8.8, in the separating gel and 0.5 M Tris/HCl, pH 6.8, in the stacking gel. These native gels were run for 70 min in running buffer and were subsequently incubated in 0.1 M DL-malic acid for 1 hour followed by staining with 0.02% ruthemium red for 2 h. AG-3 and AG-5 anastomosis tester isolates were loaded on each gel as a reference isolates, for comparison. Gels were fixed in 3mM $Na_2C0_3$, scanned and sealed in cellophane (Grosch et al. 2004).

### DNA extraction

Total genomic DNA was extracted from lyphilized mycelium of *R. solani* grown for 5 days at 25°C in potato-dextrose broth (Difco, local) using the DNeasy Plant Mini DNA extraction kit (Qiagen GmbH, Hilden, Germany), according to the specifications of the manufacturer.

### Identification with AG 3 specific primer

Amplification was performed in a 25-µl PCR reaction mixture containing 1x PCR buffer (0.2 mmol $l^{-1}$ each dNTPs, 1.5 mmol $l^{-1}$ of $MgCl_2$, 2.5 U Taq polymerase), 3.5 mmol $l^{-1}$ $MgCl_2$, 0.2 µmol $l^{-1}$of the primers PT1 and PT2 (Table 3) and approximately 5 ng of template DNA. The PCR - amplifications were carried out under the following conditions: initial denaturation at 94°C 2 min and 30s, followed by 40 cycles of denaturation at 94°C for 1 min, 63°C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Reaction products were separated on agarose gels, stained with ethidiumbromide and visualized under UV-light.

### Anastomosis group typing

Water agar coated slides were seeded with mycelial disks (5 mm in diameter) taken from the edge of growing colonies on PDA, placed on moist filter paper in large (20 cm diameter) Petri dishes and incubated for 24-30 h at 23 °C. Disks of isolates from in vitro tests and anastomosis tester isolates (Table 5.1) were placed on the coated slide in a distance of 1.5 cm. When the colonies overlapped the area was examined microscopically (100x) for hyphal anastomosis. Individual anastomosis reactions between hyphae of confronted isolates were assigned to one of eight categories (Carling et al., 2002). The four main categories are C0, C1, C2 and C3, where C0 is no reaction and C3 a self (clonal) reaction. Cataloguing a reaction into one of these categories requires the presence of five or more of the anastomosis points characteristic of that category. The additional four categories (C1–, C1+, C2–, C2+) use the definitions for C1 and C2 reactions and differ only based in the number of observed anastomosis points: C1-= 1 to 4 C1 points, C1+= 8 and more C1 points, C2– = 1 to 4 C2 points and C2+ = 8 and more C2 points (Carling, 1996).

Table 5.1 Isolates of tester isolates of *Rhizoctonia solani* used for anastomosis group typing.

| irs-code | AG | host | country | supplier | origonal code |
|---|---|---|---|---|---|
| 01-1A | 1-1A | rice | Japan | IPO | CS-KA |
| 22-03 | 2-2 IIIB | mat rush | Japan | Ogoshi | C-96 |
| 03-01 | 3 | potato | Japan | Ogoshi | ST-11-6 |
| 04-02 | 4 HG-I | peanut | Japan | Ogoshi | AH-1 |
| 05-01 | 5 | soybean | NL | Ogoshi | GM-10 |
| 08-01 | 8 | wheat | USA | IPO | W-565 |
| 10-03 | 10 | Subterranean clover | W-australia | Carling | 91778 |

### Sequence analysis of the ITS region

The ITS region of the rDNA of 15 isolates from AG 3 (AK 50, AK 10, AK 8Da, AK 8Db, SO 301, AK 14, AK 001, AK0501, 03-08, VD 302, 03-01, AK 9D) and AG 5 (AK 34, 05-01, 05-41) was amplified and sequenced (Table 5.3). Amplification of the ITS region was performed in a 50-µl PCR reaction mixture containing 1x PCR buffer (500mmol $l^{-1}$ KCl, 100 mmol $l^{-1}$

Tris/HCl, pH 8.3, 15 mmol $l^{-1}$ of Mg $Cl_2$, and 0.01% of (w/v) gelatine), 0.2 mmol $l^{-1}$ each dNTPs, 1.5 mmol $l^{-1}$ of $MgCl_2$ , 0.5 mmol $l^{-1}$ of the primers ITS 1 and ITS 4 (Table 5.2)(Isogen Life Science, the Netherlands), 0.75U Taq polymerase (Qiagen GMBH, Hilden, Germany) and 5ng template DNA. Amplifications were carried out in a PTC-200 Peltier Thermal cycler (MJ Research). The cycle parameters were an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30s, annealing at 57°C for 30 s, extension at 72°C for 1 min and final extension at 72°C for 5 min. The PCR product was purified using a Qiaquick gel extraction kit and sent to Macrogen (Korea) for custom sequencing. The sequences were analysed and aligned using Bionumerics software (Applied Maths, Belgium).

***ERIC and REP-PCR***

PCR reaction were performed in 25µl PCR reaction mixture containing 1x Gitschier buffer (16.6 mM $(NH4)_2SO4$, 67mM Tris-HCl (PH 8.8), 6.7 mM MgCl2, 6.5 µM EDTA (PH 8.8), 30mM 2-mercapto ethanol), 0.16 mg/ml BSA, 1% formamide, 2µM of each of two opposing primers (Table 5.2), 1.25mM of each dNTP, 2 U of Taq DNA polymerase and 1µl template DNA. The cycle parameters were an initial denaturation at 94°C for 2 min, followed by 35 cycles at 93°C for 3s?, at 92°C for 30s, at 40°C for 1 min and at 65°C for 8 min, then for final extension at 65°C for 10 min. Amplified products together with a 100 bp size marker up to 3 kb (Biorad, the Netherlands) were resolved by gel electrophoresis (4 V cm-1) on 3% agarose gels in 0.5 x TBE-buffer containing 0.5 mg ml-1 ethidium bromide (EB). All gels were photographed biorad Geldoc 2000 system.

Table 5.2 Primers used for PCR Amplifications

| Primers | Sequences (5'-3') |
|---|---|
| PT1 | GTTTGGTTGTAGCTGGTCT |
| PT2 | CTGAGATCCAGCTAATAC |
| ITS1 | TCCGTAGGTGAACCTGCGG |
| ITS4 | TCCTCCGCTTATTGATATGC |
| ERIC 1R | ATGTAAGCTCCTGGGGATTCAC |
| ERIC 2 | AAGTAAGTGACTGGGGTGAGCG |
| REP 1R-1 | IIIICGICGICATCIGGC |
| REP 2-1 | ICGICTTATCIGGCCTAC |

I= Iosine

Table 5.3 Isolates of *Rhizoctonia solani* from sclerotia and drycore symptoms from potato plots of different production systems in Switzerland (2003 and 2005) analysed in this study.

| Isolate | Year of Isolation | Isolated from | Location | Farming system | variety |
|---|---|---|---|---|---|
| BE 317 | 2003 | sclerotia | 3043 Uettligen | organic | Agria |
| LU 305 | 2003 | sclerotia | 6221 Rickenbach | organic | Agria |
| SO 301 | 2003 | sclerotia | 4578 Bibern | organic | Charlotte |
| BE 343 | 2003 | sclerotia | 4704 Niederbipp | integrated | Bintje |
| SG 304a | 2003 | sclerotia | 9494 Schaan | integrated | Charlotte |
| SG 304b | 2003 | sclerotia | 9494 Schaan | integrated | Charlotte |
| VD 302 | 2003 | sclerotia | 1064 St-Cierges | integrated | Charlotte |
| BE 346 | 2003 | sclerotia | 4913 Bannwil | conventional | Agria |
| FR 310 | 2003 | sclerotia | 1782 Lossy | conventional | Agria |
| ZH 309a | 2003 | sclerotia | 8303 Bassersdorf | conventional | Agria |
| ZH 309b | 2003 | sclerotia | 8303 Basserdsorf | conventional | Agria |
| AK 2 | 2003 | sclerotia | 8476 U'stammheim | organic | Agria |
| AK 3 | 2003 | sclerotia | 8486 Trüllikon | conventional | Charlotte |
| AK 5 | 2003 | sclerotia | 4578 Bibern | organic | Charlotte |
| AK 7a | 2003 | sclerotia | 4913 Bannwil | conventional | Agria |
| AK 7b | 2003 | sclerotia | 4913 Bannwil | conventional | Agria |
| AK 8 | 2003 | sclerotia | 4585 Biezwil | conventional | Agria |
| AK 11 | 2003 | sclerotia | 1607 Palézieux | organic | Agria |
| AK 12 | 2003 | sclerotia | 6221 Rickenbach | organic | Agria |
| AK 13 | 2003 | sclerotia | 3043 Uettlingen | organic | Agria |
| AK 16 | 2003 | sclerotia | 3114 O'wichtrach | integrated | Bintje |
| AK 17 | 2003 | sclerotia | 8303 Basserdorf | conventional | Agria |
| AK 18 | 2003 | sclerotia | 3212 Gurmels | conventional | Agria |
| AK 19 | 2003 | sclerotia | 8302 Kloten | conventional | Agria |
| AK 22 | 2003 | sclerotia | 6217 Kottwil | conventional | Eba |
| AK 25 | 2003 | sclerotia | 5623 Boswil | conventional | Agria |
| AK 26 | 2003 | sclerotia | 8223 Guntmadingen | conventional | Charlotte |
| AK 29 | 2003 | sclerotia | 3178 Bösingen | conventional | Agria |
| AK 31 | 2003 | sclerotia | 8917 Oberlunkhofen | conventional | Agria |
| AK 32 | 2003 | sclerotia | 2540 Grenchen | conventional | Agria |
| AK 33 | 2003 | sclerotia | 8553 Hüttlingen | organic | Charlotte |
| AK 34 | 2003 | sclerotia | 9584 Matzingen | conventional | Eba |
| AK 37 | 2003 | sclerotia | 1415 Molondin | integrated | Nicola |
| AK 40 | 2003 | sclerotia | 3186 Düdingen | conventional | Eba |
| AK 41 | 2003 | sclerotia | 6346 Altishofen | conventional | Agria |
| AK0501 | 2005 | sclerotia | 3052 Zollikofen | conventional | Agria |
| AK0502 | 2005 | sclerotia | 3052 Zollikofen | conventional | Agria |
| AK0503 | 2005 | sclerotia | 3052 Zollikofen | conventional | Innovator |
| AK0504 | 2005 | sclerotia | 3052 Zollikofen | conventional | Innovator |
| AK0505 | 2005 | sclerotia | 3052 Zollikofen | conventional | Lady Rosetta |
| AK 9S | 2005 | sclerotia | 3052 Zollikofen | conventional | Innovator |
| AK 9D | 2005 | sclerotia | 3052 Zollikofen | conventional | Innovator |
| AK 10S | 2005 | sclerotia | 3052 Zollikofen | conventional | Innovator |
| AK 12 S | 2005 | sclerotia | 3052 Zollikofen | conventional | Innovator |
| AK 11S | 2005 | sclerotia | 3052 Zollikofen | conventional | Innovator |
| AK 47 | 2003 | drycore | 4913 Bannwil | conventional | Agria |
| AK 48 | 2003 | drycore | 3212 Gurmels | conventional | Agria |
| AK 49 | 2003 | drycore | 6217 Kottwil | conventional | Eba |
| AK 50 | 2003 | drycore | 8917 O'lunkhofen | conventional | Agria |
| AK 10 | 2003 | drycore | 3083 Trimstein | integrated | Nicola |
| AK 15 | 2003 | drycore | 3083 Trimstein | integrated | Nicola |
| AK 20a | 2003 | drycore | 6221 Rickenbach | organic | Agria |
| AK 20b | 2003 | drycore | 6221 Rickenbach | organic | Agria |
| AK 21 | 2003 | drycore | 1304 Cossonay | organic | Charlotte |
| AK 30 | 2003 | drycore | 1063 Moudon | conventional | Agria |

Table 5.3 continued

| | | | | | |
|---|---|---|---|---|---|
| AK 6 | 2005 | drycore | 3052 Zollikofen | conventional | Innovator |
| AK 8D | 2005 | drycore | 3052 Zollikofen | conventional | Agria |
| AK 10D | 2005 | drycore | 3052 Zollikofen | conventional | Innovator |
| AK 11D | 2005 | drycore | 3052 Zollikofen | conventional | Innovator |
| AK 14 | 2005 | drycore | 3052 Zollikofen | conventional | Charlotte |

$^{1}$) All but one isolates revealed the typical pectic zymogram for AG 3 and reacted with a primer specific for AG 3. AK 34 was identified as AG 5 (see Table 5.4)

## RESULTS

### Pectic zymogram and AG-3 specific primer

From 60 analysed isolates (44 from sclerotia, 16 from drycore) all except one (AK 34) revealed the typical pectic zymogram for AG 3 (Fig.5.1) and reacted with a primer specific for AG 3 (Fig.5.2). AK 34 was identified as AG 5 (Table 5.3).

### Anastomosis group typing

Representative isolates of AG 3 (AK 8, AK 10, AK25, AK 20b, AK 22, AK18 and ZH 309a) and AK 34 anastomosis reactions were determined with tester isolates from different anastomosis groups (Table 5.1 and 5.4). All AG 3 isolates produced a C2 reaction with an AG-3 tester isolate. Between AK 8 and the AG 10-tester isolate and between AK 25 and the AG 8–tester isolate C2+ and C2- reactions were observed respectively. AK 34 was assigned to AG 5.

### Sequence comparison of the ITS rDNA region

A dendrogram was constructed on the basis of analysis of internal transcript spacer (ITS) regions of nuclear-encoded ribosomal DNA (rDNA) (Fig. 5.3, Appendix Table A.3). The degree of nucleotide sequence variation between AG 3 and AG 5 was greater than between isolates of AG 3. Sequence similarity among isolates of AG 3 and AG 5 ranged from 90.14 to 92.3% (Appendix Table A3), from 98.79 to 100% among the isolates of AG 3 and from 99.21-99.61 among isolates of AG 5.

Table 5.4 Anastomosis reaction of some selected isolates of *R. solani* (Table 5.3)

| | AG 1 | AG 2 | AG 3 | AG 4 | AG 5 | AG 8 | AG 10 | ZH 309b | AK 18 | AK 13 | AK 7b | AK 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AK 8 | | | C2 | | | | C2+ | | | | | |
| AK 10 | C0 | C0 | C2 | | C0 | | C2 | | | C2 | | |
| AK 25 | | | C2/C2- | | | C2- | | | | | | |
| AK 34 | C0 | C0 | C0 | C0 | C2 | C0 | C0 | | | | | |
| AK 20b | | | C2 | | | | | | C2 | | C2 | C2 |
| ZH 309a | | | C2 | | | | | C3 | | | | |
| AK 22 | | | C2+ | | | | | | | | | |
| AK 18 | | | | | | | | | C3 | | | |

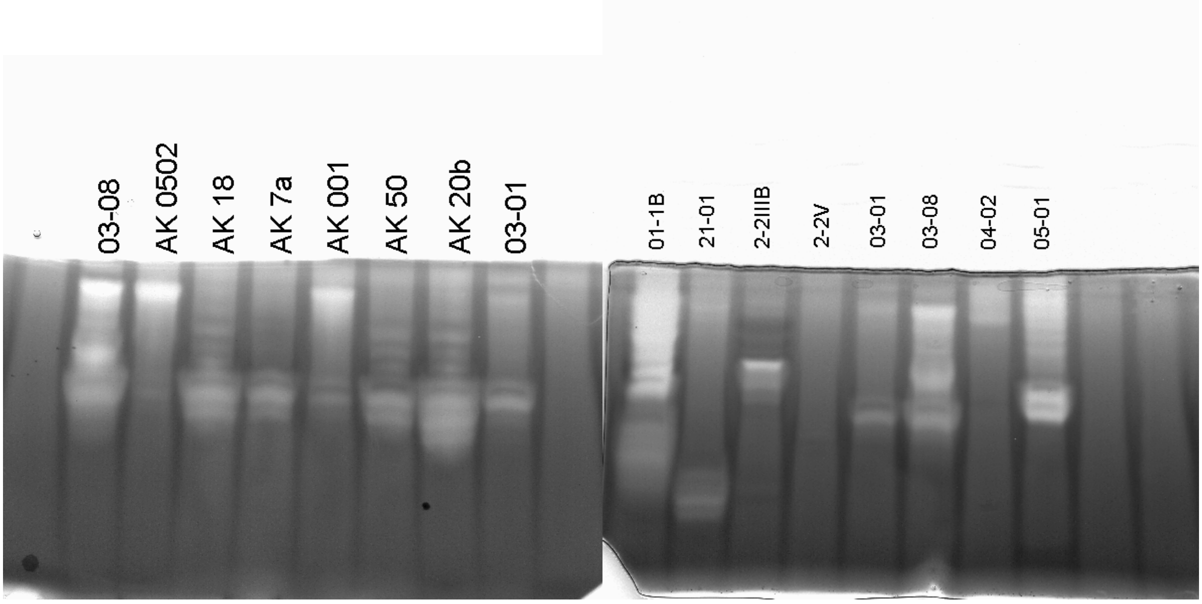


Fig. 5.1 Pectic zymograms of representative isolates of *Rhizoctonia solani* from Switzerland (Table 5.3) compared with tester strains of AG 1, 2, 3, 4 and 5.

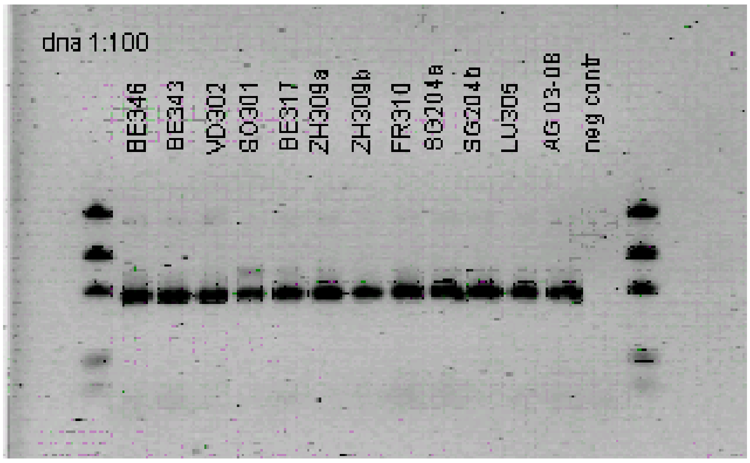


Fig. 5.2 Amplicons of purified genome of some selected isolates of *Rhizoctonia solani* AG 3 isolates (Table 5.3) with AG 3 specific primers (Table 5.2).

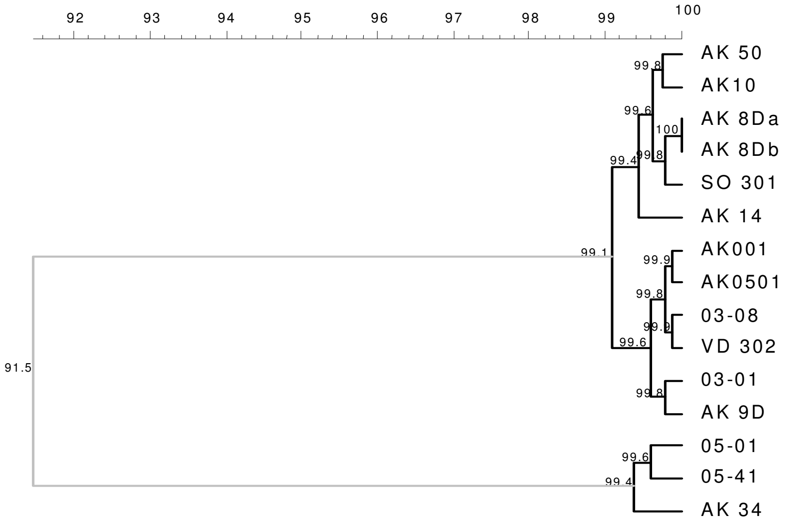


Fig. 5.3 Dendrogram based on the sequence analyses of the internal transcript spacer (ITS) with primers ITS 1 and ITS 4 of isolates of *R. solani* (see Table 5.2 and 5.3)

**ERIC and REP-PCR**

Repeated conserved sequences of ERIC and REP elements permitted the amplification of genomic DNA of *R. solani* belonging to AG 3, 5 and 10 (Fig. 5.4 and 5.5). The range of fragments for ERIC and REP was 190 bp to 3000 bp and 350 bp to 3000 respectively for AG 3 isolates and 400 bp to 2500 bp and 600 bp to 3000 bp respectively for AG 5 isolates. The total size of the marker was 3000 bp. A dendrogram was constructed using polymorphic patterns representing the relationship of the isolates (Fig.5.6). Isolates of AG 3 and AG 10 were grouped together, but not isolates of AG 5. Isolates of AG 3 dispersed in 4 clusters. Genetic similarity between isolates of AG 3 ranged from 20.4% to 86.9%. The highest genetic relatedness was observed for pairs of isolates from the same field (e.g. AK 7a and AK 7b). Isolates from different regions in Switzerland and from different varieties were found in all four clusters. Isolates from organic fields were mainly assigned to the same cluster.

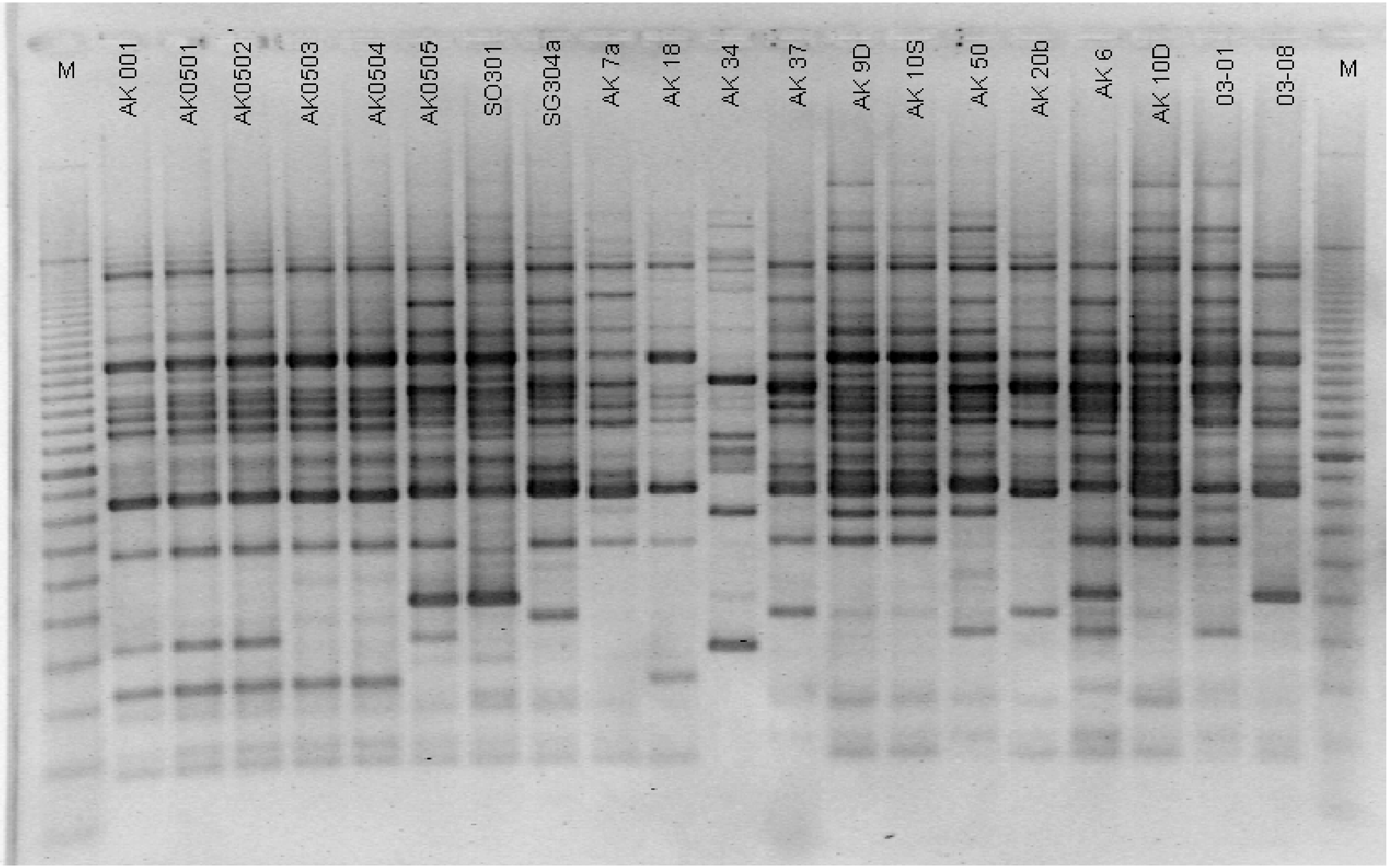


Fig. 5.4 Eric PCR fingerprints of isolates of *R.solani* belonging to AG 3 and AG 5. Origin of the isolates is given in Table 5.3
Lane M = DNA markers

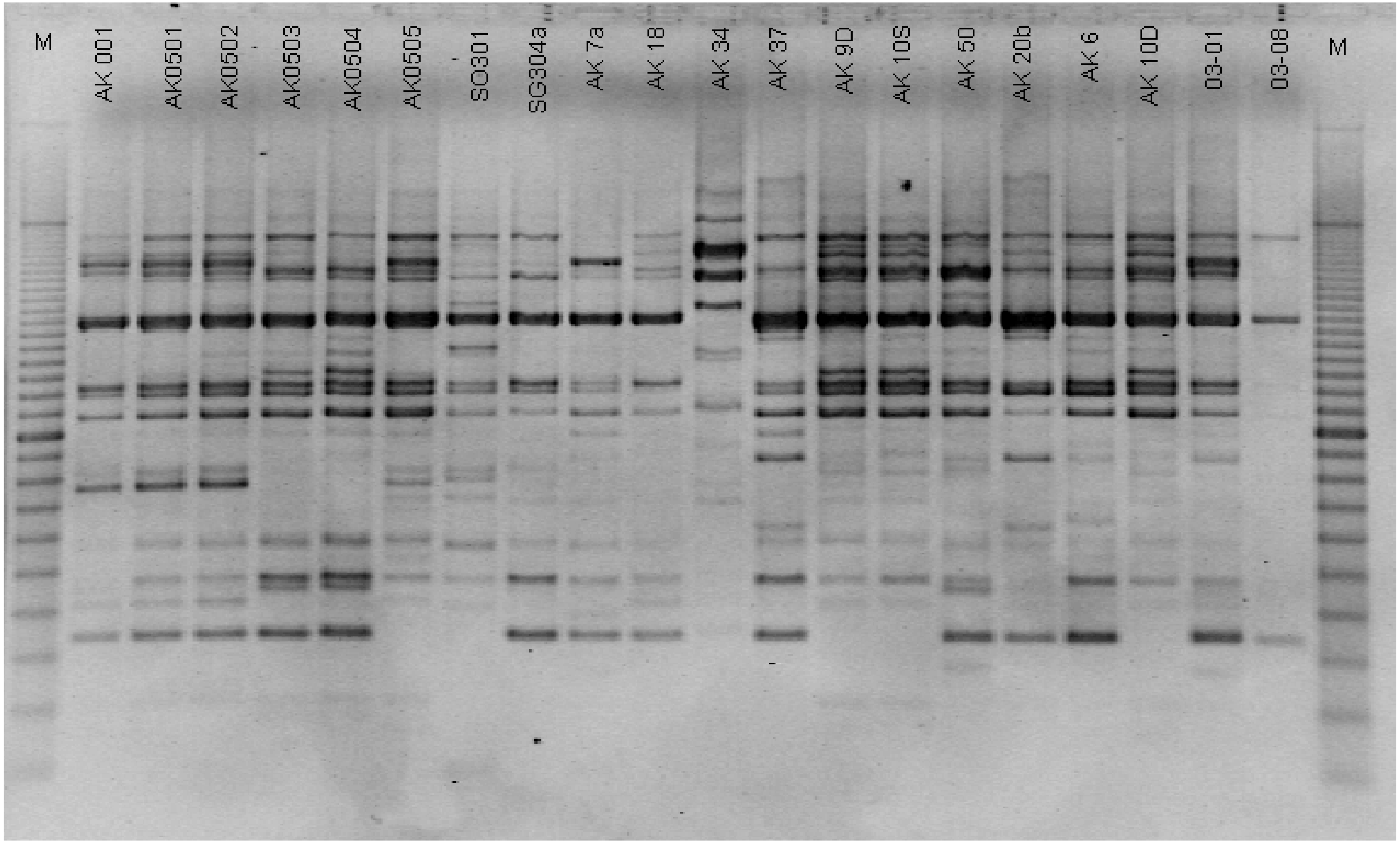
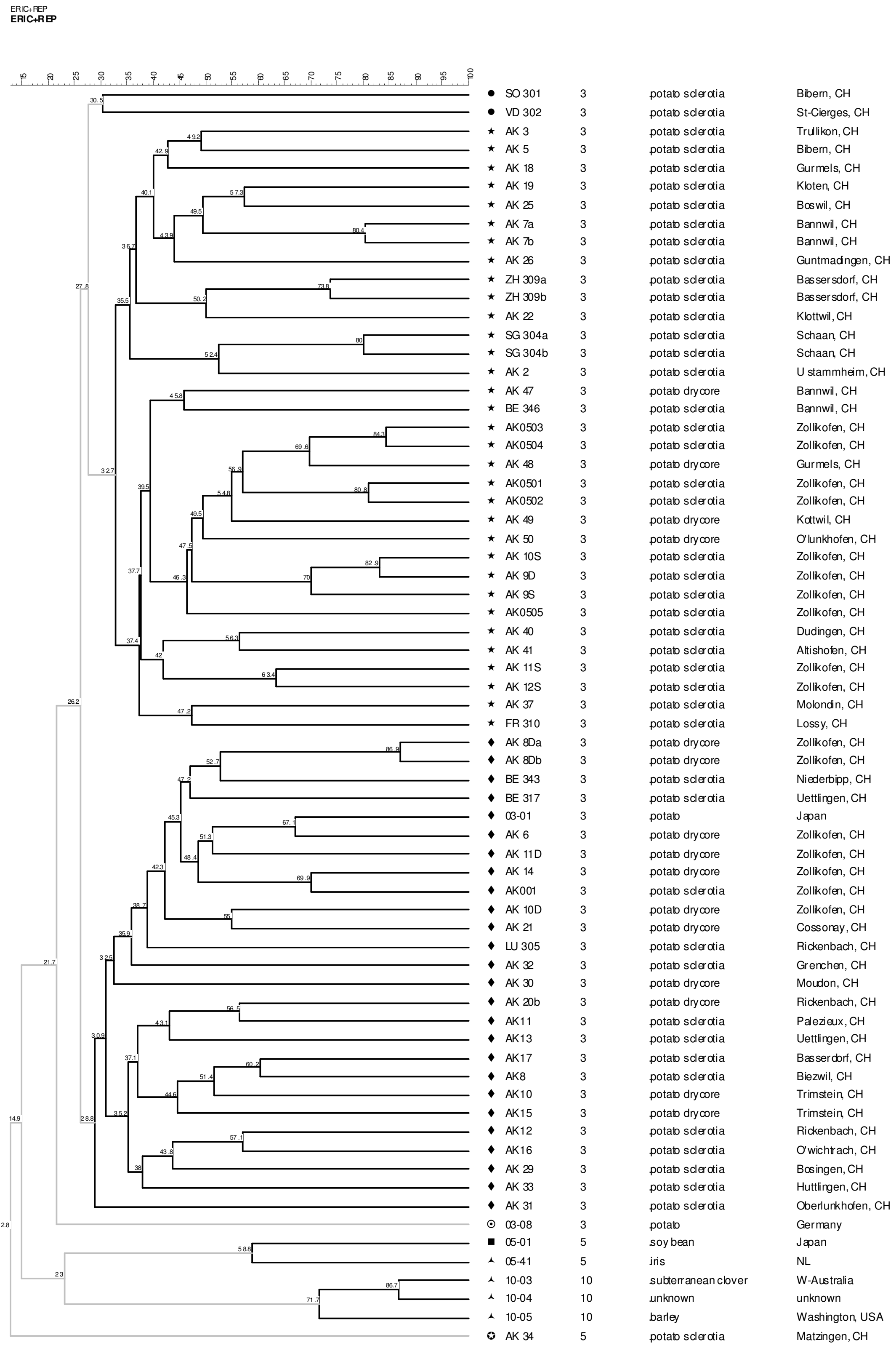


Fig. 5.5 REP PCR fingerprints of isolates of *R. solani* belonging to AG 3 and AG 5. Origin of the isolates is given in Table 5.3
Lane M = DNA markers



| | | | | |
|---|---|---|---|---|
| ● | SO 301 | 3 | potato sclerotia | Bibern, CH |
| ● | VD 302 | 3 | potato sclerotia | St-Cierges, CH |
| ★ | AK 3 | 3 | potato sclerotia | Trullikon, CH |
| ★ | AK 5 | 3 | potato sclerotia | Bibern, CH |
| ★ | AK 18 | 3 | potato sclerotia | Gurmels, CH |
| ★ | AK 19 | 3 | potato sclerotia | Kloten, CH |
| ★ | AK 25 | 3 | potato sclerotia | Boswil, CH |
| ★ | AK 7a | 3 | potato sclerotia | Bannwil, CH |
| ★ | AK 7b | 3 | potato sclerotia | Bannwil, CH |
| ★ | AK 26 | 3 | potato sclerotia | Guntmadingen, CH |
| ★ | ZH 309a | 3 | potato sclerotia | Bassersdorf, CH |
| ★ | ZH 309b | 3 | potato sclerotia | Bassersdorf, CH |
| ★ | AK 22 | 3 | potato sclerotia | Klottwil, CH |
| ★ | SG 304a | 3 | potato sclerotia | Schaan, CH |
| ★ | SG 304b | 3 | potato sclerotia | Schaan, CH |
| ★ | AK 2 | 3 | potato sclerotia | Ustammheim, CH |
| ★ | AK 47 | 3 | potato drycore | Bannwil, CH |
| ★ | BE 346 | 3 | potato sclerotia | Bannwil, CH |
| ★ | AK0503 | 3 | potato sclerotia | Zollikofen, CH |
| ★ | AK0504 | 3 | potato sclerotia | Zollikofen, CH |
| ★ | AK 48 | 3 | potato drycore | Gurmels, CH |
| ★ | AK0501 | 3 | potato sclerotia | Zollikofen, CH |
| ★ | AK0502 | 3 | potato sclerotia | Zollikofen, CH |
| ★ | AK 49 | 3 | potato drycore | Kottwil, CH |
| ★ | AK 50 | 3 | potato drycore | O'lunkhofen, CH |
| ★ | AK 10S | 3 | potato sclerotia | Zollikofen, CH |
| ★ | AK 9D | 3 | potato sclerotia | Zollikofen, CH |
| ★ | AK 9S | 3 | potato sclerotia | Zollikofen, CH |
| ★ | AK0505 | 3 | potato sclerotia | Zollikofen, CH |
| ★ | AK 40 | 3 | potato sclerotia | Dudingen, CH |
| ★ | AK 41 | 3 | potato sclerotia | Altishofen, CH |
| ★ | AK 11S | 3 | potato sclerotia | Zollikofen, CH |
| ★ | AK 12S | 3 | potato sclerotia | Zollikofen, CH |
| ★ | AK 37 | 3 | potato sclerotia | Molondin, CH |
| ★ | FR 310 | 3 | potato sclerotia | Lossy, CH |
| ♦ | AK 8Da | 3 | potato drycore | Zollikofen, CH |
| ♦ | AK 8Db | 3 | potato drycore | Zollikofen, CH |
| ♦ | BE 343 | 3 | potato sclerotia | Niederbipp, CH |
| ♦ | BE 317 | 3 | potato sclerotia | Uettlingen, CH |
| ♦ | 03-01 | 3 | potato | Japan |
| ♦ | AK 6 | 3 | potato drycore | Zollikofen, CH |
| ♦ | AK 11D | 3 | potato drycore | Zollikofen, CH |
| ♦ | AK 14 | 3 | potato drycore | Zollikofen, CH |
| ♦ | AK001 | 3 | potato sclerotia | Zollikofen, CH |
| ♦ | AK 10D | 3 | potato drycore | Zollikofen, CH |
| ♦ | AK 21 | 3 | potato drycore | Cossonay, CH |
| ♦ | LU 305 | 3 | potato sclerotia | Rickenbach, CH |
| ♦ | AK 32 | 3 | potato sclerotia | Grenchen, CH |
| ♦ | AK 30 | 3 | potato drycore | Moudon, CH |
| ♦ | AK 20b | 3 | potato drycore | Rickenbach, CH |
| ♦ | AK11 | 3 | potato sclerotia | Palezieux, CH |
| ♦ | AK13 | 3 | potato sclerotia | Uettlingen, CH |
| ♦ | AK17 | 3 | potato sclerotia | Basserdorf, CH |
| ♦ | AK8 | 3 | potato sclerotia | Biezwil, CH |
| ♦ | AK10 | 3 | potato drycore | Trimstein, CH |
| ♦ | AK15 | 3 | potato drycore | Trimstein, CH |
| ♦ | AK12 | 3 | potato sclerotia | Rickenbach, CH |
| ♦ | AK16 | 3 | potato sclerotia | O'wichtrach, CH |
| ♦ | AK 29 | 3 | potato sclerotia | Bosingen, CH |
| ♦ | AK 33 | 3 | potato sclerotia | Huttlingen, CH |
| ♦ | AK 31 | 3 | potato sclerotia | Oberlunkhofen, CH |
| ⊙ | 03-08 | 3 | potato | Germany |
| ■ | 05-01 | 5 | soy bean | Japan |
| ⅄ | 05-41 | 5 | iris | NL |
| ⅄ | 10-03 | 10 | subterranean clover | W-Australia |
| ⅄ | 10-04 | 10 | unknown | unknown |
| ⅄ | 10-05 | 10 | barley | Washington, USA |
| ✪ | AK 34 | 5 | potato sclerotia | Matzingen, CH |

Fig.5.6 Genetic relatedness of isolates of *R. solani* of AG 3, AG 5 and AG 10. The dendrogram was generated from the similarity coefficient obtained from the presence and absence of total DNA bands of ERIC and REP-PCR.

## DISCUSSION

### Identification of anastomosis groups

Fifty-nine of a total of 60 isolates of *R. solani* obtained from sclerotia and drycore of potatoes in the major potato growing area in Switzerland belong to AG 3, and only one isolate to AG 5. To the best of our knowledge this was the first time that isolates of *Rhizoctonia solani* obtained from potatoes in Switzerland were assigned to anastomosis groups. The results are consistent with Campion et al (2003) who found that 97% of 192 isolates obtained from sclerotia from potatoes in France belonged to AG 3. AG 3 is regarded as the most host-specific type of *R. solani* causing disease of potato (Carling and Leiner, 1986; Chand and Logan, 1983).

### Genetic variability

The ITS region is known to have a higher rate of molecular evolution than other ribosomal genes (Hibbett et al., 1997) and has therefore been particularly useful for identifying genetic groups at the species level. Kuninaga et al. (1997 and 2000) demonstrated the utility of rDNA ITS sequences for assessing genetic diversity and identification of AG and AG subgroups among 45 isolates of *R. solani.* Isolates belonging within the same AG shared high sequence similarity (above 96%). Between isolates of different AGs sequence similarity was between 55% and 96%. Also Gonzalez et al. (2001) found little or no divergence in ITS sequences within an AG, but higher divergence between isolates of different AGs. This is consistent with the results of this study were sequence similarity within AG 3 and AG 5 was high with 98.8% to 100% and 99.21% to 99.61% respectively, compared to 90.14% to 92.3% for isolates of different AGs.

Takeshi et al. (1999) showed that isolates of *R. solani* can be differentiated with ERIC and REP-PCR. While most subgroups or AGs isolates had a high level of genetic similarity and could be grouped together, a higher level of heterogeneity was suggested for some groups, especially 1-IC, 3, 4 and BI. The results of our study also suggest a high level of heterogeneity among 59 isolates of AG 3 with a genetic similarity ranging from 20.4% to 86.9%. Genetic similarities over 80% were only found between isolates obtained in the same field.

Tubers infested with sclerotia or mycelia are considered to be the most important factor in the epidemiology of black scurf (Anderson 1993). Potato tubers obtained from different locations may carry different genotypes of AG 3 and black scurf may develop from a combination of genetically different *R. solani*. Pre-basic and basic seed which is multiplied in Switzerland is mainly imported from France and the Netherlands depending on the variety (A. Ruegger, Swisssem, 2007, pers. comm.). It is probable therefore that seed sources may contribute to the genetic diversity detected among AG 3 isolates in Switzerland. In contrast to Takeshi et al. (1999) who reported that differences in patterns between isolates of the same region of Japan tended to be fewer, the genetic similarity of isolates of the same geographical region in Switzerland was not smaller than between isolates of different regions. This could be explained by the fact that basic seed (import and Swiss origin) is multiplied on a relative small surface by a small number of seed companies which sell their seed to all different regions in Switzerland. Also isolates which were obtained from the same variety had no higher genetic similarity than isolates from different varieties. The only factor that seems to explain the clustering of the isolates in part is the farming system. Most of the isolates obtained from potatoes of organic farms were assigned to the same cluster. Maybe this is because organic farms are obliged to use seed which has been multiplied for at least two years on organic farms. Conventional seed is only allowed if there is no more organic seed available. Because of the small surface of organic potatoes, the organic seed is produced on a very small number of farms.

All isolates obtained from drycore and all but one isolate from sclerotia analysed in this study belonged to AG 3. Thus, the higher importance of drycore in the organic farming system in Switzerland (Chapter 3) can not be explained by the occurrence of different anastomosis groups compared to the integrated and conventional farming system. The isolates analysed in this study were obtained from the same potato fields as those discussed in chapter 4 where evidence was discussed that injuries of wireworm on tubers in combination with *Rhizoctionia solani* are the major cause for drycore damage on potatoes. This constellation could therefore imply that *R. solani* AG 3 is the causal agent of drycore

## CONCLUSIONS

AG 3 is the most frequent anastomosis group of *Rhizoctonia solani* for isolates obtained from black scurf and drycore on potatoes in Switzerland. The fifty-nine AG 3 isolates analysed in this study are grouped into four clusters by ERIC and REP-PCR. The genetic similarity ranges between 20.4 and 86.9%. The genetic relatedness can not be explained by the geographical region or by the potato variety from which the isolates were obtained, but most isolates from organic fields belong to the same cluster. Further experiments under controlled conditions have to be conducted to proof that *R. solani* AG 3 is the causal agent of drycore.

# 6. Drycore symptoms on potatoes require both *Rhizoctonia solani* AG 3 infection and wounds on tubers

## ABSTRACT

Drycore is an important quality deficiency in Swiss potato production especially in organic agriculture and in crop rotations with a high percentage of grass clover leys. The hypothesis that injuries of wireworms on the tubers facilitate the penetration of *Rhizoctonia solani* AG 3 into the tuber and favour the formation of drycore was tested with open air experiments and pot experiments under controlled conditions. Isolates of *R. solani* originating from black scurf and drycore from harvested tubers were identified using pectic zymograms. ITS-rDNA primer specific amplification, ITS sequencing and ISSR-PCR fingerprinting was used to check if isolates of *R. solani* isolated from black scurf and drycore on harvested tubers shared identical fingerprint genotype to the isolates found on the seed tubers used in the field experiments and to the isolate used to inoculate the soil in the pot experiments. Drycore symptoms on harvested tubers were only observed in treatments where *R. solani* was present and potato tubers were injured either naturally by wireworm or artificially with needles. Non-wounded tubers rarely had drycore symptoms, even when they were infested with black scurf. In the treatments without *R. solani* infection black scurf or drycore was not observed. All isolates of *R. solani* from black scurf or drycore symptoms belonged to AG 3. Furthermore, all isolates obtained from black scurf and drycore in the pot experiments presented an identical fingerprinting genotype as the isolate used to inoculate the sterilized soil. These results strongly supports the hypothesis that drycore is the result of wounds caused by wireworms and *Rhizoctonia solani* AG 3.

## INTRODUCTION

*Rhizoctonia solani Kühn* (telemorph: *Thanatephorus cucumeris* (Frank) Donk) is a soil-borne plant pathogen affecting many agricultural crops worldwide (Ogoshi, 1987; Sneh et al.,1991). On potatoes (*Solanum tuberosum* L.) the disease causes delayed emergence, lesions on stems (stem canker) and stolons and sclerotial formation on tubers (black scurf) (Baker, 1970; Anderson, 1982). Infections on stolons may induce the development of malformed tubers and tubers with growth cracks (Weinhold et al., 1978; Jeger et al., 1996) (Appendix, Fig.A4 a-c, h). Besides stem canker and black scurf, other symptoms have also been attributed to *R. solani*, most notably drycore (Appendix, Fig. 4A c-g). Drycore is characterized by restricted brown cavities up to several mm deep with a diameter of 3 to 6 mm (Schwinn, 1961; Grütte, 1940; Ramsey, 1917; Müller, 1925). Other symptoms are scabby lesions on tuber skin which closely resemble netted scab symptoms caused by *Streptomyces reticuliscabiei* and some strains of *S. europaeiscabiei* (Bocheck-Mechiche et al., 2000).

The most useful system for the classification of *R. solani* is based largely on hyphal fusion with anastomosis tester isolates. To date, fourteen anastomosis groups (AG 1 to AG 13 and AG BI) have been identified and seven of them (AG 1, AG 2, AG 3, AG 4, AG 6, AG 8, AG 9) are divided into sub-groups that differ in pathology, biochemical or genetic characteristics (Anderson, 1982; Cubeta and Vilgalys, 1997; Gonzalez et al., 2001, Carling et al., 2002). AG 2-1, 3, 4, 5, 8, and 9 are capable of causing moderate to extensive damage to potato plants (Banville GJ et al., 1996). AG 3 has been known as the most host-specific type of *R. solani* causing disease on potatoes. It is by far the most important cause of black scurf because most of the sclerotia isolated from potato tubers belong to AG-3 (Carling and Leiner, 1986; Chand and Logan, 1983; Campion et al., 2003). AG 3 also causes leaf blight of tomato (Date et al., 1984) and leaf spot of tobacco (Meyer et al, 1990).

In contrast to many other rhizoctonia diseases, rhizoctonia disease of potato has an important seed-borne phase that provides a mechanism for long distance dispersal of the pathogen (Ceresini, 2002). Once established in the soil, the mycelium and sclerotia of the pathogen may provide an additional source of primary inoculum. The key for controlling this disease lies in the use of scurf-free seed tubers. However, the planting of scurf-free seed tubers alone will not guarantee a disease-free crop. The soil-borne inoculum needs to be kept minimal by the use of crop rotations (Banville et al., 1996). Three-year rotations or longer should be used (Scholte, 1992). In Alaska AG-3 was recovered from soil free of potato cropping for two years but not after five years or more (Carling et al., 1986).

In a three year survey on a total of 278 potato fields in Switzerland the occurrence of drycore was significantly more frequent and higher in all three years on organic farms. In chapter, 4 it has been shown that wireworm damage, seed quality and grass clover ley as a preceding crop significantly influenced the infestation level of drycore. Most isolate of *R. solani* from this survey were assigned to AG 3. Based on these results the hypothesis was put forward that the occurrence of drycore damage on potatoes might be caused by the association between the fungus *R. solani* AG 3 and the incidence of wireworm lesions on tubers (chapter 5). Dryocre is characterized by brown circles of 3 to 6 millimetres on the tuber surface and cavities up to several millimetres deep, whereas wireworm cause narrow tunnels in the tuber flesh. Wireworms are enhanced by grass clover ley in the crop rotation (Parker and Seeney, 1997;

Chapter 3), this might partly explain in part the higher occurrence of drycore in the organic agriculture as it is much more common to grow potatoes after several years of grass clover ley.

Wireworms are the larvae of click beetles (Elateridae). There are about 150 species of click beetles recorded in middle Europe, but only few of them can be classified as pests. By far, the most important pest species are those belonging to the genus *Agriotes*. Until recently, wireworms were regarded as a minor pest of potatoes, but locally important in mixed arable and livestock farming areas. However, in the last couple of years, wireworm damage has become an increasing problem including the occurrence of damage in fields in all-arable rotations even without long-term grassland history (Parker and Howard, 2001; Demmler, 1999). The reasons for this apparent change in pest status of wireworm are not clear, but there is some evidence that crops following long-term set-aside (1-5 years fallow) provide a suitable habit for wireworm and this may be contributing to the observed problems in arable rotations (Hancock et al., 1992; Parker, 1999). However, the claimed increase of wireworm damage in Switzerland might as well be influenced by the steady decrease of treatments with persistent insecticides that has occurred since the early 1990`s following changes in agricultural policies that favour environmentally friendly and sustainable farming systems. Another contributing factor could be the increasingly stringent quality demands from retailers, because an increasing part of washed potatoes is now sold and even smallest injuries are recognized by the consumer. The percentage of washed potatoes on the market increased in Switzerland from 30% in 1980 to more than 90% in 2005 (F. Stucki, Fenaco, Switzerland, pers. comm.).

In this study we tested the hypothesis that injuries of wireworms on potato tubers facilitate the penetration of *R. solani* AG 3 into the tuber and favour the formation of drycore symptoms. For this purpose we conducted open air experiments and pot experiments under controlled conditions in a climate chamber. We expected that *Rhizoctonia solani* causes drycore symptoms only when potato tubers were wounded either naturally or artificially by needles.

# MATERIAL AND METHODS

## Fungal isolates

*Fungal inoculum in the open air experiments*

Potatoes of the varieties “Agria” and “Innovator” either absolutely free of sclerotia or with about 25% of the tuber surface infected with sclerotia were selected from commercial seed potato lots from the seed company Semag, Lyssach, BE.

*Fungal inoculum in pot experiments in the climate chamber*

A *Rhizoctonia* isolate from the anastomosis group 3 (AK001b, Table 6.1) was used for soil infestation in the climate chamber experiments. This isolate was obtained from infested seed potatoes variety “Agria” used in the open air experiment (see below). Millet grains were soaked in water overnight, than sterilized three times for 45 minutes at 121°C, with an interval of 24-30 hours. Half of a full PDA plate colonized by *R. solani* mycelium was added per 300 ml of millet and incubated for three weeks at room temperature. Subsequently, 100 ml of infested millet grains were mixed with sterilized soil into 14 l pots and incubated for two weeks. In the control treatment sterilized millet non-infested with *R. solani* mycelium was added to the soil.

*Pathogen isolation*

From all experiments, pieces of potato tubers with drycore symptoms and sclerotia were plated on 1.5% tap water agar amended with 250 ppm chloramphenicol (WAC) and incubated for two to three days at room temperature. After a second transfer to WAC, isolates were transferred to PDA. Pure cultures of the isolates (Table 6.1) were stored at 10°C.

*Identification through Pectic zymograms*

Analysis of pectic zymograms was used as a routine method for rapid assignment of *R. solani* isolates to AG groups (Gruickshank 1990; Mac Nish et al. 1993; Schneider et al. 1997). Pure cultures were grown on pectin medium with citrus pectin (Sigma P9135, local) as a carbon source. Isolates were incubated for 10 days at 23°C in the dark. Ten µl of the culture filtrate was loaded on a native polyacrylamide gel, with 1.5 M Tris/HCL, pH 8.8, in the separating gel and 0.5 M Tris/HCl, pH 6.8, in the stacking gel. These native gels were run for 70 min in running buffer and were subsequently incubated in 0.1 M DL-malic acid for 1 hour followed by staining with 0.02% ruthemium red for 2 h. AG-3 and AG-5 anastomosis tester isolates were loaded on each gel as a reference isolates, for comparison. Gels were fixed in 3mM $Na_2C0_3$, scanned and sealed in cellophane (Grosch et al. 2004).

*Molecular discrimination between AG 3 isolates*

Total genomic DNA was extracted from lyophilized mycelium of *R. solani* grown for 5 days at 25°C in potato-dextrose broth (Difco, local) using the DNeasy Plant Mini DNA extraction kit (Qiagen GmbH, Hilden, Germany), according to the specifications of the manufacturer. Amplification was performed in a 25-µl PCR reaction mixture containing 1x PCR buffer (0.2 mmol $l^{-1}$ each dNTPs, 1.5 mmol $l^{-1}$ of $MgCl_2$, 2.5 U Taq polymerase), 3.5 mmol $l^{-1}$ $MgCl_2$, 0.2 µmol $l^{-1}$of the primers PT1 and PT2 (Table 6.2) and 5ng of template DNA. The PCR - amplifications were carried under following conditions: initial denaturation at 94°C 2 min and 30s, followed by 40 cycles of denaturation at 94°C for 1 min, 63°C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Reaction products were separated on agarose gels, stained with ethidiumbromide and visualized under UV-light.

The rDNA internal transcribed spacer (ITS including 5.8S rDNA) sequences were determined for some isolates described in Table 6.1. In total, nine isolates were sequenced using primers ITS4 and ITS5 (White et al., 1990) (Table 6.2). Sequencing was performed on an ABI 3100 Sequencer using Taq-Cycle automated sequencing with DyeDeoxy Terminators (BigDye Terminator v3.0 Cycle Sequencing Ready Reaction; Applied Biosystems) with both primers separately to ensure reliability of the sequence data. Sequences were assembled and aligned using Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, MI). BLAST searches (Altschul et al., 1997) were done against the NCBI/GenBank databases.

Two primers, -CAG and – CCA (Table 6.2) were used for amplification in independent reactions in order to improve the discrimination between clones of the fungus by increasing the number of total bands produced. Each PCR mixture contained 10-20 ng of DNA in a 20 ml reaction volume containing 10 pmol of ISSR primers, 100 mM of each nucleotide, 2 ml of 10X PCR buffer (1X PCR buffer: 10 mM KCl, 10 mM (NH4)2SO4, 20 mM Tris-HCl, 2 mM MgSO4, 0.1% Triton X-100, [pH 8.8]), and 1 U of /Taq/ DNA Polymerase (New England Biolabs, USA). The PCR amplifications were carried out under the following conditions: initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30s, 52°C for 30 s, and 72°C for 1 min and 30 s, with a final extension at 72°C for 5 min. The PCR reactions were electrophoresed for 3 h on 1.5% agarose gel and the resulting banding pattern was documented.

Table 6.1 Isolates of *Rhizoctonia solani*[1)] obtained from sclerotia and drycore symptoms in open air experiments and pot experiments in climate chambers analysed in this study.

| Isolate | year of isolation | Source | [2)]ITS-rDNA sequences deposited at GenBank(R) |
|---|---|---|---|
| Open air experiment: | | | |
| AK0501 | 2005 | sclerotia | EF370434 |
| AK0502 | 2005 | sclerotia | EF370436 |
| AK0503 | 2005 | sclerotia | |
| AK 05028D | 2005 | drycore | |
| AK 05039S | 2005 | sclerotia | EF370440 |
| AK 05039D | 2005 | drycore | EF370438 |
| AK 050310S | 2005 | sclerotia | |
| AK 050310D | 2005 | drycore | EF370448 |
| AK 050311S | 2005 | sclerotia | EF370444 |
| AK 050311D | 2005 | drycore | EF370442 |
| AK 050312 S | 2005 | sclerotia | EF370446 |
| AK 050314D | 2005 | drycore | EF370447 |
| Climate chamber experiment: | | | |
| AK001a | 2005 | sclerotia | |
| AK001b | 2005 | sclerotia | |
| AK001bR1 | 2005 | drycore | |
| AK001bR2 | 2005 | drycore | |
| AK001bR4 | 2005 | drycore | |
| AK001bR5 | 2005 | drycore | |
| AK001bR6 | 2005 | sclerotia | |
| AK001bR7 | 2005 | sclerotia | |
| AK001bR8 | 2005 | sclerotia | |
| AK001bR9 | 2005 | sclerotia | |
| AK001LV11 | 2005 | drycore | |
| AK001LV12 | 2005 | drycore | |
| AK001LV13 | 2005 | drycore | |
| AK001LV14 | 2005 | drycore | |
| AK001LV15 | 2005 | drycore | |
| AK001LV16 | 2005 | drycore | |
| AK001LV17 | 2005 | drycore | |
| AK001LV31 | 2005 | drycore | |
| AK001LV32 | 2005 | drycore | |
| AK001LV33 | 2005 | drycore | |

[1] All isolates revealed the typical pectic zymogram for AG 3 and produced a positive PCR amplification with AG 3 specific primers (see chapter 5).

[2] All these sequences were similar (by BLASTN searches; Altschul et al., 1997) to sequences of ITS-5.8S rDNA from AG 3 of *R. solani*.

Table 6.2 Primers used for PCR amplifications and DNA sequencing

| Objectives | Primers | Sequences (5’-3’) |
|---|---|---|
| PCR detection of AG 3 by ITS-rDNA primer specific amplification | PT1 | GTTTGGTTGTAGCTGGTCT |
| | PT2 | CTGAGATCCAGCTAATAC |
| PCR amplification and ITS-rDNA sequencing | ITS1 | TCCGTAGGTGAACCTGCGG |
| | ITS4 | TCCTCCGCTTATTGATATGC |
| ISSR fingerprinting | CAG | CAG CAG CAG CAG CAG |
| | CGA | DHB1) CGA CGA CGA CGA CGA CGA |

1) DHB = three degenerated bases at the 5’-end.

***Wireworm (Agriotes ssp.)* inoculum**

Seven hundred wireworms were collected from a field in Wallenstalden, BE, with history of a massive wireworm damage. The wireworms were stored at 10°C in pots filled with soil from the field, until ready for using as inoculum. Oats were sown to feed the wireworms.

**Open air experiments**

*Location*

In 2005 two field trials were conducted on a farm field in Zollikofen, BE. There were no grass clover leys on this field since 1990 and no potatoes were planted on it since 1995. Therefore the soil should be free of wireworms (*Agriotes* ssp.) and soil-borne *R. solani*. The soil was a sandy loam with 3.5% organic matter and a pH of 6.5. The soil nutrient status was slightly enriched for P and K and adequate for Mg with 104 mg/kg, 274 mg/kg and 103 mg/kg ammoniumacetat-EDTA extractible content respectively.

*Experiment A*

Two potato tubers of the variety Agria (either absolutely free of sclerotia or with 25% of the tuber surface infested with sclerotia) were planted per plot, on April 6th. The plots with a surface of 0.6 x 0.4 m were enclosed with metal frames which were put 0.25 m deep in the soil to make sure that the wireworms would not escape from the plot. Twenty wireworms were released in half of the metal frames at the beginning of tuberization, on June 6th 2005. The experimental design was a complete-randomized block design with eight repetitions.

Chemical haulm destruction was done on July 28th. Potatoes were harvested on August 24th. Disease rating was done just after harvest.

*Experiment B*

One potato tuber of the variety Innovator absolutely (either absolutely free of sclerotia or with 25% of the tuber surface infested with sclerotia) were planted in 12 l pots with a diameter of 0.33 m, on April 20th. The pots were filled with the soil of the field (see location) and put in the ridges of the potato field so that the potatoes could grow under field conditions. Ten wireworms were released in half of the pots at the beginning of tuberisation, on June 12th. Chemical haulm destruction was done on July 28th, potatoes were harvested on August 31st. Disease rating was done just after harvest.The experimental design was a complete-randomized block design with six repetitions.

### Climate chamber experiments

Potatoes of the variety Charlotte absolutely free of sclerotia were used in these pot experiments. Instead of using wireworms, in half of the potatoes 20 holes with the depth of 1 mm were made with a thin sterilized needle, the other half of the potatoes were kept unwounded. These trials were meant to test if mechanical injuries favour the penetration of *R. solani* into the tuber. Four potato tubers either with or without holes were planted per pot. The pots were distributed in a randomized complete block design with four replications in a climate chamber at 20°C and 85% relative humidity with a day/night regime of 16 and 8 hours. After 10 days of incubation, the potatoes were checked for sclerotia and drycore symptoms. The experiment was repeated three times (September 2005, January 2006 and March 2006) with the same potato stock.

### Disease rating and statistical analysis

In the climate chamber experiments the incidence of *Rhizoctonia* disease on potatoes was visually rated considering the presence of sclerotia and drycore. Presence of sclerotia was expressed on a scale from 1 to 7 (1= no sclerotia, 7 > 75% of the tuber surface with sclerotia). Percentage of tubers with drycore and numbers of drycore per tuber were noted. In the open air experiments also malformed tubers were counted and yield (expressed in g per plant) was measured. Incidence of wireworms was rated in all experiments counting the number of holes per tuber.

The data were statistically analysed for normal distribution and comparable variability and subjected to analysis of variance (ANOVA). To compare the treatment means, Tukey-Kramer-Test was conducted at P = 0.001. All statistical analysis was done with NCSS 2004 (Number Cruncher Statistical Software, Kaysville, Utah 84037 Utah, USA).

## RESULTS

### Open air experiments

In both experiments (A and B), a significant amount of drycore symptoms (37% and 53% damaged tubers respectively) were only found when *R. solani* infested seed tubers were planted and wireworms were added (Fig. 6.1B and 6.2B). Treatments with rhizoctonia infested seed tubers (R+W-, R+W+) had a high level of black scurf with 78% and 73%, respectively in experiment A and 96.5% and 88%, respectively in experiment B (Fig. 6.1A and 6.2A). Malformed tubers (Fig. 6.1C and 6.2C) were only found when *R .solani* infested seed was planted. Absolutely no symptoms of *R. solani* were found in the treatments with clean potato seeds. Wireworms alone caused the typical symptoms of wireworm damage (6.1D and 6.2D). In both trials, tuber yield was significantly higher in the control treatments without *R. solani* and wireworm (data not shown).

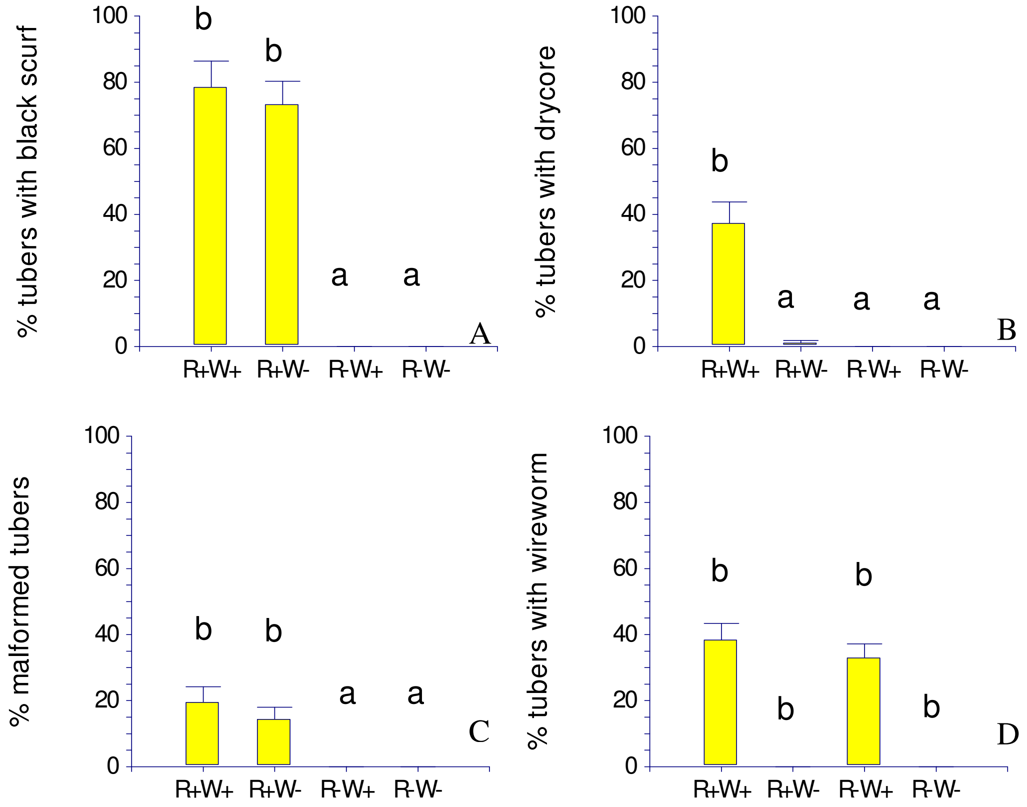


Fig. 6.1 A-D. The effect of seed healthy quality and wireworm on the incidence of black scurf (A), drycore symptoms (B), malformed potato tubers (C) and wireworm damage (D) in the open air experiment A. The treatments consisted of: R+W+: seed tubers with sclerotia + wireworm, R+W-: seed tubers with sclerotia, no wireworms, R-W+: clean seed + wireworm, R-W-: clean seed, no wireworm. Bars indicate the standard error of the mean. Treatments with different letters are statistically different at p=0.001.

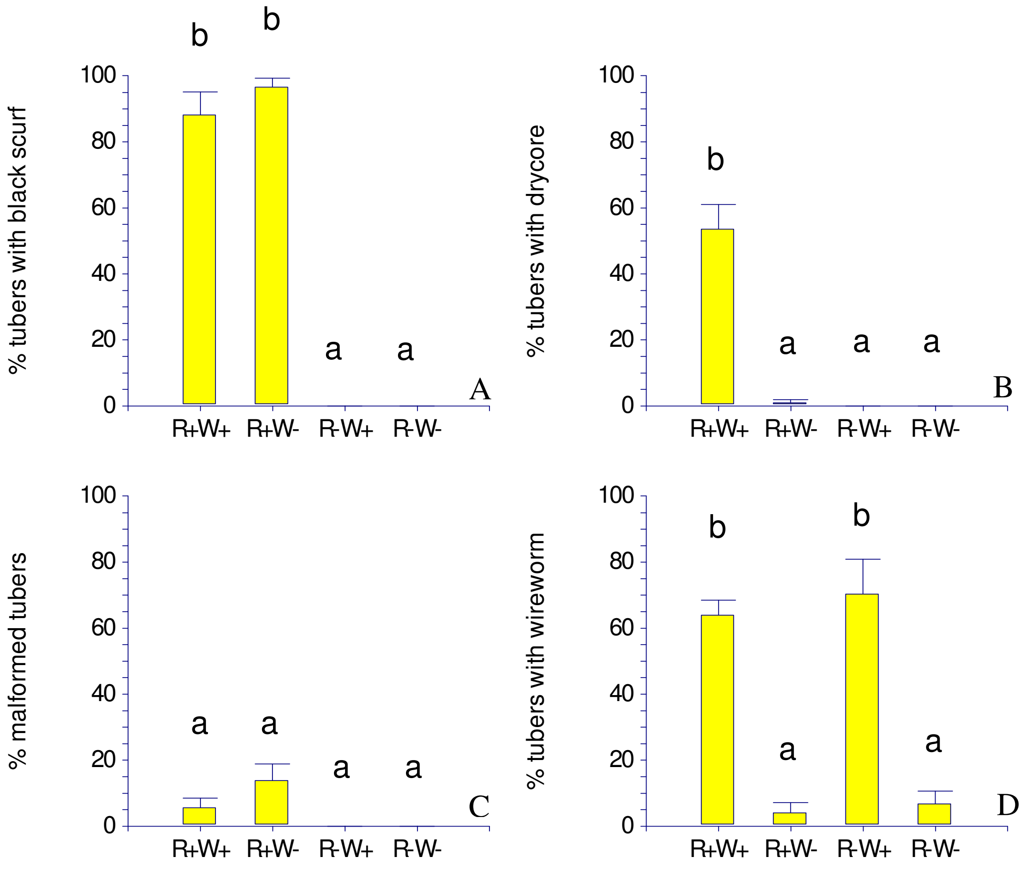


Fig. 6.2 A-D The effect of seed healthy quality and wireworm on the incidence of black scurf (A), drycore symptoms (B), malformed potato tubers (C) and wireworm damage (D) in the open air experiment B. The treatments consisted of: R+W+: seed tubers with sclerotia + wireworm, R+W-: seed tubers with sclerotia, no wireworms, R-W+: clean seed + wireworm, R-W-: clean seed, no wireworm. Bars indicate the standard error of the mean. Treatments with different letters are statistically different at p=0.001.

## Climate chamber experiments

In all three pot experiments *R. solani* AG 3 isolate AK001b formed drycore symptoms within 10 days if the tubers were wounded with needles (Fig. 6.3 A+B). In the first and second experiment (Fig. 6.3 A) drycore symptoms occurred only around the needle holes and not on another area of the tuber and no drycore symptoms on non-wounded tubers. In the third trial (Fig. 6.3 B) thirteen percent of the drycore symptoms were observed apart from the needle holes and in contrast to the first to trials drycore symptoms occurred also in the treatment with non-wounded tubers. Like in the field experiments, absolutely no drycore symptoms or black scurf were found in the treatments without *R. solani.* The isolate of *R. solani* produced black scurf with a score between 2 (0.1-5% of tuber surface) and 3 (5.1-10%). Formation of black scurf was similar on both treatments (with and without needle holes) in both first and second trials, whereas in the third trial black scurf in the treatment with needle holes was significantly higher (p=0.001).

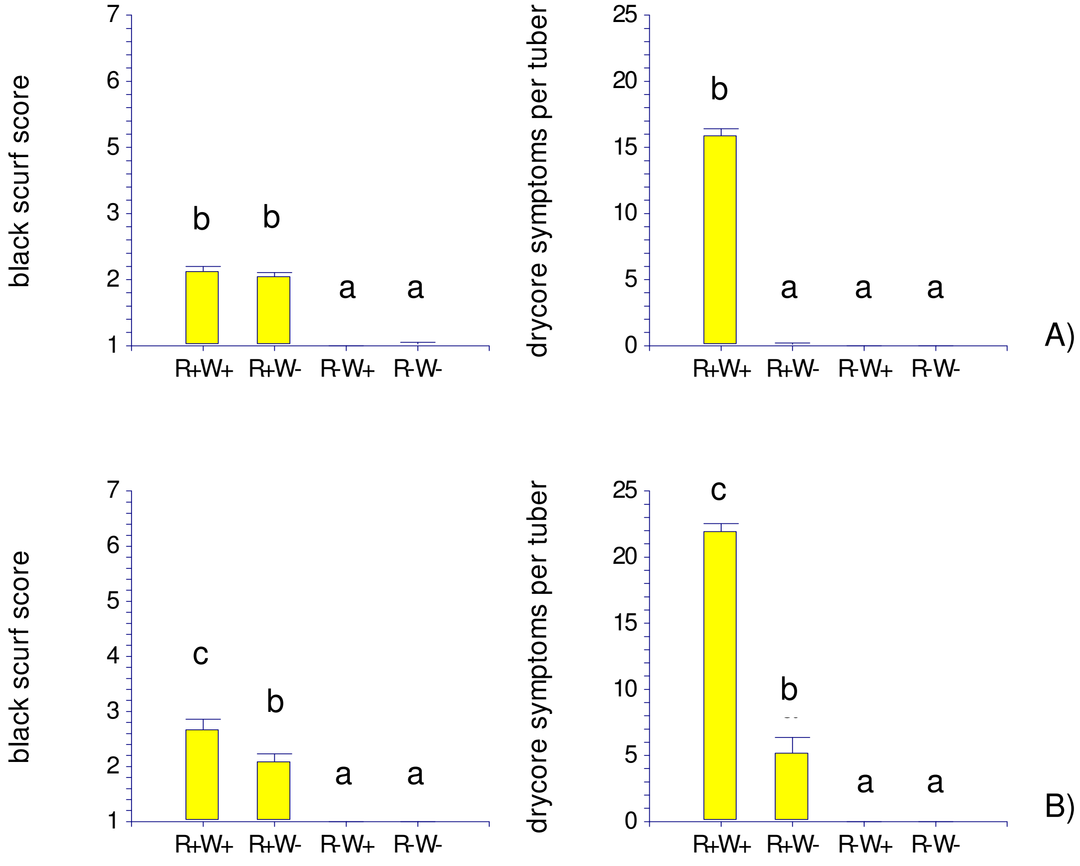


Fig. 6.3 Black scurf and drycore symptoms on tubers of potato cv. “Charlotte” 10 days after planting in infested soil. A) Results of the first and second experiment (Sept. 2005, Jan. 2006). B) Results of the third experiment (March 2006). The treatments were as follows: R+W+ = soil infested with *R. solani*, each tuber with 20 needle holes, R+W-= soil infested with *R. solani*, non-wounded tubers, R-W+: sterile soil, each tuber with 20 needle holes; R-W-: sterile soil, non-wounded tubers. The black scurf incidence varied from 1 = 0% to 7 = 75-100% of the tuber surface covered with sclerotia. Treatments with different letters are statistically different at p=0.001. Error bars indicate the standard error of the mean.

## Discrimination between isolates

### *Pectic zymograms and AG-3 specific primers*

All isolates obtained in the open air and climate chamber experiments from either drycore symptoms or from sclerotia present on the tubers surface (Table 6.1) showed pectic zymogram pattern typical for AG 3. For all isolates an amplicon was produced with AG 3 specific primers. Figures of positive PCR-amplification from representative isolates of AG 3 are presented in Chapter 5. Sequences of the ITS-5.8S rDNA from few of these isolates were similar to sequences of *R. solani* AG 3 deposited in the GenBan(R) / NCBI. Similarity of these isolates was between 99.08 and 100%. Isolates obtained from sclerotia and drycore on the same tuber were identical (AK0503_9S / AK0503_9D and AK0503_10S / AK0503_10D respectively).

### *ISSR- PCR Fingerprinting*

#### *Open-air experiments*

Based on ISSR – PCR (Fig. 6.4), three distinct fingerprint patterns were observed with both primers amongst the isolates of AG 3 obtained from black scurf and drycore on the tubers harvested in open-air experiment B: Pattern 1 = isolate AK0503_11D and AK0503_11 S, pattern 2 = isolates AK0503_9S, AK0503_9D, AK0503_10S and AK0503_10D, pattern 3 = isolate AK0503_12S). Fingerprint patterns of isolates obtained from drycore symptoms and sclerotia on the same tuber were identical (e.g. AK0503_9S and AK0503_9D). The fingerprint from isolate AK0503 obtained from sclerotia on a seed tuber used in this experiment was different from the fingerprints of isolates on harvested tubers. In the control treatments where seed tubers absolutely free of sclerotia were planted, absolutely no symptoms of *R. solani* were found.

#### *Pot experiments in a climate chamber*

The fingerprint patterns of the isolate AK001b (which was used to infest the sterile soil) and the fingerprints of the isolates from drycore symptoms and sclerotia in all three pot experiments were identical (Appendix, Fig. A2 and A3). Thus all drycore symptoms and black scurf found in all three pot experiments were caused by the isolate AK001b of *R. solani* AG 3.

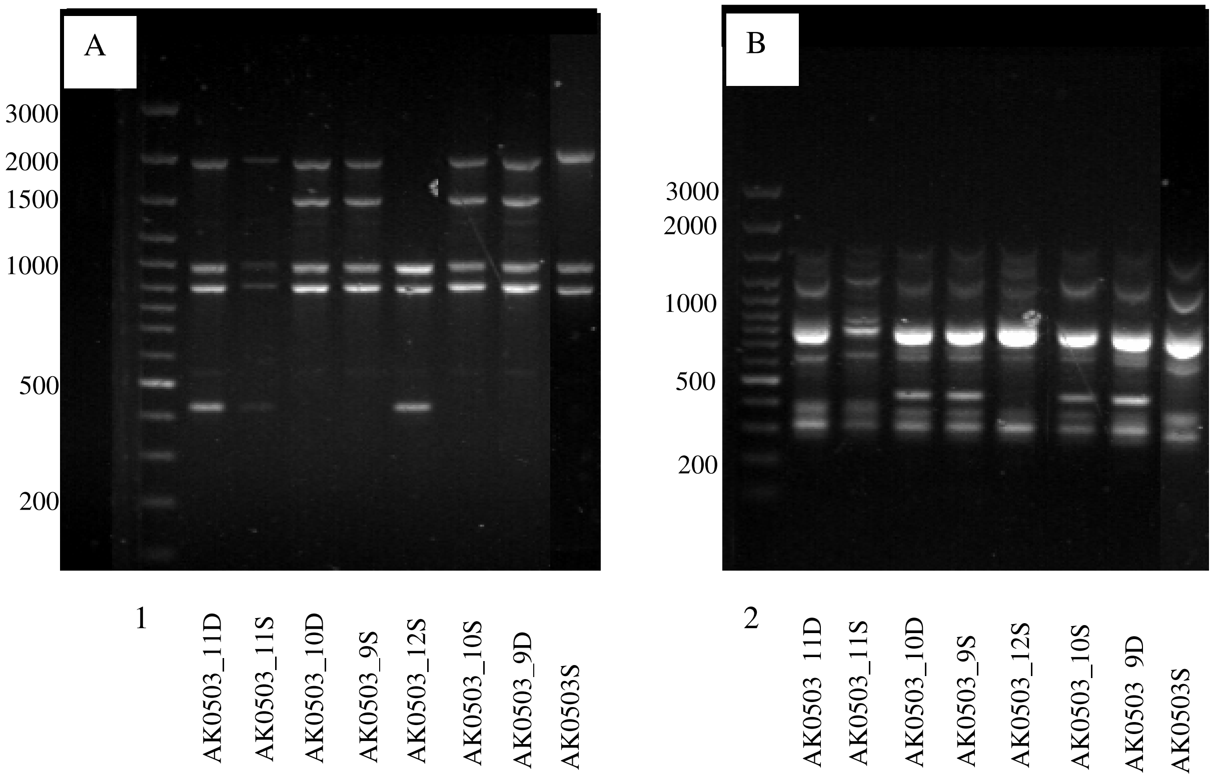


Fig. 6.4 Fig. 6.4 ISSR-PCR fingerprints obtained of *R. solani* isolates from seed (AK0503) and harvested tubers from the open-air experiment B. S = obtained from sclerotia, D = obtained from drycore. Lane 1 and 2 are 100-bp ladders Plus (PeQlab). For the ISSR fingerprinting primers CAG (A) and CGA (B) were used. Based on pectic zymogram profile, all isolates belonged to AG 3. Isolates with the same number (e.g. AK0503_9S and 9D) were isolated from the same tuber.

## DISCUSSION

The hypothesis that injuries on tubers caused by wireworm or other means may facilitate the penetration of *R. solani* in to the tuber and favour the formation of drycore symptoms was tested with open-air and climate chamber experiments. The observations in our experiments are in line with the results in chapter 4 which showed that drycore damage was only observed when seed potatoes infested with black scurf were planted in fields where wireworm damage occurred. The general accepted opinion is that drycore symptoms are caused by *R. solani* only if lenticels tend to form excrescences under wet conditions, especially in soils with higher clay content. However, we found that drycore damage was on the same level in all three years of a survey conducted in Switzerland between 2001 and 2003 (Chapter 3). This level of incidence of drycore was not reduced in the exceptionally dry and hot year 2003 (600 to 750 mm annual precipitation in all regions, compared to 1150 – 1450 mm and 1000 – 1300 mm in 2001 and 2002 respectively (Bundesamt für Meteorologie und Klimatologie, 2006). The interaction between *R. solani* and wireworm observed in our field experiments, could explain the important drycore damage in the extremely dry year 2003 and also the higher occurrence of drycore in organic agriculture (chapter 3). In organic agriculture potatoes are often grown after grass clover leys and wireworm populations are enhanced by grass clover in the crop rotation (Parker and Seeney, 1997; chapter 3). In addition, no effective seed treatment against *R. solani* is available in organic agriculture and the initial level of seed-borne inoculum is higher as organic seed lots often exceed the threshold for black scurf, (chapter 3).

The results of pot experiments where tubers were wounded by needles suggest that wounds caused by other means than wireworm also favour the formation of drycore symptoms by *R. solani* AG 3. Drycore symptoms occurred only where the tuber was injured by the needle. Only in the third experiment (where potatoes had been stored for five month before usage) drycore symptoms were found apart form needle holes. It could be that the tuber skin got weaker during storage and facilitated the penetration of the fungus. Also Schwinn (1961) presented evidence from greenhouse trials that infections through mechanical wounds can cause drycore symptoms, but unfortunately the anastomosis group of the isolates he used is unknown.

Considering other possible wound agents on potato tubers, we found no relation between slug damage and drycore on the same tuber (chapter 4).

Pectic zymogram, positive PCR- amplification with AG 3 specific primers and sequences of the ITS-5.8S show that all isolates obtained from black scurf or drycore symptoms in the open air experiment belonged to *R. solani* AG 3. Considering the DNA-fingerprinting techniques used in this study (ISSR-PCR fingerprints) the isolates showed three distinct fingerprint genotypes on the harvested tubers, which were different from the fingerprint of the isolate obtained from sclerotia on the seed used in the experiment. Considering that absolutely no symptoms of *R. solani* were found in the control treatments with clean seed, the distinct genotypes of AG 3 were probably imported through the infested seed tubers used in the experiments (Ceresini, 2003). The 15 year-rotation could definitely reduce the inoculum of the pathogen in the soil. Carling et al. in Alaska (1986) recovered isolates of AG 3 from soil free of potato for two years but not from soil free of potato for five years and more. Furthermore, all isolates obtained from black scurf and drycore in the pot experiments presented an identical fingerprinting genotype as the isolate used to inoculate the sterilized soil. This indicates that sclerotia and drycore on tubers from the pot experiments were caused by the same isolate of *R. solani* AG 3.

## CONCLUSIONS

Our observations from this study strongly support the hypothesis that injuries on potato tubers by wireworm or other means can cause drycore damage even under dry soil conditions if the potato seed tubers are infested with *R. solani* AG 3. Important measures to reduce the risk for drycore and to avoid important losses to the potato crop are the use of clean seed tubers without black scurf and the reduction of wireworm incidence by optimized crop rotations without grass clover leys or other crops which harbour the insects. Further experiments under controlled conditions should be conducted to test *R. solani* isolates of other AGs for their potential to cause drycore symptoms and to investigate whether injuries on tubers caused by other pests than wireworm favour the formation of drycore.

# 7. General discussion and conclusions

The aim of this thesis was to investigate the relative importance of quality deficiencies caused by wireworms (*Agriotes* ssp.), slugs (*Deroceras reticulatum, Arion ssp.*), drycore and black scurf (*Rhizoctonia solan)* as well as the influence of farming systems, specific cultivation methods and site parameters on the occurrence of these outer quality deficiencies in an on-farm survey. Furthermore possible relations between these quality deficiencies were analysed. Additional open air and pot experiments were conducted to check the hypothesis that the occurrence of drycore damage on potatoes might be caused by the association between the fungus *R. solani* AG 3 and the incidence of wireworm lesions on tubers, which was based on the results of the survey. Outer quality deficiencies have become more important in Switzerland because today more than 90% of the sold potatoes are washed, compared to 30% in 1980. In the quality assessment performed by the trade quality deficiencies caused by wireworm, drycore and slugs are not distinguished in detail but subsumed under the generic term “outer quality deficiencies”. Therefore the relative importance and the influencing factors for the claimed quality problems are unknown.

The results of the on-farm survey (chapter 3 and 4) clearly showed that outer quality deficiencies caused by wireworm, slugs and drycore were responsible for important economic losses in all farming systems in Switzerland. For all three deficiencies the quality damage was higher in the organic farming system. For drycore this difference was significant. For 36% of the organic fields the quality of the harvested potatoes was below standard (limit = 18% of damaged tubers for washed potatoes) due to these outer quality deficiencies compared to 13% and 12% in the integrated and conventional farming system respectively. For all other quality deficiencies assessed in the on-farm survey (e.g. powdery scab, *Spongospora subterranea*) no influence of the farming system was observed. In the past, symptoms of these outer quality deficiencies were probably often mixed up, because quality assessment in trade is mainly done with unwashed tubers. The relative importance of slugs as a potato pest was much higher in our survey than expected. Whereas slugs are an important pest for potatoes in Britain (Hommay 1995), they have been considered a minor problem on the continent so far. No clear influence of the soil characteristics was observed.

On 29% of the organic fields drycore damage alone led to a price reduction, compared to 3% in the integrated and conventional farming systems. Thus drycore was the most important outer quality deficiency in the organic farming system. Only few scientific studies were conducted about drycore and most of them 1961 or earlier. Drycore damage seems to be of regional importance, as it is more or less unknown in many potato growing areas. But discussions with researchers and farmers during the last five years confirm that drycore is also an increasing problem especially on organic farms in some regions in Germany and Austria. Drycore, restricted brown cavities up to several mm deep with a diameter of 3 to 6 mm, was first described by Ramsey (1917) and Müller (1925) and has been attributed to *Rhizcotonia solani*. Based on the results of the on-farm survey we hypothesized that drycore is caused by *R. solani* and that the higher occurrence of drycore in the organic farming system is caused by an interaction of fungus and wireworms (*Agriotes ssp.*).

Schwinn (1961) presented evidence that *Rhizoctonia solani* is the causal agent of the drycore disease of potato tubers. Infections with the mycelium of *R. solani* were successful both through mechanically wounded normal lenticels as well as naturally grown lenticel excrescences. Unfortunately, the anastomosis group is unknown for the isolates that Schwinn used; since then, nobody successfully repeated Schwinn's experiments. Occurrence of drycore in our survey was on the same level in all three years and could not be explained by the soil type or meteorological conditions. These results are in contradiction to Schwinn (1961) who postulated that the formation of drycore symptoms is favoured by wet and heavy soils. In the organic farming system drycore damage was significantly higher when more than 20% of the seed tubers were infested with black scurf and drycore symptoms on tubers were never observed on plants without any symptoms of *Rhizoctonia solani.* In all three farming systems important drycore damage was never observed on fields with both a low occurrence of black scurf on the seed tubers and a low occurrence of wireworm damage. Even on fields with heavy black scurf infestation on progeny tubers, drycore occurred very rarely without wireworm wounds on the tubers. These results were confirmed by the calculation of the relative risk for drycore from data of 32'000 assessed tubers of 40 fields with important drycore damage. Tubers had a higher risk for drycore if black scurf or wireworm damage were on the same tuber. In contrast, no higher risk for drycore was observed on tubers with slug damage. The analysis of variance for drycore showed a significant influence of wireworm damage, black scurf level on seed tubers and grass clover as preceding crop on the infestation level of drycore. Abiotic factors like solid manure use, organic matter, texture and pH of the soil had no significant influence on the level of drycore (chapter 4). Wireworms were enhanced by grass clover ley in the crop rotation (chapter 3). This is consistent with observations of Parker and Seeney (1997). This explains in part the higher occurrence of drycore in the organic farming system where it is much more common to grow potatoes after several years of grass clover ley. Grütte (1940) also observed that occurrence of drycore was higher after ploughing of grassland. The higher proportion of seed lots that exceeded the threshold for black scurf in the organic system (Chapter 3) and the lack of an effective seed treatment could be other reasons for the severe drycore problem in organic agriculture. This underlines the importance of improved seed quality. Our results suggest that a promising issue for future research might be to develop methods to produce seed free of black scurf in organic production and to find biological control methods against wireworm.

The variable significance of drycore in the three farming systems (Chapter 3) cannot be explained by the occurrence of different anastomosis groups. AG 3 seems to be the most frequent anastomosis group in Swiss potato production independently from the farming systems. Genetic characterization of 60 isolates of *Rhizoctonia solani* obtained from sclerotia and drycore symptoms (2003 and 2005) with pectic zymogram and AG-specific primer showed that all but one isolate (AG5) belonged to AG 3 (Chapter 5). This is consistent with (Chand and Logan, 1983) who regard AG 3 as the most important anastomosis group of *Rhizoctonia solani* on potatoes. The analysed AG 3 isolates showed a high level of heterogeneity among 59 isolates of AG 3 with a genetic similarity ranging from 20% to 87% and were grouped into four clusters based on polymorphic patterns of ERIC and REP-PCR (Chapter 5). In contrast to Takeshi et al. (1999) no higher genetic similarity could be observed among isolates of the same region or the same variety. Tubers infested with sclerotia or mycelia are the most important factor in the epidemiology of black scurf (Anderson 1993) and therefore seed sources may contribute to the genetic diversity detected among AG 3 isolates. Most basic seed is produced in Switzerland and only relatively small quantities are imported mainly from France and Netherland. Furthermore, certified seed is produced on a relative small surface by a small number of seed companies, which sell their seed across the whole country. Thus a genetic differentiation among isolates of different parts of the country seems unlikely. Genetic differences between isolates of the organic system tended to be less than between isolates of different farming systems. Maybe this is because the organic seed is produced on a very small number of farms in Switzerland and organic potato growers are obliged to use seed which has been multiplied for at least two years on organic farms.

By randomized open air experiments and pot experiments in the climate chamber (Chapter 6) with isolates of AG 3 the hypothesis based on the results of the survey was proven that drycore is caused by *Rhizoctonia solani* AG 3 and that wounds on tubers caused by wireworms favour the formation of drycore. Drycore symptoms on harvested tubers were only observed in treatments where *R. solani* was present and potato tubers were injured either naturally by wireworm or artificially with needles. Non-wounded tubers very rarely had drycore symptoms even when tubers were infested with black scurf. In the treatments without *R. solani* infection black scurf or drycore was never observed. All isolates of *R. solani* obtained from black scurf or drycore symptoms belonged to AG 3 and all isolates obtained in the pot experiments presented an identical fingerprinting genotype to the isolate used to inoculate the sterilized soil.

The results of this thesis strongly suggest that the higher importance of drycore in the organic farming system and crop rotations with grass clover ley is caused by an interaction of *R. solani* and wireworms (*Agriotes ssp.*). Further research is needed to investigate whether wounds on tubers caused by other organisms or other isolates of other anastomosis groups can also cause drycore symptoms.

Independently from the farming system the risk for quality damages caused by wireworm, slugs and drycore was clearly higher in rotations with a high proportion of grass-clover leys and when potatoes were grown in the first two years after grass clover ley. The crop rotation should therefore be optimized on mixed farms where milk production is based on grass clover leys in the crop rotation. This is especially important on organic farms, where grass clover leys preceding the potato crop are regarded as an important source of nitrogen. On these farms the possibility to reduce grass clover leys in the crop rotation is often limited. Common crop rotations with one or several farms and a specialisation in potato production are possible options to reduce the risk for insufficient quality.

The increasing risk for slug damage with increasing soil cover over winter (catch crops, grass-clover leys) also indicates that conflicting aims exist between the quality standards set by the trade and ecological aims of an environmentally friendly production. Research is needed to find plants with a low acceptability for slugs which are suitable for catch crops or green manure.

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# Appendix

Table A1. Chi square test to check for binomial distribution of black scurf, drycore, wireworm and slugs within the plots.

| | | | **Black scurf** | | | **Drycore** | | |
|---|---|---|---|---|---|---|---|---|
| Plot | N tubers | N plants | % infested tubers | Chisquare | p-value | % infested tubers | Chisquare | p-value |
| 1 | 4 | 65 | 0.9 | 36.56 | 0.000 | 4.2 | 0.54 | 0.763 |
| | 6 | 67 | 2 | 108.10 | 0.000 | 5 | 208.10 | 0.000 |
| | 7 | 83 | 2.2 | 34.40 | 0.000 | 4.4 | 185.51 | 0.000 |
| | 8 | 37 | 3.1 | 4.95 | 0.175 | 3.8 | 4.49 | 0.106 |
| 2 | 9 | 27 | 81.8 | 173256.53 | 0.000 | | | |
| | 10 | 31 | 74.8 | 125051.60 | 0.000 | | | |
| | 11 | 35 | 73.7 | 617601.69 | 0.000 | | | |
| | 12 | 29 | 78.3 | 3235988.11 | 0.000 | | | |
| | 13 | 29 | 76.3 | 306796.37 | 0.000 | | | |
| | 14 | 31 | 78.2 | 551.43 | 0.000 | | | |
| 4 | 6 | 47 | 22.4 | 2721.53 | 0.000 | 13.9 | 26.24 | 0.000 |
| | 8 | 83 | 16.3 | 387041.68 | 0.000 | 7.9 | 111.60 | 0.000 |
| | 10 | 51 | 21.6 | 357970.52 | 0.000 | 10 | 160.42 | 0.000 |
| | | | **Wireworm** | | | | | |
| 1 | 4 | 65 | 57.5 | 25.65 | 0.000 | | | |
| | 6 | 67 | 53.5 | 62.68 | 0.000 | | | |
| | 7 | 83 | 49.7 | 241.76 | 0.000 | | | |
| | 8 | 37 | 52.8 | 25.18 | 0.001 | | | |
| 4 | 6 | 47 | 4.6 | 0.72 | 0.871 | | | |
| | 8 | 83 | 2.9 | 4.99 | 0.173 | | | |
| | 10 | 51 | 3.7 | 360.97 | 0.002 | | | |
| 2 | 12 | 24 | 3.8 | 1.24 | 0.538 | | | |
| | 13 | 23 | 3.7 | 4.29 | 0.118 | | | |
| | 14 | 23 | 1.6 | 1.09 | 0.580 | | | |
| | 15 | 24 | 1.4 | 0.63 | 0.730 | | | |
| | 16 | 24 | 1.3 | 0.64 | 0.727 | | | |
| | 17 | 26 | 2.8 | 42.50 | 0.000 | | | |
| | 18 | 26 | 2.7 | 37.58 | 0.000 | | | |
| | 19 | 19 | 4.7 | 16.69 | 0.000 | | | |
| | | | **Slugs** | | | | | |
| 1 | 4 | 65 | 46.5 | 4.27 | 0.234 | | | |
| | 6 | 67 | 41 | 67.45 | 0.000 | | | |
| | 7 | 83 | 38.9 | 146.56 | 0.000 | | | |
| | 8 | 37 | 34.7 | 156.62 | 0.000 | | | |
| 3 | 9 | 27 | 11.5 | 12.79 | 0.012 | | | |
| | 10 | 31 | 8.7 | 4.70 | 0.319 | | | |
| | 11 | 35 | 9.9 | 7.82 | 0.099 | | | |
| | 12 | 29 | 8.4 | 218.83 | 0.000 | | | |
| | 13 | 29 | 5.3 | 39.99 | 0.000 | | | |
| | 14 | 31 | 6 | 4.11 | 0.391 | | | |
| 2 | 12 | 24 | 12.5 | 2.80 | 0.423 | | | |
| | 13 | 23 | 11.4 | 27.12 | 0.000 | | | |
| | 14 | 23 | 8.5 | 84.86 | 0.000 | | | |
| | 15 | 24 | 8.6 | 50.49 | 0.000 | | | |
| | 16 | 24 | 13 | 11.64 | 0.009 | | | |
| | 17 | 26 | 19.8 | 137.50 | 0.000 | | | |
| | 18 | 26 | 8.4 | 131.11 | 0.000 | | | |
| | 19 | 19 | 11.9 | 19.20 | 0.004 | | | |

Table A2. Lower (p=2.5%) and upper confidence level (p=97.5%) for assessed means in % damaged tubers for sample sizes of 200 to 1400 tubers. One thousand random samples for each sample size were simulated.

| | | **wireworm** | | | **slugs** | | | **black scurf** | | | **drycore** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Field 1** | N tubers | mean | LCL 2.5% | UCL 97.5% | mean | LCL 2.5% | UCL 97.5% | mean | LCL 2.5% | UCL 97.5% | mean | LCL 2.5% | UCL 97.5% |
| | 200 | 48.0 | 40.9 | 54.5 | 37.2 | 30.2 | 44.2 | 3.1 | 1.0 | 6.0 | 3.8 | 1.5 | 6.5 |
| | 400 | 48.0 | 43.1 | 52.9 | 37.2 | 32.2 | 41.7 | 3.1 | 1.8 | 4.8 | 3.8 | 2.0 | 5.8 |
| | 600 | 48.0 | 44.0 | 52.0 | 37.2 | 33.3 | 41.4 | 3.1 | 1.8 | 4.7 | 3.8 | 2.4 | 5.4 |
| | 800 | 48.1 | 44.7 | 51.8 | 37.2 | 33.8 | 40.7 | 3.2 | 2.0 | 4.4 | 3.8 | 2.5 | 5.3 |
| | 1000 | 48.0 | 44.9 | 51.2 | 37.1 | 34.1 | 40.2 | 3.1 | 2.1 | 4.2 | 3.8 | 2.6 | 5.1 |
| | 1200 | 48.1 | 45.3 | 50.9 | 37.2 | 34.4 | 39.9 | 3.2 | 2.3 | 4.2 | 3.8 | 2.8 | 5.0 |
| | 1400 | 48.1 | 45.5 | 50.8 | 37.1 | 34.6 | 39.7 | 3.2 | 2.3 | 4.0 | 3.8 | 2.9 | 4.9 |
| **Field 2** | 200 | 3.5 | 1.0 | 6.5 | 14.1 | 9.5 | 19.1 | | | | | | |
| | 400 | 3.6 | 1.8 | 5.3 | 14.1 | 10.6 | 17.8 | | | | | | |
| | 600 | 3.5 | 2.2 | 4.9 | 14.0 | 11.1 | 17.0 | | | | | | |
| | 800 | 3.5 | 2.4 | 4.9 | 14.1 | 11.7 | 16.8 | | | | | | |
| | 1000 | 3.6 | 2.5 | 4.7 | 14.1 | 12.0 | 16.4 | | | | | | |
| | 1200 | 3.6 | 2.5 | 4.6 | 14.1 | 12.2 | 16.1 | | | | | | |
| | 1400 | 3.5 | 2.3 | 4.5 | 14.0 | 12.3 | 16.0 | | | | | | |
| **Field 3** | 200 | 0.6 | 0.0 | 2.0 | 9.5 | 5.5 | 13.6 | 80.1 | 74.0 | 85.4 | | | |
| | 400 | 0.6 | 0.0 | 1.5 | 9.6 | 6.8 | 12.6 | 80.2 | 76.3 | 83.8 | | | |
| | 600 | 0.6 | 0.2 | 1.3 | 9.5 | 7.4 | 11.9 | 80.1 | 76.8 | 83.5 | | | |
| | 800 | 0.6 | 0.1 | 1.3 | 9.5 | 7.6 | 11.5 | 80.2 | 77.5 | 82.4 | | | |
| | 1000 | 0.6 | 0.2 | 1.1 | 9.5 | 7.8 | 11.3 | 80.2 | 77.6 | 82.7 | | | |
| | 1200 | 0.6 | 0.3 | 1.1 | 9.5 | 7.8 | 11.2 | 80.2 | 78.1 | 82.5 | | | |
| | 1400 | 0.6 | 0.3 | 1.1 | 9.5 | 8.0 | 11.1 | 80.2 | 78.2 | 82.2 | | | |
| **Field 4** | 200 | 3.6 | 1.5 | 6.1 | 4.8 | 2.0 | 7.6 | 22.5 | 16.5 | 28.3 | 12.3 | 8.0 | 17.0 |
| | 400 | 3.7 | 2.0 | 5.8 | 4.8 | 2.8 | 7.0 | 22.3 | 18.1 | 26.4 | 12.4 | 9.1 | 15.8 |
| | 600 | 3.6 | 2.2 | 5.3 | 4.8 | 3.2 | 6.7 | 22.4 | 19.0 | 25.7 | 12.4 | 9.7 | 15.1 |
| | 800 | 3.6 | 2.4 | 5.0 | 4.8 | 3.5 | 6.3 | 22.3 | 19.5 | 25.0 | 12.4 | 10.0 | 14.8 |
| | 1000 | 3.6 | 2.5 | 4.8 | 4.9 | 3.5 | 6.2 | 22.3 | 19.8 | 24.9 | 12.4 | 10.3 | 14.5 |
| | 1200 | 3.6 | 2.6 | 4.8 | 4.8 | 3.7 | 6.0 | 22.3 | 19.9 | 24.7 | 12.3 | 10.5 | 14.2 |
| | 1400 | 3.6 | 2.7 | 4.7 | 4.8 | 3.7 | 6.0 | 22.3 | 20.1 | 24.4 | 12.4 | 10.7 | 14.1 |

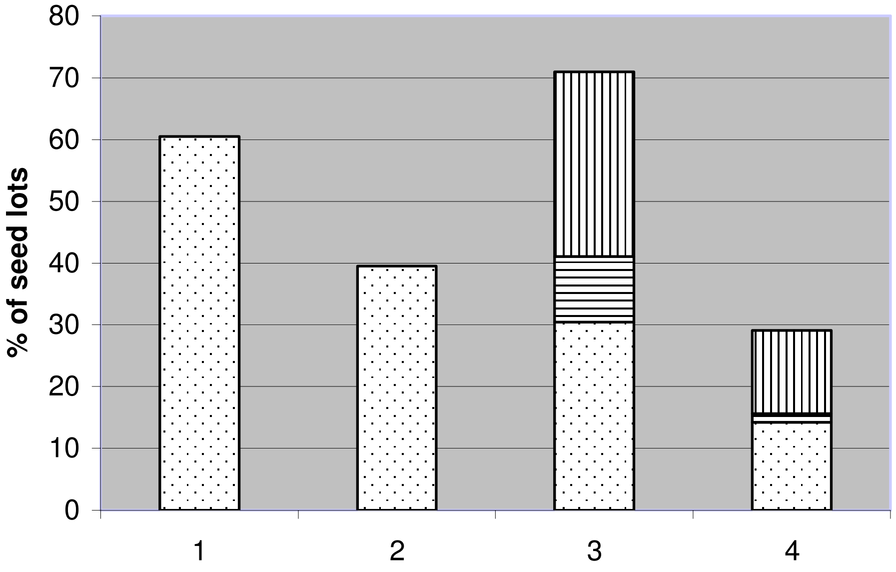

Fig. A1 Seed tuber quality in the fields of the on-farm program 2002 and 2003. 1 = organic farming system, less than 20% of the tubers infested with black scurf, 2= organic farming system, more than 20% of tubers infested with black scurf, 3 = integrated and conventional farming system, less than 20% of the tubers infested with black scurf, 4 = = integrated and conventional farming system, more than 20% of the tubers infested with black scurf.
⬚ = no fungicide seed treatment, ▤= fungicide seed treatment at storage, ▥ = fungicide seed treatment during planting.

Table A3 Similarity matrix for rDNA ITS 1 and 2 of some isolates of *R.solani* AG 3 and AG 5 (Table 5.3) based on ITS sequencing

| | AK 50 | AK10 | AK 8Da | AK 8Db | SO 301 | AK 14 | AK001 | AK0501 | 03-08 | VD 302 | 03-01 | AK 9D | 05-01 | 05-41 | AK 34 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AK 50 | 100.00 | | | | | | | | | | | | | | |
| AK10 | 99.75 | 100.00 | | | | | | | | | | | | | |
| AK 8Da | 99.57 | 99.61 | 100.00 | | | | | | | | | | | | |
| AK 8Db | 99.57 | 99.61 | 100.00 | 100.00 | | | | | | | | | | | |
| SO 301 | 99.65 | 99.68 | 99.79 | 99.79 | 100.00 | | | | | | | | | | |
| AK 14 | 99.51 | 99.54 | 99.36 | 99.36 | 99.43 | 100.00 | | | | | | | | | |
| AK001 | 99.19 | 99.22 | 99.04 | 99.04 | 99.11 | 99.51 | 100.00 | | | | | | | | |
| AK0501 | 99.19 | 99.22 | 99.04 | 99.04 | 99.11 | 99.51 | 99.89 | 100.00 | | | | | | | |
| 03-08 | 99.04 | 99.08 | 98.90 | 98.90 | 98.97 | 99.40 | 99.79 | 99.79 | 100.00 | | | | | | |
| VD 302 | 99.01 | 99.04 | 98.87 | 98.87 | 98.93 | 99.36 | 99.75 | 99.75 | 99.89 | 100.00 | | | | | |
| 03-01 | 99.04 | 99.08 | 98.90 | 98.90 | 98.97 | 99.25 | 99.65 | 99.65 | 99.61 | 99.61 | 100.00 | | | | |
| AK 9D | 99.08 | 98.97 | 98.79 | 98.79 | 98.87 | 99.15 | 99.54 | 99.54 | 99.51 | 99.51 | 99.79 | 100.00 | | | |
| 05-01 | 90.42 | 90.67 | 90.42 | 90.42 | 90.32 | 90.67 | 90.44 | 90.44 | 90.49 | 90.64 | 90.29 | 90.14 | 100.00 | | |
| 05-41 | 91.86 | 91.59 | 91.87 | 91.72 | 91.65 | 92.05 | 92.30 | 92.30 | 92.19 | 92.15 | 92.05 | 91.93 | 99.61 | 100.00 | |
| AK 34 | 91.85 | 91.87 | 91.41 | 91.85 | 91.78 | 91.89 | 92.14 | 92.14 | 92.14 | 92.14 | 92.00 | 91.89 | 99.21 | 99.57 | 100.00 |

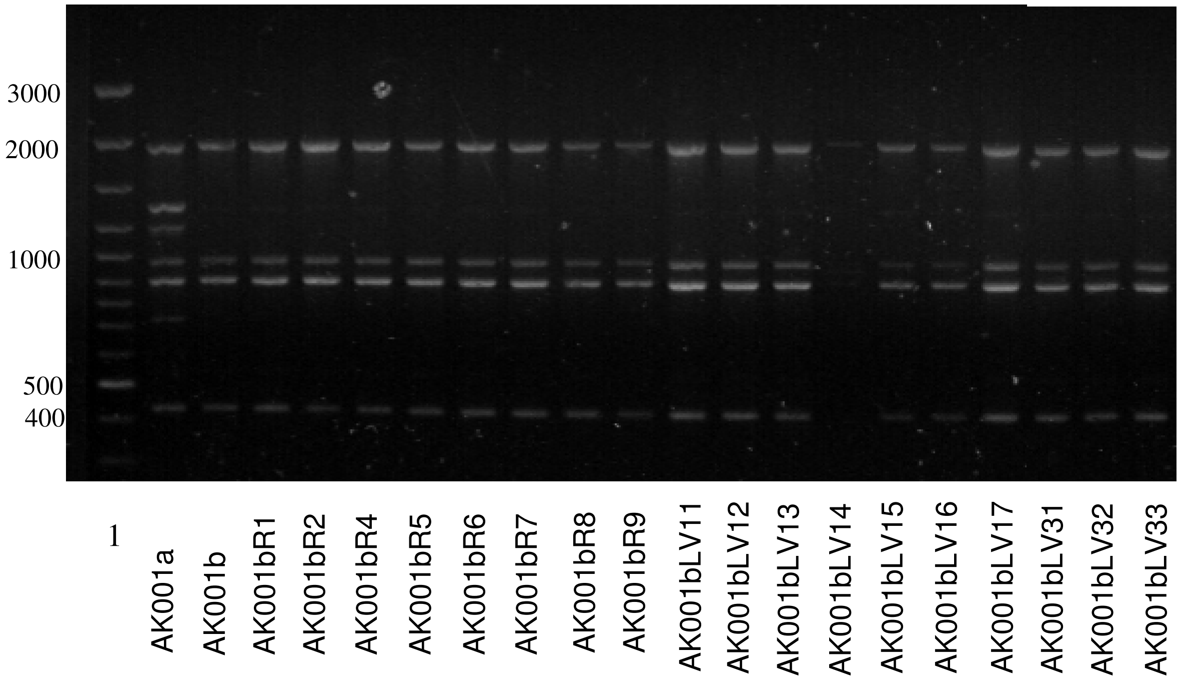

Fig. A2 DNA-fingerprints obtained by ISSR-PCR with CAG – primer of *R. solani* isolates from sclerotia and drycore symptoms on harvested tubers from three pot trials under controlled conditions. Lane 1 is a 100-bp ladder Plus (PeQlab). In all climate chamber experiments the sterilized soil was infested with the isolate AK001b (AG 3). The following isolates were fingerprinted: from the first trial, the isolates AK001LV11 to LV17; from the second trial, the isolates AK001R1 to R9; from the third trial, isolates AK001LV31 to 33.

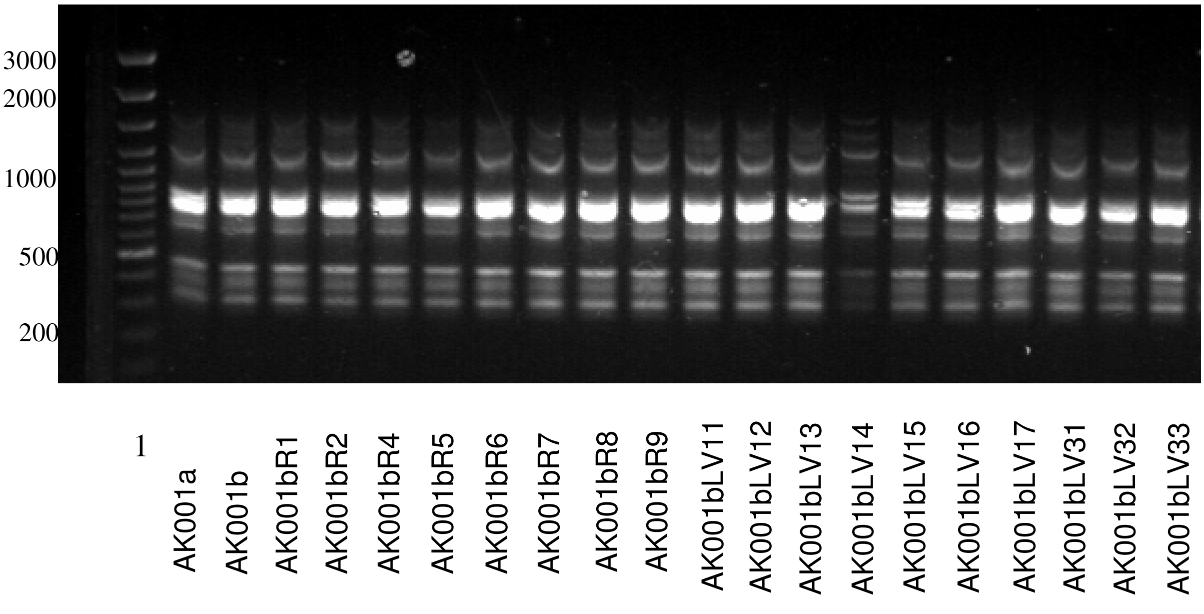

Fig. A3 DNA-fingerprints obtained by ISSR-PCR with CGA – primer of *R. solani* isolates from sclerotia and drycore symptoms on harvested tubers from three pot trials under controlled conditions. Lane 1 is a 100-bp ladder Plus (PeQlab). In all climate chamber experiments the sterilized soil was infested with the isolate AK001b (AG 3). The following isolates were fingerprinted: from the first trial, the isolates AK001LV11 to LV17; from the second trial, the isolates AK001R1 to R9; from the third trial, isolates AK001LV31 to 33.

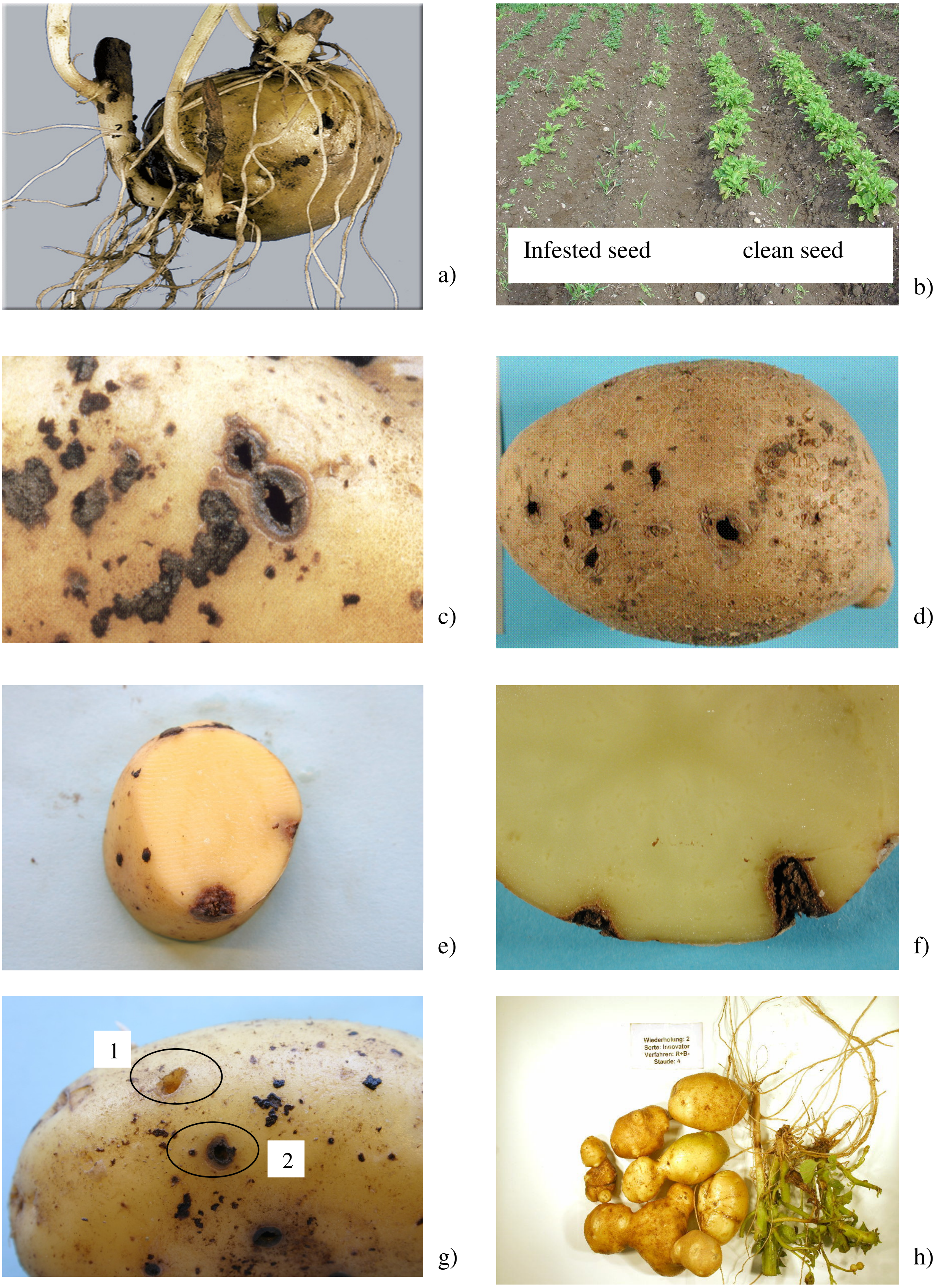

Fig. A4 Symptoms of *Rhizoctonia solani* on potatoes: a) stem canker, b) infested seed leeds to irregular and late emerge, c)+d) black scurf and drycore symptoms of progeny tubers, e)+f) cross section of a drycore hole, g) wireworm damage (1) and drycore (2) on the same tuber, h) malformed tubers.

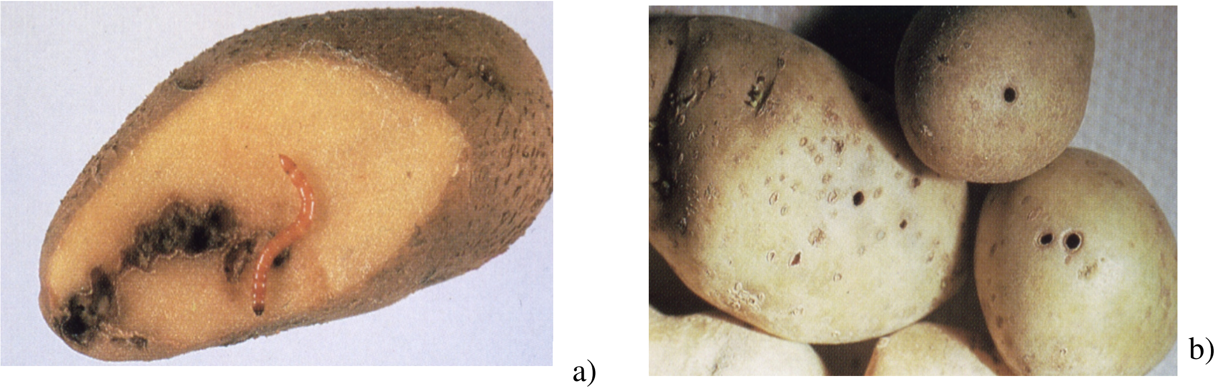

Fig. A5 a) Wireworm damage on potato tubers a cross section of wireworm damage with click beetle (larvae of Agriotes ssp.), b) wireworm holes on tubers.

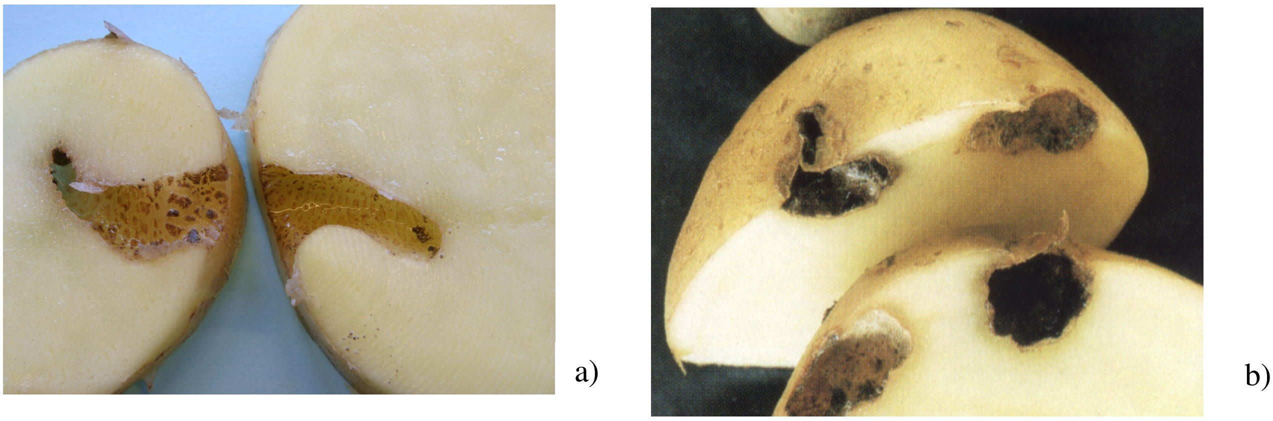

Fig. A6 a) + b) cross sections of slug damage on potato tubers.

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## Curriculum vitae

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| | |
|---|---|
| 2004 - 2007 | PhD candidate ETH Zurich, based at the Swiss College of Agriculture in Zollikofen BE. |
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| 1989 – 1999 | Head of the extension service for plant production and crop protection at the Agricultural School Wallierhof in Riedholz SO. |
| 1988 | Assistant at the extension service for crop protection at the Agricultural school Rheinhof in Salez SG during 9 months. |
| 1988 | Diploma thesis at the IITA in Benin: “Ertragsbildung der Kuherbse *Vigna unguiculata* (Walp.) unter dem Einfluss von Trockenheitsstress und Befall durch die Insekten *Megalurothrips sjostedti* Trybom und *Maruca testulalis* (Geyer) in Benin.“<br>Examiner: Prof. Dr. V. Delucchi |
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| 1981 | General qualification for university entrance („Matura Typus C“), Gymnasium Münchenstein BL. |