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Modelling of potato virus pathosystems by means of quantitative epidemiology: An exemplary case based on virus degeneration studies in Peru

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Addendum

Fig. 1. 8. on page 32 was not reproduced satisfactorily by offset printing. Therefore, a postscript printout is added below.

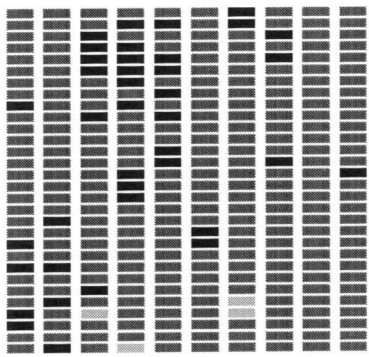

Fig. 1.8. Spatial pattern of infected plants at harvest in a plot, with 2% of PLRV infected seed tubers, in Sta. Ana 1988/89 (3250 m.a.s.l.). Grey, black and light grey rectangles represent healthy, infected and missing plants respectively. Infection was detected by the analysis of 3 tubers per plant with ELISA. The modern cultivar Yungay was used (*Solanum tuberosum ssp. tuberosum x Solanum tuberosum ssp. andigena*). Plot size was 10 x 10 m.

Corrigenda

Page 61, Table 2.3: dimension of tsi and tpi: $[N^{-1}]$.

Page 66, paragraph on *aphid-transmitted viruses*: replace "binomial distribution" by "negative binomial distribution".

Zürich, August 4th, 1992

To my wife Luzia, and to my parents

CONTENTS

SUMMARY

Potato is an important crop for human consumption in the developing world. It is often grown in subsistence-oriented food systems with a productivity which is low compared with that in developed countries. The production and distribution of improved seed (less than 4% of pathogen-infected tubers) has been a successful strategy in developed countries to avoid degeneration of the seed tubers by viruses and other pathogens. Data on the importance of viruses (incidence and yield reduction) are often scarce in developing countries. Such information is important, however, for demarcating zones appropriate for the multiplication of improved seed in which the infection risk is low. A simulation model which is adaptable to different agroecological zones and genotypes and which predicts harvest infection (percentage of infected tubers) in the respective zone would be helpful for the demarcation of seed multiplication zones, and would avoid laborious and long-term virus studies. The research presented was divided into three parts: first, epidemiological data for PVX, APMV, PVY and PLRV were generated in contrasting agroecological potato growing zones of Peru; secondly a computer simulation model was developed and its sensitivity studied; thirdly, the model was validated and applied to agroecological conditions of Peru.

The epidemiological experiments were realised from 1986 to 1989 in three agroecological zones of Peru (112, 3280, and 4000 m.a.s.l.). The harvest infection was determined separately for each virus in plots with low, medium and high seed infection (approximately 2, 20 and 50% of infected seed tubers and 192 to 300 plants per plot). The spatial pattern of infected plants was monitored at harvest. The efficiency of autoinfection (percentage of tubers which are infected among those produced by secondarily-infected plants) was quantified as well as the percentage of primarily-infected plants and the percentage of infected tubers of such plants for each plot. In the work presented, the expression "efficiency of autoinfection" is deduced and justified since this mechanism was not identified previously for the studied viruses.

The largest increases between seed and harvest infection with aphid-transmitted viruses (PVY and PLRV) were obtained in plots at 112 m.a.s.l. whereas at higher altitudes low increases of infection or statistically significant reductions were determined, particularly in plots with medium to high seed infection. Since the aphid-transmitted viruses are the most damaging to the crop, it is concluded that improved seed should be multiplied only in the highlands in the appropriate valleys, but not in the coastal zone. With contact-transmitted viruses (PVX and APMV) the tendencies were similar but less expressed. The reductions of infection are explained by low efficiencies of autoinfection and by few primary infections. For PVX, APMV, PVY and PLRV the efficiencies ranged from 41 to 83, 30 to 84, 54 to 83, and 33 to 88% respectively.

A biologically significant and explanatory simulation model (EPIVIT) was developed from the common knowledge on potato virus epidemiology and the findings of the research conducted in Peru. It predicts harvest infection with a contact- or aphid-transmitted virus and is sensitive and adaptable to changing agroecological conditions. Its principal input variable is daily temperature, temperature being the most relevant variable for virus transmission and multiplication besides plant genotype and vector activity and characteristics. For aphid-transmitted viruses quantitative weekly data on the composition of the aphid population are required in addition. State variables are the

efficiency of autoinfection, the percentage of primarily-infected plants and the percentage of infected tubers of such plants. The model uses the beta function for the simulation of temperature-sensitive parameter variables of an epidemic such as the efficiency of an aphid in transmitting virus and of rates related to the simulation of the plant's physiological age, the latent period, the susceptibility of a plant to an infection, and virus multiplication and translocation towards the tubers. EPIVIT's adaptability to different agroecological conditions and plant genotypes is reflected in a considerable number of required parameter variables. The model was implemented for IBM-compatible computers. It may be run for multiple seasons assuming that the harvest of one season serves as a source for seed selection for the following season. Based on a coarse and a fine sensitivity analysis, the model code was considered to be reasonable and consistent. The model's agreement with the objectives of its development and the prospects for application are discussed.

A first approach of model validation was undertaken. The efficiency of autoinfection could be simulated accurately ($P<0.05$). With respect to the simulation of the primary infection of plants no measured values from the real system were available for several of the parameter variables. Empirical estimates were assigned to some of these parameters. Others were estimated by stepwise changes in their values until a determined level of accuracy was obtained. EPIVIT was not further calibrated for PVY due to paucity of data provided by the historical data set for this virus. It was considered that additional parameter fine-tuning is required for the simulation of primary infection with PVX and APMV because the model predictions with the selected parameter value sets did not meet the criteria of precision established. Simulations for PLRV, however, were precise enough. Consequently, EPIVIT was applied for the estimation of the long-term trend of harvest infection with PLRV assuming that seed multiplication is started with improved seed and the harvest is used as source for seed selection during consecutive years. It was concluded that the model validation proves the correctness of EPIVIT's basic structure and strongly supports and specifies the assumptions made on pathogen x host genotype x environment interactions.

The conclusions and prospects presented refer to i) how the findings of this study may help explaining phenomena related to the incidence and pathogenicity of viruses in the Andes, ii) how these findings may be suitable for improving techniques related to plant breeding, iii) which requirements need to be satisfied for an application of EPIVIT to other environments and plant genotypes, and iv) the model's suitability for being applied to what it was principally developed for: the estimation of when the farmer needs to refresh his seed stock with some improved seed which he is multiplying further by traditional crop management.

RESUMEN

La papa es un importante cultivo de consumo humano en el mundo en desarrollo, donde suele sembrarse en sistemas de autoconsumo cuya productividad es baja en comparación con la observada en los países industrializados. En esos países, la producción y distribución de semilla con escasa infección patógena (semilla mejorada con menos del 4% de tubérculos infectados) es una estrategia eficaz que evita la degeneración de los tubérculos-semillas por virus u otros tipos de patógenos. Ahora bien, en los países en desarrollo suele haber poca información relevante sobre los virus (incidencia y reducción de rendimiento), la cual sin embargo es indispensable para definir zonas apropiadas para la multiplicación de semilla mejorada. Sería útil para la delimitación de estas zonas contar con un modelo de simulación que fuera adaptable a los diferentes genotipos y áreas agroecológicas, y que sirviera para predecir el nivel de infección que se observar en cada zona en el momento de la cosecha (número de tubérculos infectados en la cosecha). Por otra parte, obviaría la necesidad de efectuar estudios laboriosos y prolongados sobre los virus.

La presente investigación se dividió en tres partes: en la primera, se generaron datos epidemiológicos sobre PVX, APMV, PVY y PLRV en zonas agroecológicas contrastantes del Perú; en la segunda, se desarrolló un modelo de simulación computarizada y se observó su sensibilidad; en la tercera, se hizo la validación del modelo y se le aplicó en las condiciones agroecológicas del Perú.

Se efectuaron de 1986 a 1989 experimentos epidemiológicos en tres zonas agroecológicas del Perú (a 112, 3280 y 4000 msnm). El grado de infección con cada virus en el momento de la cosecha se determinó en parcelas sembradas con tubérculos-semilla de infección baja, intermedia y alta en cada zona (estaban infectados aproximadamente 2, 20 y 50% de los tubérculos-semillas por parcela con un número de plantas entre 192 y 300). Se cuantificó también la eficiencia de la autoinfección (o sea, el porcentaje de tubérculos infectados entre los producidos por plantas con infecciones secundarias), así como el porcentaje de plantas con infecciones primarias y el porcentaje de tubérculos infectados que éstas produjeron en cada parcela. En el trabajo presentado, el uso del término eficiencia de autoinfección se justifica en vista de que hasta ahora a este mecanismo no se le ha asignado ningún nombre en la virología de la papa.

En cuanto a los virus transmitidos por áfidos (PVY y PLRV), los mayores incrementos entre los niveles de infección en semilla y los encontrados en la cosecha se obtuvieron a 112 msnm, mientras que en altitudes más elevadas se registraron incrementos bajos de infección o incluso reducciones estadísticamente significativas, sobre todo en parcelas que presentaron niveles de infección en semilla entre intermedios y altos. Como los virus transmitidos por áfidos son los más nocivos para el cultivo, se llegó a la conclusión de que la semilla mejorada debe multiplicarse sólo en los valles altos, no en la zona costera. En el caso de los virus transmitidos por contacto (PVX y APMV), se observaron tendencias similares, pero su expresión fue menor. La baja eficiencia de autoinfección y pocas infecciones primarias en las zonas altas provocaron las reducciones de los grados de infección. En el caso de PVX, APMV, PVY y PLRV, la eficiencia de autoinfección fluctuó entre 41 y 83%, 30 y 84%, 54 y 83% y 33 y 88%, respectivamente.

El modelo de simulación EPIVIT fue desarrollado con base en lo que se sabe comúnmente de la epidemiología de los virus que atacan la papa y en los resultados de

las investigaciones realizadas en el Perú. Este modelo estima el grado de infección con un virus particular (porcentaje de tubérculos infectados) en el momento de la cosecha y es sensitivo y adaptable cambios de las condiciones agroecológicas. EPIVIT requiere datos de la temperatura diaria, ya que ésta constituye la variable más importante para la transmisión y multiplicación del virus, después del genotipo de la planta y la actividad y las características del vector. En cuanto a los virus transmitidos por áfidos, además de los datos arriba anotados, se requieren datos cuantitativos semanales sobre la composición de la población de los áfidos. Las variables de estado incluyen la eficiencia de autoinfección, el porcentaje de plantas con infección primaria y el porcentaje de tubérculos infectados producidos por esas plantas.

El modelo utiliza la función beta para simular parametros de una epidemía que varían según la temperatura como la eficiencia de un áfido en transmitir el virus y tasas de incremento de la edad fisiológica de la planta, del período de latencia, de la susceptibilidad de la planta y de la multiplicación y del desplazamiento del virus hacia los tubérculos. Un considerable número de variables paremétricos refleja que EPIVIT se adapta a distintos genotipos y condiciones agroecológicas. El modelo fue creado para usarse en computadoras compatibles con IBM y puede correrse durante varios ciclos, siempre que se seleccione semilla de la cosecha anterior para sembrarla en el ciclo siguiente. Se presenta un análisis detallado de sensibilidad del modelo, y se examina hasta qué punto el modelo cumple los objetivos para los que fue creado, así como sus posibles aplicaciones.

Se efectuó una primera validación del modelo, y fue posible simular en forma precisa la eficiencia de autoinfección ($P < 0.05$). En respecto a la simulación de la infección primaria, no existen mediciones reales de algunos parámetros que EPIVIT utiliza. A algunos se les asignaron estimaciones empíricas; otros parámetros se estimaron modificando paso a paso los valores hasta obtener un determinado nivel de precisión. No fue posible validar EPIVIT para las infecciones primarias con PVY porque en los experimentos efectuados en el Perú no se obtuvieron suficientes datos sobre este virus. Resultó que se require calibrar aún más el modelo para los virus PVX y APMV dado que las predicciones para infecciones primarias con estos virus no cumplieron los criterios establecidos de precisión. Las simulaciones para PLRV se consideraron precisas, y en consecuencia se aplicó EPIVIT para estimar la tendencia a largo plazo de la infección con ese virus en el momento de la cosecha, dando por supuesto que la multiplicación se ha iniciado con semilla mejorada y que se selecciona semilla de la cosecha anterior para sembrarla en el ciclo siguiente. En conclusión, la validación del modelo indica que la estructura básica de EPIVIT es correcta y que apoya y especifica los supuestos respecto a las interacciones patógeno x genotipo x ambiente.

Las conclusiones y perspectivas presentadas se refieren a 1) las explicaciones que ofrece el modelo estudiado de fenómenos que se relacionan con la incidencia y patogenicidad de los virus que atacan la papa en los Andes; 2) la utilidad de lo encontrado por intermedio de esta investigación para perfeccionar las técnicas relacionadas con el mejoramiento de la papa; 3) lo que se requiere para aplicar EPIVIT a otros ambientes y cultivos; y 4) la aptitud del modelo para estimar la frecuencia con que el agricultor debe renovar su semilla con semilla mejorada que después multiplicará utilizando las prácticas tradicionales de cultivo.

ZUSAMMENFASSUNG

Die Kartoffel ist eine landwirtschaftliche Kulturpflanze mit grosser Bedeutung für die Ernährung der Bevölkerung in Entwicklungsländern. Ihre Ertragskraft ist dort im allgemeinen allerdings gering. In entwickelten Ländern hat Qualitätspflanzgut, das wenig mit Pathogenen infiziert ist (ca. 4% der Knollen), einen wesentlichen Beitrag zur signifikant höheren Ertragskraft in diesen Ländern geleistet. Der Vermeidung von Virusinfektionen wird bei der Produktion von solchem Pflanzgut besondere Beachtung geschenkt.

Wenig ist bekannt über die Verbreitung und ertragsschädigende Wirkung der Viren in Entwicklungsländern. Das erschwert die Entwicklung angepasster Anbaustrategien und Anstrengungen zur Verbesserung der Ertragskraft der Kartoffel. Ein Simulationsmodell für die Vorhersage des Anteils virusinfizierter Knollen im Erntegut in einer bestimmten Anbauzone wäre in diesem Zusammenhang von besonderem Nutzen und könnte die Entwicklung solcher Strategien wesentlich beschleunigen.

Die präsentierte Forschungsarbeit gliederte sich in drei Teile: 1. Erhebung umfangreicher Felddaten zur Epidemiologie der Kartoffelviren PVX, APMV, PVY und PLRV. 2. Entwicklung eines Simulationsmodells und anschliessende Sensitivitätsanalyse. 3. Erste Validierung des Modells und Anwendung für verschiedene agroökologische Zonen in Peru.

Die epidemiologischen Studien wurden in den Jahren 1986 bis 1989 in drei agroökologischen Zonen Perus durchgeführt (112, 3280 und 4000 m.ü.M.). Die Erntegutinfektion (Anteil infizierter Knollen) wurde für Versuchsflächen bestimmt, die mit wenig, mittel oder stark infiziertem Pflanzgut (2%, 20% oder 50% infizierte Saatknollen in Parzellen mit 192 bis 300 Pflanzen) bepflanzt worden waren. Für jede Versuchsfläche wurde ebenfalls die Autoinfektionseffizienz (infizierter Anteil der von sekundär infizierten Pflanzen produzierten Knollen), der Anteil primär infizierter Pflanzen und der Anteil infizierter Knollen von solchen Pflanzen bestimmt. Der Ausdruck "Autoinfektionseffizienz" wird im Rahmen der vorgelegten Arbeit abgeleitet und begründet, da bis anhin dieser Mechanismus für die entsprechenden Viren nicht als solcher identifiziert und beschrieben worden war.

Die höchsten Zuwachsraten der Infektion mit den durch Blattläuse übertragenen Viren (PVY und PLRV) wurden auf 112 m.ü.M. gemessen, während im Hochland die Zunahmen viel geringer waren oder sogar eine statistische Abnahme der Infektion gemessen wurde. Da PVY und PLRV den Ertrag am stärksten schädigen, sollte Qualitätspflanzgut nur im Hochland vermehrt werden. Die beobachteten Tendenzen mit mechanisch übertragbaren Viren (PVX und APMV) waren ähnlich, aber weniger ausgeprägt. Eine Reduktion des Anteils infizierter Knollen vom Pflanz- zum Erntegut konnte durch die geringe Autoinfektionseffizienz der Pflanzen im Hochland und wenig Primärinfektionen erklärt werden. Die gemessenen Autoinfektionseffizienzen lagen in folgenden Bereichen: 41 bis 83% für PVX, 30 bis 84% für APMV, 54 bis 83% für PVY sowie 33 bis 88% für PLRV.

Aufbauend auf den allgemein bekannten Grundsätzen der Kartoffelviren-Epidemiologie und den während der Studien in Peru gemachten Erfahrungen wurde das Modell EPIVIT entwickelt. Das Modell errechnet den Anteil der Knollen im Erntegut, der mit einem bestimmten Virus infiziert ist, und ist sensitiv für und anpassbar an Änderungen

der agroökologischen Bedingungen einer Anbauzone. Es basiert auf der Simulierung der einzelner Komponenten des Kartoffelviren-Pathosystems und deren mechanistischen Verknüpfung und hat somit erklärenden Charakter. Haupteingabevariablen sind die tägliche Temperatur und, im Fall der durch Blattläuse übertragenen Viren, wöchentliche Angaben zum Umfang und der Zusammensetzung der Blattlauspopulation. Zustandsvariablen sind die Autoinfektionseffizienz, die Primärinfektion von Pflanzen und die Knolleninfektion derselben Pflanzen. Das Modell verwendet die Beta-Funktion zur Simulierung mehrerer temperatursensitiver Parameter einer Epidemie, wie etwa die Übertragungseffizienz verschiedener Blattlausarten oder die Zuwachsraten des physiologischen Alters der Pflanzen, der Latenzperiode, der Infektionsanfälligkeit der Pflanze sowie der Virusvermehrung und des Virustransportes in der Pflanze. Dadurch wird das Modell anpassbar an verschiedene Umweltbedingungen. EPIVIT wurde implementiert für IBM-kompatible Personalcomputer. Das Modell hat verschiedene Ausgabefunktionen. Unter anderem kann die Zunahme der Infektion des Erntegutes simuliert werden unter der Annahme, dass das Pflanzgut einer Anbauperiode aus dem Erntegut der vorherigen Periode selektioniert wird. Als Resultat einer detaillierten Sensitivitätsanalyse des Modells wurden dessen Reaktionen auf Änderungen der Modellparameter als vernünftig und konsistent beurteilt.

EPIVIT wurde ein erstes Mal validiert. Die in Peru gemessenen Autoinfektionseffizienzen wurden vom Modell mit guter Genauigkeit reproduziert ($P<0.05$). Für PVY war für eine weitere Validierung zuwenig Datenmaterial verfügbar. Die Schätzungen des Modells für Primärinfektionen mit PVX und APMV genügten den aufgestellten Präzisionskriterien nicht und verlangen nach einer erweiterten Modellkalibrierung. Die Vorhersagen für PLRV wurden hingegen als genügend präzise beurteilt. EPIVIT wurde folglich angewandt für die Schätzung der Zunahme der Erntegutinfektion bei mehrjährigem Nachbau desselben Pflanzgutes in derselben Agroökozone. Klima- und Blattlausdaten von Peru wurden dafür verwendet. Aufgrund dieser Validierung wurde die Struktur des Modells als realistisch beurteilt. Die Resultate der Validierung unterstützen insbesondere die Annahmen, die das Modell macht, bezüglich der Interaktion zwischen Pathogen, dem Genotyp der Pflanze und der Umwelt.

Die Bedeutung der Resultate dieser Arbeit für ein verbessertes Verständnis des Kartoffelviren-Pathosystems wird diskutiert. Es wird dargelegt, welche Relevanz die ge-wonnenen Erkenntnisse haben für die Erklärung von Beobachtungen der Virusverbreitung und -pathogenität in den Anden und für die Verbesserung von Selektionstechniken der Pflanzenzüchtung. Es wird ausserdem besprochen, welche Voraussetzungen für eine erfolgreiche Anwendung EPIVITs erfüllt sein müssen, und wie geeignet das Modell ist für eine Schätzung der Häufigkeit, mit welcher ein Bauer sein Pflanzgut mit Qualitätssaatgut erneuern sollte, welches er anschliessend gemäss traditioneller Anbautechnik Jahr für Jahr vermehrt.

List of Tables

List of Figures

List of Figures ...../continued

List of Abbreviations

Af	Attraction factor
ACMV	African cassava mosaic virus
APMV	Andean potato mottle virus
bd	Beta-degrees
bdd_a	Beta-degree-days related to the efficiency of autoinfection
bdd_{at}	Triggered beta-degree-days related to the primary infection of plants
bdd_b	Beta-degree-days related to the primary infection of plants
C	Randomisation constant
CIP	Centro Internacional de la Papa (International Potato Center)
COTESU	Cooperación Técnica Suiza (Swiss Development Cooperation)
C_{wr}, C_{br}	Physiological age when canopy closes within a row and between rows respectively
DH	Developmental heat sum
dr	delay range of temperature degrees
ELISA	Enzyme linked immunosorbent assay
eh	Emergence of healthy seed tubers (%)
esi	Emergence of secondarily-infected seed tubers (%)
h_1, h_2, h_3	Hour of the day related to aphid behaviour
He	Number of non-infectious plants (including latently infected plants)
HeE	Number of emerged He
Hf	Number of virus-free plants
hi	Harvest infection (percentage of infected tubers in the harvest)
i	Index for week
In	Simulated, performed inoculations in the field
Inp	Simulated, performed inoculations per plants
INIAA	Instituto Nacional de Investigación Agraria y Agroindustrial (Peru's national institute for agricultural and agroindustrial research)
k	Index for season
k_b	Parameter of the negative binomial distribution
kp	Scaling factor for r_p
Lp	Latent period (in physiological time units)
Lpw	Latent period transformed to weeks
m, n	Parameters of the beta function
M	Average number of moves which an aphid makes within a field before leaving
Mri	Physiological age at the initialisation of mature plant resistance

List of Abbreviations ...../continued

N	Number of seed tubers planted in a field
Ne	Number of planted tubers in the simulated field
NH	Number of daughter tubers of a healthy plant
NHe	Number of daughter tubers of a non-infectious plant
NPi	Number of daughter tubers of a primarily-infected plant
Ns	Number of emerged tubers in the simulated field
NSi	Number of daughter tubers of a secondarily-infected plant
pa	probability of a landing aphid being viruliferous
pc	Probability with which a contact transmitted virus spreads from a source plant to an adjacent healthy plant
Pi	Number of primarily-infected plants which are infectious
PiH	Number of primarily-infected and healthy plants
PiP	Number of primarily-infected plants (latently infected and infectious)
PLRV	Potato leafroll virus
P_{max}	Physiological age of a cultivar accumulated until harvest
P-time	Physiological time
PVX	Potato virus X
PVY	Potato virus Y
q	Scaling parameter for REF and Af
REF	Relative efficiency factor
r_a	Rate parameter of the monomolecular function for tsi=f(DH)
r_{mi}	Rate parameter of the multiple infection transformation
r_{mr}	Rate parameter of the logistic function related to mature plant resistance
r_p	Rate of advancement of physiological age with t
Sf	Susceptibility factor
SFIT	Swiss Federal Institute of Technology in Zürich
Si	Secondarily-infected plant
SiE	Number of emerged Si
Sp	Number of aphid species
Su_c	Constitutive susceptibility factor between 0 and 1.0.
Su_{mr}	Susceptibility which is related to mature plant-resistance (temperature sensitive)
r_{mr}	Rate parameter of the logistic function related to mature plant resistance
t	Season time

List of Abbreviations ...../continued

T	Temperature
Ta	Average daily temperature at which T is at its maximum
Tc	Weekly aphid trap catches
T_{min}, T_{max}	Cardinal temperatures of a determined mechanism
TDDCM	Two-dimensional distance class model
TH	Trigger developmental heat related to the efficiency of autoinfection
TMV	Tobacco mosaic virus
tpi	Percentage of tubers, which are infected among those produced by all Pi in a plot
tpih	Percentage of tubers, which are infected among those produced by all PiH in a plot
tsi	Percentage of tubers, which are infected among those produced by all Si in a plot (efficiency of autoinfection)
tsi_0	y-axis intercept of the monomolecular function for tsi=f(DH)
TVP	Trapped vector pressure
Vp	Simulated vector pressure
YWT	Yellow water trap
z	index

INTRODUCTION

About potatoes and viruses. Potato is a commercialised and industrialised crop in developed countries, whereas, in the developing world, it is often grown in a traditional way in subsistence-oriented food systems (3). In the latter case, the potato crop plays an important direct role in human nutrition: the increase in percent of its total production during the last 20 years, is, together with wheat, the highest in this part of the world. It ranks sixth in terms of total fresh weight production behind rice, wheat, maize, sweet potato and cassava, and is among the top-ranking crops in terms of edible energy and protein production/ha/d (3). However, potato yields are generally low in these countries and large efforts are made at national and international levels to improve potato productivity.

Viruses are considered to be one of the most restricting problems of the potato crop. These pathogens are systemic and are carried through successive generations in the tubers, which are saved for seed (seed potato). Loss of yield potential due to infection of seed potatoes with pests, diseases or of an inappropriate tuber handling during storage, is called degeneration. Degeneration increases if the harvest of a plot is used for seed during consecutive seasons. Under temperate climate conditions, the increase of the proportion of virus-infected tubers in such a seed lot is an important reason for this phenomenon.

Strategies for potato virus control. Strategies to avoid virus infection are different in developed and developing countries. Plants or tubers cannot be directly protected or cured from infection, since there are no biological or chemical pesticides against viruses. In developed countries, large amounts of so-called improved seed are produced. Seed production is separated from cropping areas to facilitate control of the spread of pathogens such as viruses and fungi. The production of improved seed requires a package of crop management practices to ensure maximum yield, appropriate tuber size and tuber health. This is guaranteed in developed countries by strictly organised seed multiplication and certification systems (20). The large majority of farmers buy such seed annually to change totally the tubers used for potato production. Since aphids are known to be the vectors of potato leafroll virus (PLRV) and potato virus Y (PVY), seed production specialists established methods to relate aphid population to tuber infection some 30 to 40 years ago (9). Haulm destruction in plots for seed tuber production is recommended at specific dates, in order to avoid exposure of the crop to large aphid populations and to late blight inoculum. Destruction dates are fixed, principally based on information of aphid population behaviour, e. g. the first appearance of winged aphids, and the intensity of aphid mass flight. In the developing world, the situation is different: based on excellent experience with improved seed in developed countries in temperate climate zones, many developing countries established an official seed production system for improved seed, using modern methods such as thermotherapy and

rapid multiplication. Numerous factors, however, such as lack of experts, poor financial resources and infrastructure, make it difficult in these countries to set up an efficiently controlled seed production and certification system which supplies the whole country. The Andean region illustrates another crucial difficulty which such systems may face. The majority of potato growers there are small farmers who are poorly linked to commercial production and official seed systems (13). They cannot afford to buy large amounts of new seed every year. These farmers mainly conserve their own seed from season to season using seed selection and storage techniques. They move seed informally between fields at different altitudes as well as between different zones as a means of maintaining seed quality (13). Recent attempts to develop production and distribution systems of improved seed, which are compatible with the above-mentioned constraints, aim at the institutionalisation of only a small system for the production of high quality seed. Such seed is then distributed periodically, building on existing informal seed flows and seed management techniques rather than by trying to replace them by official and formal procedures (11). Through such a distribution system, farmers would periodically obtain fresh seed in small quantities for multiplication in order to replace the older seed and to "flush" highly degenerated seed out of the production system in the respective agroecozone (13).

Peru's potato research programme. Such innovative approaches are the result of agricultural research programs. In Peru, in 1983, a joint project of the Peruvian National Institute of Agricultural and Agroindustrial Research (INIAA) and the International Potato Center (CIP) started to produce high quality seed with innovative modern methods (2). As well as producing seed, this programme initiated studies of the quality of the seed which is commonly used and produced by farmers themselves (farmers' seed), and comparisons of improved with farmers' seed. Under cool climate highland conditions, farmers could increase their yields on average by 20 percent using improved seed instead of farmers' seed. The programme studied the farmers' reactions to direct sales of small quantities of improved seed (20 kg). Although the price of this seed was double to triple that of farmers' seed, the farmers wanted to buy it again one season after having purchased such seed for the first time. This shows their strong interest in the agronomic characteristics of improved seed. Many farmers bought different cultivars to start the multiplication and, to extend the benefits of the seed on a broader basis (13). For the programme, it was important to know the frequency with which seed of a particular cultivar should be renewed. This frequency should reflect the rate of degeneration in each agroecological zone. Such a concept called for reliable data to estimate correctly the importance of viruses and improved seed for potato production in Peru.

Virus research of the Peruvian potato programme. The programme described above initiated extensive surveys in different zones, recording that virus incidence in farmers' seed is very high in Peru (5). Similar information has been published for other developing countries (1, 8).

Detailed investigations of the yield reduction due to virus infection suggested that damage from viruses is probably lower than expected under the cool climate conditions

in the Peruvian Highlands, compared with loss data from temperate climate zones in developed countries (4, 7, 18). An interaction of climate and virus pathogenicity was suggested by reports of field data from India, where yield losses due to virus infection were higher in warmer than in cooler seasons (16). Referring to degeneration, preliminary experimental results from the Peruvian programme suggested a much lower increase of virus infection in a seed lot planted in the highlands, compared to one planted in the arid coastal region (unpublished data). From an agronomic point of view, it would therefore seem reasonable that seed is changed in the highlands less frequently than on the coast, and probably also less than in temperate climate zones. Such dependence of the seed renewal rate on agroecological conditions would alter the goals for seed production and distribution in a country with such diverse agroecological conditions as Peru. Quantitative data on degeneration velocity are required to prove the above assumptions. Such data would also help answer questions such as: Why is virus incidence not 100 % in seed of native varieties, despite the fact that virus-free seed had never been available in the past (5)? Why is virus incidence of PLRV and PVY very low in susceptible varieties in the highlands, despite of high aphid populations in the respective zone (5, 14)? It was expected that studies on seed degeneration in different agroecozones in Peru would broaden knowledge on potato virus epidemiology in the Andes.

About epidemiology and modelling of potato virus pathosystems. Epidemiology studies interactions between components of a pathosystem over time. A diagram of the potato-virus pathosystem is presented in Fig. 1. Improving the understanding of such interactions facilitates comprehension of biological mechanisms, which in turn is essential for crop improvement. Such mechanisms may include resistance of host plants against pathogens, and the interaction of plant and pathogen genotype with agroecological conditions.

Modelling is an iterative process, which provides increasingly closer approximation of a limited section of reality with each iteration (17). Simulation has been called "the use of mathematical models to quantitatively reproduce some aspects of the real world as realistically as possible" (6). For simulation modelling, computers offer numerous advantages. According to the authors of the first published simulator in phytopathology (19), computer simulation modelling has been applied to epidemics of plant pathogens because of its potential role in "deciphering, testing, reasoning and predicting". Crop protection against plant pathogens may be optimised by using simulation models (21). Simulation models on pest and disease epidemics have been proposed increasingly in the last few years for finding decision rules that are necessary for crop management, and in particular, for crop protection practices (12). In potato production, computer-based models which simulate tuber-sprouting and crop growth (10), or the incidence of various diseases, have been proposed as tools for the understanding of crop ecology. So far, only one simulation model has been published for a potato-virus pathosystem. This model forecasts the incidence of potato virus Y^O in the tuber harvest of a potato field in

Sweden. It is thought to improve understanding of the complex interactions between virus, vectors, host and environment (15).

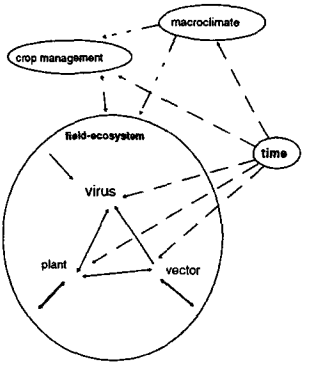

Fig. 1: The composite potato-virus pathosystem.

Possible benefits of a model of the potato-virus pathosystem. A model which predicts the proportion of virus-infected tubers in the harvest of a potato field would be beneficial for potato production in both the developing and developed world. In developed countries, seed production specialists are looking for secure methods to determine the haulm destruction date in order to avoid major infection by aphid-transmitted viruses. In developing countries, the model would be useful for policy makers as well as for seed producers; forecasting the increase of the proportion of infected tubers in a seed lot which is used during consecutive seasons in the same agroecological environment, would help to determine for how many years a farmer could continue to multiply seed in the traditional way from a single, small introduction of virus-free high quality seed. Such a model would also facilitate demarcation of zones which are appropriate for seed production, in countries where knowledge of the sanitary conditions relating to potato viruses is still scarce.

The objectives of the presented research. The research presented had the following objectives:

First, seed tuber degeneration by the most important viruses in terms of incidence in the seed had to be recorded for important, potato growing agroecozones of Peru. Three experimental sites were selected for this purpose, located in zones representing the commercialised production system on the arid coast; the partially commercialised production system on the inter-andean valley bottoms in the cool climate highland between 2900 and 3300 m.a.s.l.; and the subsistence-oriented production system between approximately 3000 and up to 4000 m.a.s.l on the steep slopes of mountains which demarcate these valleys (Fig. 2.).

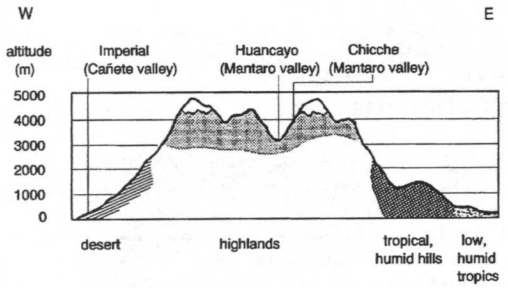

Fig. 2: Cross-section (west-east) of the Central Peruvian Andes: Agroecological zones and experimental sites for the study of seed potato degeneration PVX, APMV, PVY and PLRV (source: International Potato Center, modified).

Secondly, a theoretical model had to be developed, based on knowledge retrieved from the literature and from experiments in Peru.

Thirdly, the model had to be implemented on a micro-computer in a user-friendly way, in order to design a practical tool for seed production specialists and policy makers who are familiar with the basic biological principles involved in potato virus epidemiology.

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I. Quantification of seed potato degeneration by PVX, APMV, PVY and PLRV in three agroecological zones of Peru

Abstract

The percentage of virus-infected tubers in the harvest of individual experimental plots for PVX, APMV, PVY and PLRV in Peru was determined between 1987 and 1989, using the modern cultivar Yungay (*Solanum tuberosum ssp. tuberosum x Solanum tuberosum ssp. andigena*). Experimental sites at 112, 3280 and 4000 m.a.s.l. respectively, represented three agroecological zones. A special standardised field design was used for plots with up to 300 plants, with a low, moderate or high percentage of infected tubers (1-2, 10-20 and 44-73%) in the respective seed tuber lot. The spatial pattern of infected plants at planting and at harvest was mapped. Aphid populations were monitored with yellow water traps. Regarding the percentage of virus-infected tubers (incidence) in a seed lot and its respective tuber harvest, results can be grouped into three categories: incidence in the tuber harvest was lower, equal or higher than incidence in the respective seed tuber lot. Lower infections were obtained only in plants of moderate or high seed infection. Seed degeneration with PVY and PLRV, in terms of the difference between the proportions of virus-infected tubers in a seed tuber lot (seed infection) and in its respective tuber harvest (harvest infection), progressed by far the fastest at 112 m.a.s.l., whereas degeneration with PVX and APMV was similar at 112 and 3280 m.a.s.l., but slower at 4000 m.a.s.l. Four mechanisms were evaluated in relation to their relevance for harvest infection: a complex of phenological variables (e. g. emergence); the efficiency of autoinfection (percentage of infected tubers which is produced by a secondarily-infected seed tuber); the primary infection of plants; and the primary infection of tubers. For the first time, the efficiency of autoinfection was determined to be lower than 100 % for the four viruses as well as being one of the key mechanisms which determine harvest infection in Peru. The efficiency of autoinfection was high (among 80% for all viruses) at 112 m.a.s.l., but significantly lower at the other elevations, explaining the reduction of incidence in the respective plots. In contrast to the other viruses, more primary infections of plants were determined for PVX in plots with similar seed infection at 3280 m.a.s.l. than at 112 m.a.s.l. Primary infection of plants with PVY and PLRV was much higher at 112 m.a.s.l. than at higher elevations mainly due to differences in the capacity of virus transmission of the prevalent aphid populations, and it was particularly decisive for harvest infection with these viruses. Several vector pressure indices did not correlate well with PVY primary infection of plants or with infection of the tuber harvest, whereas a good correlation was found for PLRV primary infection. The spatial pattern of infected plants at harvest was analysed with a two-dimensional distance class model. Differences in the characteristics of the spread of contact- and aphid-transmitted viruses were detected by the model. The results suggest that autoinfection, primary infection, and mechanisms of virus transmission and propagation respond strongly to changing temperature conditions. The results are discussed in relation to their relevance for the multiplication of high quality seed in Peru.

Introduction

Potatoes are planted on more than 200 000 ha in Peru, which is the third highest acreage per country in the developing world (46). The per capita consumption and the consumed food calories per capita are among the top ranking of developing countries

(46). The Peruvian farmers produce most of their own tuber seed of native and modern cultivars by traditional methods (farmers' seed). This seed is heavily infected with viruses (7). At 8 t/ha, average yield is reported to be very low (46), partly due to viruses. In 1983, the Peruvian National Institute of Agricultural and Agroindustrial Research (INIAA) and the International Potato Center (CIP), with the financial support of the Swiss Development Cooperation (SDC/COTESU), initiated a joint potato project to improve potato productivity in Peru (23). It was of crucial importance for this programme to know the zones which are appropriate for multiplication of improved seed. This knowledge is essential to avoid a rapid virus infection of improved seed during field exposure. The difference between the proportions of virus-infected tubers in a seed tuber lot (seed infection) and its respective tuber harvest (harvest infection) is subsequently referred to as "degeneration". Seed multiplication zones had not been demarcated in Peru based on scientific evidence on degeneration velocity until this programme was started.

In the fifties some research efforts were made to determine the importance of potato viruses in Peru and to deduce practical suggestions on how to manage the problem (68, 69). However, these studies could not be followed up to the expected extent to the benefit of Peruvian agriculture. Preliminary results from degeneration studies in the highlands did not demonstrate a statistically significant difference between seed infection (percentage of infected tubers in a seed lot) and the percentage of infected tubers in the respective harvest (CIP-INIAA-COTESU, unpublished data). More information was required on the rate of seed degeneration in different agroecological zones, on the mechanisms involved in degeneration, and on their interactions with the environment. It should help to justify scientifically where improved seed should be multiplied and to understand how virus incidence in farmers' seed could have built up.

Epidemiology has been called the science which studies disease dynamics in natural states, i.e. "populations of pathogens in populations of hosts, and the disease resulting therefrom under the influence of environment and human interferences" (51). Epidemiological studies of plant viruses require special methods for reliable and rapid detection of the pathogen and monitoring of virus behaviour in its ecosystem (2). Modern, sensitive methods tend to be expensive and can often not be applied for testing a large number of samples. Enzyme-linked immunosorbent assay (ELISA) is a serological technology which has been proposed as the method of choice for "large-scale routine determination of plant viruses" (76). It is now widely used in plant virology, simple to handle, and requires relatively limited laboratory infrastructure and financial resources. With the initiation of the above-mentioned programme, this technique became available to many Peruvian scientists and could be used for routine testing as well as for research needs.

The objective of this study was to document, during several seasons, seed degeneration by the most important viruses in the principal potato-growing agroecozones of Peru. A uniform experimental design was to be developed and used in each site, to

allow comparison of results from different zones, to facilitate the monitoring of virus spread from plant to plant in a field, and to allow for an analytical interpretation of the virus incidence determined in the tuber harvest of the respective plot.

Some components and mechanisms of the investigated pathosystem which are mentioned repeatedly are represented by symbols. For a clear separation of variable symbols from the text these are written in italic letters except in formulae. Symbols which represent integer numbers start with a capital letter, whereas symbols for percentages are written entirely in lower case.

Materials and Methods

Viruses. The agronomic importance of a plant pathogen may be expressed by its incidence in a certain zone and by the severity of disorders which it produces in the plant and its product. In Peru, the mechanically-transmitted PVX and APMV are the most widely spread viruses, among those which are known to date, in commonly used seed potatoes (7). In terms of yield reduction, aphid-transmitted PVY and PLRV are the most severe viruses (65). Consequently, PVX, APMV, PVY and PLRV were selected to be studied. Damage from PVY is especially high if the plant is co-infected with PVX (33). The probability of a co-infection with PVX in potato fields in the Andes is very high, as PVX incidence normally ranges from 50 to 95% (7, 55). Consequently, PVY infected seed tubers in experimental plots were co-infected with PVX. Among the 4 selected viruses, differences between strains are reported to be most pronounced for PVY and PVX. It was determined in CIP that over 90% of the PVY infected seed tubers which were utilised for this research were infected with a strain which belongs to the PVY^N strain group, and up to 10% with a strain which belongs to the PVY^O group (Fernandez-Northcote, unpublished). PVX infections belonged to the O serotype (common). Therefore the strains present in the utilised infected seed tubers reflected predominant strains in the Andean Highlands (24, 25).

Research sites and experimental concept. In Peru, 4 major agroecozones can be distinguished in which potatoes are grown: the coastal valleys in the desert belt which borders the Pacific Ocean, with an irrigated, well-commercialised cropping system; the inter-andean valley bottoms between approximately 2900 and 3300 m.a.s.l., with partially commercialised, mostly rainfed agriculture and one growing season per year with heavy rainfall; the slopes of the mountains which demarcate these valleys, between approximately 3000 and 4300 m.a.s.l., with mainly traditional, subsistence-oriented agriculture; and the valleys which are open to the Amazon basin, between approximately 2500 and 3800 m.a.s.l., with 2 growing seasons and rainfall which is distributed over the whole year. Reported results come from degeneration experiments carried out between 1987 and 1989 in 3 locations, each of them characteristic for an agroecological

zone: Nuevo Imperial (subsequently called Imperial) in the Cañete valley of the coastal desert belt at 112 m.a.s.l.; Sta. Ana near Huancayo, which is a city in the bottom of the Mantaro valley, a zone in the Central Andes at 3280 m.a.s.l., being agronomically most productive; and Chicche, an Indian community at 4000 m.a.s.l. on the Eastern slope of the mountain range which demarcates the Mantaro valley from the jungle (Table 1.1.).

TABLE 1.1: Information on experimental sites and crops in Peru.

Name of Site	Imperial	Imperial	Sta. Ana	Sta. Ana	Chicche	Chicche
Season	1987	1988	1987/88	1988/89	1987/88	1988/89
Department	Lima	Lima	Junin	Junin	Junin	Junin
Province	Cañete	Cañete	Huancayo	Huancayo	Jauja	Jauja
District	Nuevo Imperial	Nuevo Imperial	El Tambo	El Tambo	Apata	Apata
Latitude (S)	13°0'	13°0'	12°1'	12°1'	11°5'	11°5'
Longitude (W)	76°2'	76°2'	75°1'	75°1'	75°2'	75°2'
Elevation (masl)	112	112	3280	3280	4000	4000
Season information						
Average daily mean (°C)	18	19	16	12	8	8
Rainfall planting-harvest (mm)	3	1	642	707	-[a]	-[a]
Radiation (MJ/m^2/day)	9.6[b]	12.1[c]	22.2[d]	19.9[e]	-[f]	-[f]
Planting information[g]						
Week of the year	29	35	49	45	45	41
Average max. temp. (°C)	25	19	26	20	14	20
Average min. temp. (°C)	14	13	6	5	8	3
Harvesting information						
Week of the year	50	4	21	17	21	17
Week after planting	21	21	24	24	28	28
Average max. temp. (°C)	29	27	26	21	13	13
Average min. temp. (°C)	15	19	4	3	2	3

[a] Incomplete data.
[b] CIP Annual Report 1988, average of weeks 18-48, 12°05' latitude (S), 240 m.a.s.l.
[c] CIP Annual Report 1989, average of weeks 18-48, same site as for [b].
[d] CIP Annual Report 1988, average of weeks 48-21, same site as for [b].
[e] CIP Annual Report 1989, average of weeks 48-21, same site as for [b].
[f] No data available.
[g] The modern potato cultivar Yungay was planted.

Plots with a low (1-2%), moderate (10-20%) and high (44-73%) proportion of infected seed tubers were planted at each selected site. Planting distances and fertilisation were the same in all locations and within the common practice of each respective agroecological zone. The rest of the crop management (hilling, spraying with fungicides, acaricides and insecticides, irrigation) was representative for each zone. Four tubers per plant were harvested (including 1 spare tuber), stored, and three of them subsequently submitted to analysis by enzyme-linked immunosorbent assay (ELISA) for virus detection. Data of 2 growing seasons per research site are presented for PVX, APMV and PLRV, and of 1 season for PVY. As a consequence of careful data evaluation (see below: special data testing), PVY data of another season were not included in the data set. The modern cultivar Yungay (*Solanum tuberosum ssp. tuberosum* x

Solanum tuberosum ssp. andigena) was chosen, because it is widely grown in all zones. It is reported to be susceptible to all selected viruses (47).

Seed tubers and plot design. Healthy seed tubers were produced by INIAA (basic seed with less than 2% of infected tubers). Secondarily-infected seed tubers came from experiments in the Mantaro Valley or had been multiplied there for this purpose in the field. They were tested by ELISA to ensure that they were infected only by the particular virus which was to be studied in the respective plot. Plot size varied between 5x8 m (120 plants), 8x8 m (192 plants), 6x12 m (216 plants) and 10x10 m (300 plants), depending on the number of secondarily-infected seed tubers available. The position of secondarily-infected tubers within the plots of low, moderate and high seed infection, were distributed in a uniform, or recently so-called regular (14), way: the distance between secondarily-infected tubers was always the same within a row, but they did not neighbour each other between rows (Fig. 1.1.). Distance between rows was 1 m, and between plants within a row 1/3 m. Two border rows at both plot sides, and 2 m borders at the heads of the rows with healthy seed tubers were planted to prevent border effects (e.g. damage by animals (cow, sheep), or extremely high virus input due to abnormally high infection pressure). Plots of a particular experiment which had the same infection level (low, moderate or high), but different seed infections (PVX, APMV, PVY or PLRV), were grouped in a rectangular joint-plot of the experiment. Joint-plots of different levels of seed infection were separated by at least 25 m. They were protected by a wire fence where damage by animals was to be expected.

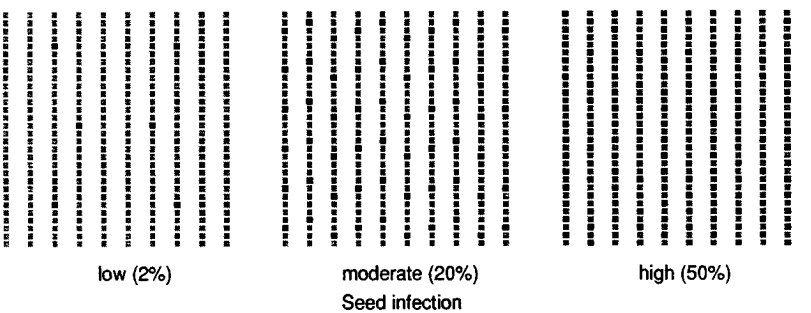

Fig. 1.1. Experimental design of plots of 300 plants with a low, moderate and high percentage of secondarily-infected seed tubers (2, 20, and 50% respectively) to study seed potato degeneration by PVX, APMV, PVY and PLRV in three agroecological zones of Peru. Grey and black rectangles represent healthy and infected plants respectively. Plot size is 10 x 10 m.

Data recording of crop management practices and climate. The position of secondarily-infected tubers was registered at planting. Crop management practices were always started in plots with the lowest initial infection. The plots were fertilised with 90-180-180 kg N-P_2O_5-K_2O at planting and 90 kg N at hilling. Each experiment was visited several times during the season to register main phenological stages (emergence,

flowering, 100% senescence), crop management (e.g. irrigation, hilling, fertilisation, spraying with pesticides, etc.), climatic (e.g. frost, hail) and other influences (eventual animal damage etc.).

Climate characterises in part an agroecological zone and also crop growth and pathogen development. Parameters of mesoclimate (29) of each research site were assessed. Daily minimum and maximum temperature were measured with a thermometer, continuous temperature and relative humidity with a permanently measuring thermohygrograph, and precipitation with a pluviometer. Recordings were made in a shady exposition, 1 to 2 m above the soil surface and within 500 m of the respective experiment. Missing data for up to 3 days were treated as described elsewhere (17).

Monitoring the aphid population. Winged populations of aphids (alatae) were monitored during each experiment at 112 and 3280 m.a.s.l. with yellow water traps (YWT) of 10x60x60 cm or 6x30x42 cm. Each trap was placed in the centre of a jointplot of the respective experiment, i.e. within the plot border rows of the chosen jointplot. The YWT was set directly on the ground and later mounted on a rack which was adjusted periodically to crop height. The traps were emptied every second day. Alatae were saved in flasks with 65% alcohol, changed weekly. According to preliminary results from 1986, aphid flights were rare in Chicche at 4000 m.a.s.l., and prevention from trap robbery and daily counting of insects was difficult. However, apterous aphids were found on the crop and other host plants (unpublished data). Therefore, no YWT was installed in this zone, but the colonising population was estimated, 103 and 134 days after 50% emergence, by counting aphids on leaves. One leaf from 112 and 300 randomly-chosen plants was chosen, consecutively from the lower, middle or upper part of each plant. Leaves were clipped, collected in a plastic bag, and a cotton tip with methyl-iso-butyl ketone added to make aphids retract their stylets and to kill them (42). Leaves were washed intensively the same day in a funnel of 60 cm diameter on a sieve at the funnel top, the aphids filtered off through a nylon screen cloth in the water outlet on the funnel bottom, and then saved in flasks with 65% alcohol for later counting and identification. All trapped and collected aphids were sent to Institute Pasteur, Paris, to be identified by G. Remaudière.

The population of flying aphids above a field correlates with the amount of virus spread in this field (44). A method has been proposed for calculating the correlation between PVY^O incidence in the tuber harvest of a potato field in Sweden and aphid catches with YWTs (67). According to this method, weekly YWT-catches of each trapped aphid species are multiplied by relative efficiency factors (REF) for virus transmission by the respective species. These corrected aphid counts are further multiplied by a factor which represents susceptibility of the potato plant to an infection and which depends on mature plant resistance in the respective week. The results of such corrections were totalled over all species to compute weekly vector pressure indices. This method was also used for the study presented: indices for virus transmission were calculated as described above with YWT data between 50% emergence and 100%

senescence of the crop. The index was called trapped vector pressure (TVP). Total TVPs were computed weekly. TVPs were multiplied by a factor for susceptibility (Sf) of the potato plant at the respective age, to yield an index which was called TVP_{c1}. In addition to this method from the literature (67), TVP_{c1} indices of each species were further corrected for selective attraction of aphid species by the yellow trap colour (74) by multiplying them with a species-specific attraction factor (Af), representing the relative attraction of the respective species compared to a reference species. These corrected aphid counts were multiplied by 100 to obtain an index which was comparable in size to TVP_{c1} indices. It was called TVP_{c2} and computed as $TVP_{c2} = (TVP_{c1}/Af)*100$.

REF values for PVY^N were taken from the literature (12). If several references existed for the same species and strain (13, 43), average values were calculated and rounded to the first decimal if the average was >=0.1, or to the second decimal, if this average was <0.1. If a species for which only qualitative data were available from literature was reported to be a vector as well as a non-vector (i.e. *Lipaphyis erysimi*; 45, 50), REF was fixed at 0.01. If no controversial reports in this respect had been published, REF values were set according to the available qualitative data (4, 8, 50), as for *Cavariella pastinacae, Myzaphis rosarum, Myzus ornatus* and *Rhopalosiphoninus staphyleae* (for specific values see Table 1.2.). For PLRV, no REF values have been published so far. All REF values were estimated according to qualitative data from the literature (4, 9, 50, 62; for specific values see Table 1.2.)

Sf values were fixed for each week after 50% emergence to 1.0, 1.0, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, and 0.0 respectively, distributing the factors which were proposed in literature (67) over a wider range of weeks, reflecting the longer growing season of Andean cultivars compared with European cultivars. For experimental sites at 3280 m.a.s.l., additional values of 0.9, 0.8, 0.7 were inserted in this range for the respective weeks.

Published Af values were used (9, 22) and calibrated to meet values obtained by Eastop (1955). For some scarcely trapped species, no Af values were found in the literature. Their attraction factor was estimated according to personal observation with yellow water traps in Peru and according to their feeding behaviour: species which feed on grasses and sedges are generally not attracted by the yellow colour (74).

Simple linear and multiple regressions were calculated to test for correlation between virus infection and the alatae population. As suggested in the literature (67), the percentages of primarily-infected plants and the percentages of infected tubers in the harvest of particular plots were considered as dependent variables. As an independent variable, the total counts of trapped alatae were used, as well as total indices TVP, TVP_{c1} or TVP_{c2}. Other simple regressions were computed by using the above-mentioned dependent variables and the infection of the tuber seed in the respective plot as an independent variable. It was subsequently tested whether the additional consideration of aphid indices for the calculation of a multiple regression improves the significance

level of the respective regression (67). Independent variables were used untransformed, as well as transformed by the multiple-infection transformation (35).

TABLE 1.2: Relative efficiency factors for PVY^N and PLRV transmission and relative attraction factors by a canary yellow colour, for different aphid species.

Species	Relative efficiency factor		Attraction factor [a]
	PVY^N	PLRV	
Acyrthosiphon pisum	0.1	-	1.0 [b]
Aphis fabae	0.1	0.1	6.6 [b]
Aulacorthum solani	0.1	0.1	4.2 [b]
Brachycaudus helichrysi	0.1	-	19.0 [b]
Brachycaudus ssp.	0.4	-	19.2 [c]
Cavariella pastinacae	0.05	-	10.0 [c]
Dysaphis ssp.	0.07	-	30.0 [c]
Hyadaphis foeniculi	0.2	-	25.0 [c]
Hyperomyzus lactucae	0.2	-	5.8 [b]
Lypaphis erysimi	0.01	-	5.4 [b]
Macrosiphum euphorbiae	0.1	0.5	29.4 [b]
Metopolophium dirhodum	0.05	-	1.0 [c]
Myzaphis rosarum	0.01	-	30.0 [c]
Myzus ornatus	0.5	0.3	50.0 [b]
Myzus persicae	1.0	1.0	12.8 [b]
Rhopalosiphoninus latysiphon	-	0.1	3.0 [c]
Rhopalosiphoninus staphyleae	0.01	0.01	10.0 [c]
Rhopalosiphum padi	0.1	-	2.0 [d]
Sitobion avenae	0.01	-	1.6 [b]
Uroleucon ssp.	0.1	-	10.0 [c]

- Non-vector species
[a] Factors are calibrated to meet values obtained by Eastop (1955)
[b] Eastop (1955)
[c] Personal observation
[d] Boiteau (1990)

Virus testing. The standard ELISA test procedure (16) was adapted to the available laboratory conditions and facilities (6). Assays were performed with polyclonal immunoglobulin G (IgG) antisera (supplied by CIP) for PVX, PVY and APMV detection, and monoclonal antibodies for PLRV (Bioreba AG, Switzerland). Polyclonal conjugates had been produced with alkaline phosphatase (AP) and glutaraldehyde according to standard protocols, using dialysis for removing excess glutaraldehyde, to meet final concentrations of 2580 U AP/0.646 mg IgG/ml. Optimal concentrations for plate coating with IgG and conjugate solution were determined for each antibody production lot by comparing the reaction of dilution series, and then choosing the combination with the lowest non-specific reaction and the highest sensitivity. For coating, polyclonal IgG concentration was between 0.66 and 1.33 µg/ml, depending on the antibody production lot. The conjugate was diluted 750 to 1500 fold. Monoclonal antisera were diluted 1000 fold. Per plate well, 230, 180 and 200 µl of IgG solution, plant extract and conjugate solution were applied and incubated at 30°C during 5 hours, at 4°C over night, and at

30°C during 4 hours respectively. Nitrophenyl-phosphate was applied at 1 mg/ml substrate solution, 160 µl per well. Special additives were egg albumin (2% w/v) for extraction and conjugate buffer (instead of expensive bovine serum albumin in the latter case), and 1 mmol of $MgCl_2$/L of conjugate buffer (38). Plates were visually evaluated.

Testing of secondarily-infected seed tubers: The seed tubers were stored at 20°C in darkness, and more than 60% relative humidity during at least 5 weeks. The tubers' sprout sap was subsequently tested. It was extracted by grinding 2 sprouts per tubers in extraction buffer (1:5 w/v) in a plastic bag (40).

Testing of harvested tubers: For each harvested plant, up to 4 tubers were stored in open paper bags placed in wooden trays for 1 to 2 months after harvest, protected from wind and rainfall, but under ambient temperature conditions. They were then treated with Rindite (250 ml/m^3) or Bromethane (200 ml/m^3) during 48 hours at 20-25°C, in a black box which had an internal ventilation system and a capacity of 8 trays with a total of approximately 50 kg of tubers. Trays were maintained afterwards during 5 weeks, at 20-25°C and 60-70% relative humidity, in the dark. The tubers were tested by analysing sprout sap, which was extracted as mentioned above; or tuber sap, which was extracted at the rose end of the tuber with a plant sap extractor (Tecan AG, 8634-Hombrechtikon, Switzerland) and applied together with extraction buffer (1:10) directly to the plate well (38, 39). In order to check this procedure permanently for its sensitivity to virus detection, 100 tubers were selected of each treatment lot of 8 trays and tested again by ELISA. These tubers were chosen, whenever possible, from plants that had produced 1 or 2, but not 3 ELISA positive tubers. The sprout sap of the selected tubers was tested again, as described above, or the tubers were planted in an aphid-proof screen house, and leaf extract of the corresponding plants was tested by ELISA 4 to 5 weeks later. If more than 5% of initially ELISA negative tubers were detected to be infected, all check results of the respective treatment lot were considered to be missing data.

Methods for data analysis and comparison. Statistical methods were required for two purposes: first, the mass of spatial and qualitative ELISA data needed to be evaluated, in order to detect undesired biased data (see below: Special data testing), i.e. to test for their suitability to be considered for further analysis. Secondly, the significance of differences between treatment results had to be tested.

Spatial pattern analysis: Several qualitative data were mapped for each plant position in a particular plot (e.g. emergence, tuber production, infection of a plant; see below). The spatial distribution of such data which characterise each plant position was tested for randomness with a model (34) which is called two-dimensional distance class model (TDDCM). The TDDCM tests differences between frequencies in distance classes. These are categories to which selected plants, separated by a determined distance in a plant-lattice, belong. The distance is expressed in separation units between plants within a row and across a row (two-dimensional). The selection criterion for a plant pair to be selected and assigned to a distance class may be any discriminating criterion of which the spatial distribution is to be tested, such as an infection, non-

emergence, etc. A computer programme was written for this model in Pascal (Turbo-Pascal compiler, version 6.0, Borland International, 4585, Scotts Valley Drive, Scotts Valley, CA 95066), to be run on a micro-computer (IBM PC or compatible, no graphics card required). The model can be applied flexibly to any r x p lattice plot, with r rows and p plants in each row. It allows for missing positions (e.g. non-emerged plants in studies of the pattern of infected plants).

Comparison of percentages: Many of the variables studied are expressed as percentages (emergence, proportion of infected tubers in the seed or in the harvest, etc.). These percentages are not averages, because no plot replicates were used in the chosen experimental design. The number of cases (plants, tubers) on which the percentages were based was not necessarily uniform for each treatment. Such frequency data were compared by a Chi-square test, testing all possible 2x2 tables of the particular data matrix. If the total of cases of a particular treatment was fewer than 30, the exact treatment of 2x2 tables according to Fisher (Fisher's exact test) was applied (26).

The significance of the difference between a given, exact percentage and a percentage related to a sample (e.g. the comparison of the exact percentage of planted infected tubers in the seed of a particular plot and the corresponding calculated percentage for the respective tuber harvest which is based on ELISA results of a tuber sample of this harvest) was determined by testing whether the exact percentage lay within the 95% confidence limits of the binomial distribution for the calculated percentage (64).

Special data testing. For some plants it was not possible to obtain test results from three tubers, due to several, unforeseen reasons: some can be attributed to sources of variance which farmers also experience, and others to experimental variation, the applied test procedure and special cases, i.e. reasons for tuber loss which do not affect farmers to the same extent. Plants which did not emerge, and plants which did not produce tubers, or which produced rotten tubers, and theft of tubers of particular plants belong to the first class. To the second class belong tuber lots which were abnormally highly infected with pathogens such as *Fusarium sp., Macrophomina phaseolina, Pythium sp., Pseudomonas* (the last three only at the coast, where temperatures may be particularly high) or others, or heavily post-harvest infected with pests such as potato tuber moth (*Pthorimaea operculella*). Infection of the saved tuber harvest with these diseases and pests destroyed in determined cases tubers which had been damaged during transportation from the field to the testing facilities, the particular microclimate in the respective paper bags, and the storage conditions for tuber incubation between harvest and tuber test (high temperatures and humidity). *Macrophomina sp.* and *Pythium sp.* especially could sporulate readily under such conditions. Tuber loss may bias percentages which are calculated with qualitative ELISA results of a tuber lot. Therefore, data of each experimental plot were carefully evaluated by the subsequent procedure to avoid wrong interpretation of test results.

Plots were rejected, if more than 25% of the plants with tuber harvest did not yield test results because of tuber loss between harvest and test. If the spatial distribution of

such plant positions was not at random according to the TDDCM, the size of the plot which was submitted to further analysis was adjusted until spatial random distribution was met.

The proportions between the total number of harvested and tested tubers of healthy plants and of plants which were not secondarily-infected but yielded ELISA positive test results (primarily-infected plants), were compared with the Chi-square or Fisher's exact test, to detect selective tuber loss of primarily-infected or healthy plants. If significantly more tubers were lost from primarily-infected plants, the plot was not accepted for further analysis.

Frequency distribution of lost plant positions on secondarily-infected and initially healthy plants was analysed by Chi-square or Fisher's exact test. Plots which did not meet independence were not accepted for further analysis. In determined experiments, secondarily-infected seed tubers had been cut to obtain the necessary amount of infected seed. Seed tubers had been fungicide treated. Results from such plots were accepted for comparison with other plots only if the above-mentioned conditions were fulfilled and if the density of plants with tuber harvest was not less than 90% of the highest planting density which was observed in all experimental plots in any experimental site corresponding to the respective virus. In order to exclude incorrect interpretation of results in the few plots where cut seed tubers had been used for secondarily-infected seed (see above), the percentage of infected tubers in the seed was determined by back-correcting the number of emerged plant positions with site and season specific emergence to compute infection in the respective seed lot. The spatial pattern of infected plants was analysed by the TDDCM only in plots which fulfilled all these conditions.

Determination of virus infection in the tuber harvest. The percentage of virus-infected tubers in the harvest of an experimental plot of season *k*, harvest infection *hi*(k), was determined based on qualitative results of ELISA analysis of sampled tubers of the respective tuber lot. Since in some cases few tubers were lost between harvest and ELISA test (see above), *hi*(k) was not calculated directly by dividing the number of infected tubers which had been detected by ELISA test through the total number of tested tubers in order to avoid biased results. Harvest infection was calculated according to equation 1.

$$hi(k) = [SiE(k)*tsi(k)*NSi + PiH(k)*tpih(k)*NPiH] / [SiE(k)*NSi + PiH(k)*NPiH] \qquad \text{eq.1}$$

SiE(k) is the number of emerged and harvested plants with a secondarily-infected seed tuber; *tsi*(k) (subsequently called "the efficiency of autoinfection"; for explanations see discussion) the percentage of infected daughter tubers among those which were produced by secondarily-infected plants; *PiH*(k) the number of harvested plants with a healthy seed tuber (including healthy and primarily-infected plants); *tpih*(k) the percentage of infected tubers among those which were produced by all *PiH*(k); and *NSi* and *NPiH* the average number of tubers which were produced by a plant with the

PiH(k) and *tpih*(k) are variables only for purposes of calculation. It would be biologically more meaningful to calculate with a variable related purely to primarily-infected plants (and not include the healthy plants as for *PiH*). However, with an ELISA test of 3 tubers per plant, the probability of sampling 3 healthy tubers of a primarily-infected plant increases, the lower the percentage of infected tubers produced by this plant. This makes the detection of an individual primarily-infected plant difficult and decreases the accuracy of values obtained for primary infection of plants and tubers. In conjunction with the problem of tuber loss between harvest and ELISA test (see above) this procedure could yield biased results. Therefore, the above-mentioned approach of using the artificial variable *PiH*, was preferred.

The variables for the number of tubers which are produced per plant (*NSi* and *NPiH*) were set to a constant value (1), since previous investigations of the Peruvian programme indicated that the number of produced tubers is not affected by the health state of the respective plant (unpublished data).

The efficiency of autoinfection *tsi*(k) was determined by pooling the test results of plants of an experiment for a particular virus in a particular site and season which were secondarily-infected and of which 3 tubers had been tested. Results for *tsi*(k) were compared by submitting the pooled respective tuber numbers to a Chi-square test or Fisher's exact test.

The value of *tpih*(k) was determined separately for each plot of a particular experiment by pooling the results of all *PiH*(k)-plants of this plot, of which 3 tubers had been tested, and dividing the number of tested, infected progeny tubers of these plants by their total tested tuber number. The separate determination of this variable for each plot is necessary because the percentage of infected tubers which is produced by an individual primarily-infected plant depends on mature plant resistance, i.e. the earliness of infection within a season (3), and consequently *tpih*(k) does so if it is calculated as an average of all primarily-infected plants which were exposed to infection in a plot. In plots with a high proportion of secondarily-infected seed tubers, this averaged percentage was expected to be higher than in plots with few infected seed tubers, because in the first case, the probability of a massive early primary infection is higher, and early infections contribute more to the average in such plots.

Calculation of variables decisive for virus infection of the tuber harvest. Several variables determine *hi*(k) and must be considered for a biologically meaningful interpretation of obtained degeneration results:

1) emergence, tuber producing plants, and plants with non-rotten tubers at harvest, which may all be called, for reasons of simplicity, phenological variables for plants with an infected or a healthy seed tuber,

2) the efficiency of autoinfection *tsi*(k),

3) the percentage of primarily-infected plants (*Pi*(k), and

4) the percentage *tpi*(k) of infected daughter tubers which are produced by primarily-infected plants.

These variables were calculated and compared as follows: emergence was mapped for each plant 5 to 7 weeks after planting and expressed in percent of planted tubers. Tuber-producing plants and plants with non-rotten tubers at harvest were monitored at harvest, and expressed as a percentage of emerged and tuber-producing plants, respectively, both for plants with a secondarily-infected and with a healthy seed tuber. The values of these variables which belong to one virus, site, and season, were compared with the Chi-square test. If the differences were not significant between plots, the data were pooled for the respective site and season. Furthermore, if the differences between plants with a secondarily-infected and a healthy seed tuber were not significant, an overall averaged value was calculated for all plots which had been planted in the respective site and season for degeneration studies of one particular virus.

Because the percentage of infected tubers which is produced by primarily-infected plants may be less than 100%, a number of primarily-infected plants was expected to escape detection by the chosen sampling procedure. The number of primarily-infected plants, of which 3 healthy tubers had been sampled, was estimated based on the assumption that the frequencies of primarily-infected plants, with 1, 2 and 3 infected tubers out of 3 tested tubers, follows a binomial distribution. The estimated number could therefore be deduced from the binomial distribution, corresponding to the observed number of plants with 1, 2 and 3 infected tubers. The total number of primarily-infected plants (*Pi*) was then calculated. With *Pi*, the averaged percentage of primarily-infected tubers which is produced by all *Pi* (*tpi*), was subsequently computed.

Results

Degeneration with PVY and PLRV was much faster in the coastal zone compared to the highlands. With APMV this tendency was less evident. Degeneration with PVX was faster at 3280 m.a.s.l. compared to 112 m.a.s.l., and slowest at 4000 m.a.s.l. Regarding the change of the percentage of virus-infected tubers (incidence) between planting and harvest, results can be grouped into 3 categories: virus incidence in the tuber harvest of a particular season *k*, *hi*(k), was statistically higher, equal, or lower than incidence in the utilised seed. Emergence was always higher than 92%, more than 93% of the plants produced tubers in any case, and more than 98% of the latter plants produced a tuber harvest which was not rotten at the time of harvesting (Table 1.3.). In relation to these variables, differences were obtained between secondarily-infected plants and with plants which were grown from a healthy seed tuber only in a few cases: in two experiments for PLRV (Sta. Ana and Chicche in 1988/89), secondarily-infected seed had worse emergence than healthy seed, and in three experiments (APMV at Imperial in

1987 and 1988 and PLRV at Chicche in 1988/89), fewer plants from secondarily-infected seed tubers produced tubers. However, differences between the respective values for plants from secondarily-infected and healthy seed tubers ranged only from 4 to 7%.

TABLE 1.3: Phenological variables in experimental potato plots for the study of seed degeneration by viruses in 3 agroecological zones of Peru (cultivar Yungay [a]).

Plot	Site	Elevation (masl)	Season	Emergence (%)[b,c]	Tuber producing plants (%)[c,d]	plants with non-rotten tubers (%)[e]
PVX	Imperial	112	1987	95.9	99.5	100
			1988	96.0	96.4	98.5
	Sta. Ana	3280	1987/88	-	98.9	100.0
			1988/89	99.8	100.0	100.0
	Chicche	4000	1987/88	-	94.3	98.7
			1988/89	97.3	99.5	99.8
APMV	Imperial	112	1987	-	92.7/99.1	100.0
			1988	92.2	93.3/99.0	99.9
	Sta. Ana	3280	1987/88	-	99.9	100
			1988/89	96.4/99.8	100.0	99.9
	Chicche	4000	1987/88	-	95.7	100.0
			1988/89	94.7	99.9	99.7
PVY[f]	Imperial	112	1988	95.6	98.2	99.5
	Sta. Ana	3280	1988/89	98.5	99.7	100.0
	Chicche	4000	1988/89	97.3	99.4	100.0
PLRV	Imperial	112	1987	-	99.3	100.0
			1988	96.8	99.3	100.0
	Sta. Ana	3280	1987/88	-	98.4	100.0
			1988/89	96.8/99.3	100.0	99.3
	Chicche	4000	1987/88	-	98.8	100.0
	Chicche	4000	1988/89	95.6/99.4	96.7/99.6	99.8

[a] *Solanum tuberosum ssp. tuberosum x Solanum tuberosum ssp. andigena.*
[b] Percent of planted tubers. Seed tubers in the corresponding experiments were of 60-80 g; emergence was evaluated 5-7 weeks after planting.
[c] Two percentages separated by a slash indicate that the difference is significant between frequencies which correspond to plants grown from a secondarily-infected seed tuber (first value) and from a healthy seed tuber (Chi-square test, P<=0.05).
[d] Percent of emerged plants.
[e] Percent of tuber producing plants.
[f] Secondarily-infected seed tubers were co-infected with PVX.
- No emergence is given for experiments where cut seed tubers had been used.

Degeneration results are first presented separately for each studied virus and each plot which fulfilled the conditions established above. The efficiency of autoinfection (*tsi*), primarily-infected plants (*Pi*), and the percentage of tubers which are infected and produced by all *Pi* (*tpi*) are also presented to facilitate interpretation of harvest infections. Harvest infections are graphically summarised in Fig. 1.2. The complete set of variables which were used for the calculation of *hi* according to equation 1 is displayed in Table 1.4. Results which are related to some particular components of the potato

virus pathosystem (e. g. vector population) and which are important for the comprehension of the pathosystem are subsequently presented.

PVX. Virus incidence in the tuber harvest of 10 plots of a total of 17 was higher than in the respective seed lot (Fig. 1.2., Table 1.4.). In 6 plots it remained the same, and in one plot a significant reduction of virus incidence was observed. The latter plot was in the highlands at 3280 m.a.s.l. (high seed infection of 73%). The tendencies were similar in both seasons. Autoinfection was high at 112 m.a.s.l. (approximately 80%), but significantly lower at 3280 and 4000 m.a.s.l. (Fig. 1.3.). Plots with increased seed infection yielded more primarily-infected plants, and this correlated to a higher proportion of infected tubers of such plants (Fig. 1.4. and 1.5.). In plots with 2% seed infection, primary infection of plants was similar in all sites. In plots with moderate or high seed infection however, highest percentages of primarily-infected plants were observed in Sta. Ana at 3280 m.a.s.l. (Fig. 1.4.).

APMV. In the tuber harvest of only 1 plot of a total of 18, virus incidence was higher than in the respective seed lot (Fig. 1.2., Table 1.4.). In 8 plots it was the same, and in 9 plots a reduction of virus incidence was observed. Incidence increased in one plot at 112 m.a.s.l. with a low seed infection and one of moderate seed infection at 3280 m.a.s.l. In plots with a proportion of infected seed tubers of approximately 50%, the lowest reductions were obtained at the lowest elevation, i.e. conditions for APMV tuber infection were most favorable at 112 m.a.s.l. This tendency was similar in both seasons. Autoinfection was high at 112 m.a.s.l. (approximately 80%), but significantly lower at 3280 m.a.s.l. and at 4000 m.a.s.l. (Fig. 1.3.). At 4000 m.a.s.l., a higher seed infection yielded a higher primary infection in the respective plot, and also a higher proportion of infected tubers of primarily-infected plants, whereas at 3280 and 112 m.a.s.l., such correlation was not observed (Fig. 1.4. and 1.5.). In plots of approximately 20% and 50% seed infection, more healthy plants were primarily-infected in the highlands compared with the coastal zone (Fig. 1.4.).

TABLE 1.4: Harvest infection (hi, % virus-infected tubers) with PVX, APMV, PVY and PLRV in experimental plots in 3 agroecological zones of Peru, and auxiliary variables for the calculation of hi.

Site	Elevation (masl)	Season	Plot	Planted tubers	S[a]	Si[b]	tsi(%)[c]	PiH[d]	tpih(%)[e]	hi(%)	Plot	Planted tubers	S[a]	Si[b]	tsi(%)[c]	PiH[d]	tpih(%)[e]	hi(%)
Imperial	112	1987	PVX	300	2.0	6	83.3	281	1.2	2.8	PVY	/	/	/	/	/	/	/
				300	20.0	57	83.3	229	9.0	23.9		/	/	/	/	/	/	/
				300	50.0	143	83.3	143	23.9	53.6		/	/	/	/	/	/	/
		1988		300	2.0	5	80.1	268	2.3	3.9		216	1.9	2	83.3	198	4.1	5.6
				300	20.0	55	80.1	219	11.1	25.0		216	9.7	20	83.3	182	22.2	28.2
				300	50.0	137	80.1	137	37.0	58.9		216	19.4	39	83.3	163	32.9	42.7
Sta. Ana	3280	1987/88		300	2.0	6	75.0	290	3.2	4.7		/	/	/	/	/	/	/
				300	18.6	54	75.0	237	17.3	28.1		/	/	/	/	/	/	/
				300	73.3	203	75.0	74	47.3	67.6		/	/	/	/	/	/	/
		1988/89		300	2.0	6	71.8	293	4.4	5.7		216	1.9	4	54.4	208	1.8	2.8
				300	20.0	60	71.8	239	18.6	29.2		216	19.9	42	54.4	170	10.5	19.2
				300	50.0	150	71.8	150	53.7	62.8		216	50.0	106	54.4	106	19.1	36.7
Chicche	4000	1987/88		300	2.0	6	40.7	272	7.4	8.0		/	/	/	/	/	/	/
				300	15.5	41	40.7	222	8.3	13.4		/	/	/	/	/	/	/
		1988/89		300	2.0	6	58.3	284	1.7	2.8		216	1.9	4	58.0	211	8.1	9.0
				300	20.0	58	58.3	232	6.1	16.5		216	12.5	27	58.0	188	11.2	17.1
				300	50.0	145	58.3	145	44.2	51.3		/	/	/	/	/	/	/
Imperial	112	1987	APMV	300	1.3	4	84.2	268	5.1	6.1	PLRV	300	1.7	6	88.2	283	4.4	5.9
				270	19.7	45	84.2	197	2.0	17.4		240	19.0	44	88.2	185	45.3	53.5
				300	50.0	128	84.2	137	4.0	42.7		300	43.8	113	88.2	145	65.8	75.6
		1988		300	2.0	5	76.8	268	0.0	1.5		300	2.0	6	83.3	283	5.4	7.0
				300	20.0	52	76.8	219	6.8	20.1		300	10.0	29	83.3	260	21.2	27.4
				300	50.0	129	76.8	137	2.0	38.3		150	20.0	29	83.3	116	66.7	70.0
Sta. Ana	3280	1987/88		300	2.0	6	71.1	283	1.9	3.3		192	1.6	3	32.1	183	2.4	2.8
				300	20.0	58	71.1	231	11.2	23.2		192	18.6	26	32.1	151	5.1	9.0
				240	74.6	170	71.1	58	24.8	59.4		/	/	/	/	/	/	/
		1988/89		300	2.0	6	44.3	294	1.6	2.4		300	2.0	6	33.2	290	5.2	5.8
				300	20.0	58	44.3	240	6.5	13.9		300	20.0	58	33.2	237	3.5	9.3
				90	50.0	43	44.3	45	15.9	29.9		300	50.0	144	33.2	148	8.7	20.8
Chicche	4000	1987/88		300	2.0	6	31.5	280	1.0	1.5		300	2.0	6	34.3	286	2.0	2.7
				300	15.5	42	31.5	228	2.2	6.7		300	15.2	41	34.3	234	0.9	6.0
				140	47.4	60	31.5	67	14.6	22.6		180	47.4	79	34.3	88	9.5	21.3
		1988/89		300	2.0	6	30.3	291	1.1	1.7		300	2.0	6	43.2	291	0.5	1.3
				300	20.0	59	30.3	238	6.7	11.4		150	20.0	28	43.2	119	3.1	10.7
				230	50.0	114	30.3	114	37.5	33.9		300	50.0	129	43.2	138	11.7	26.9

[a] Seed infection (% infected tubers).

[b] Tuber producing plants with a secondarily infected mother tuber and not-rotten progeny tubers.

[c] Average efficiency of autoinfection (% infected tubers produced per Si).

[d] Tuber producing plants with a healthy mother tuber and non-rotten progeny tubers.

[e] Average tuber infection of plants with a healthy mother tuber (% infected tubers, produced per PiH).

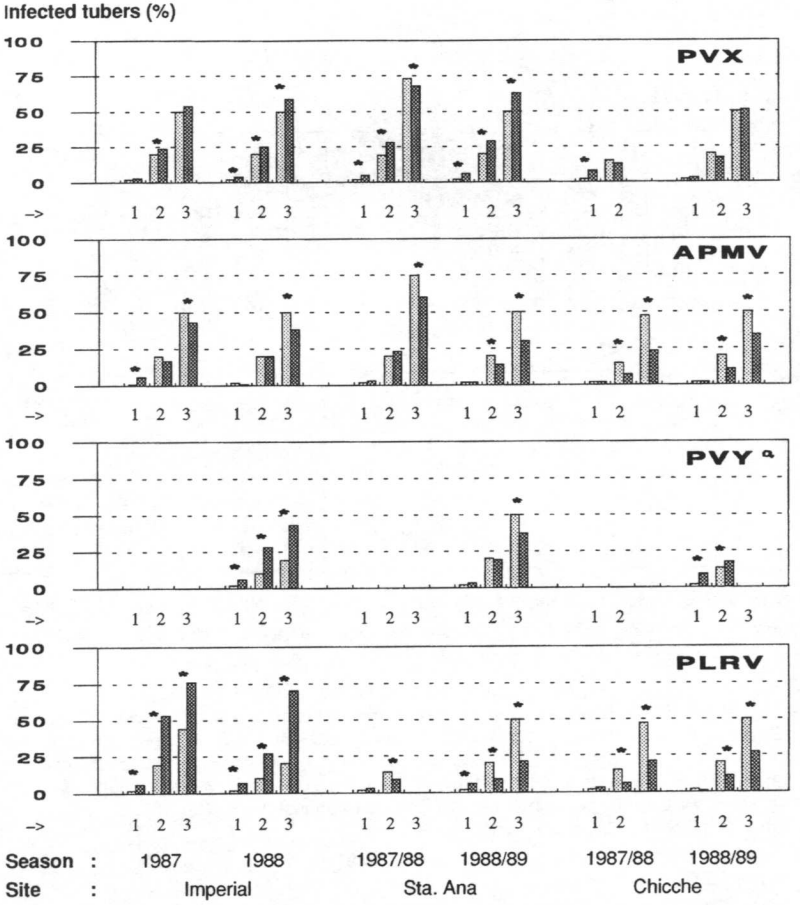

Fig. 1.2. Harvest infection (% infected tubers) in experimental plots with determined seed infection (% infected tubers) with PVX, APMV, PVY and PLRV respectively in three agroecological zones of Peru. The cultivar Yungay *(Solanum tuberosum ssp. tuberosum x Solanum tuberosum ssp. andigena)* was used. Harvest infection was detected with ELISA of three tubers per plant.

▒: Seed infection, ■: Harvest infection.

* Column pairs with an asterisk indicate that seed infection is not included in the 95% confidence interval of the binomial distribution for harvest infection.

[a] Seed tubers were co-infected with PVX.

-> Numbers represent the seed infection level in the particular plot (the seed infection is represented by the bright column).

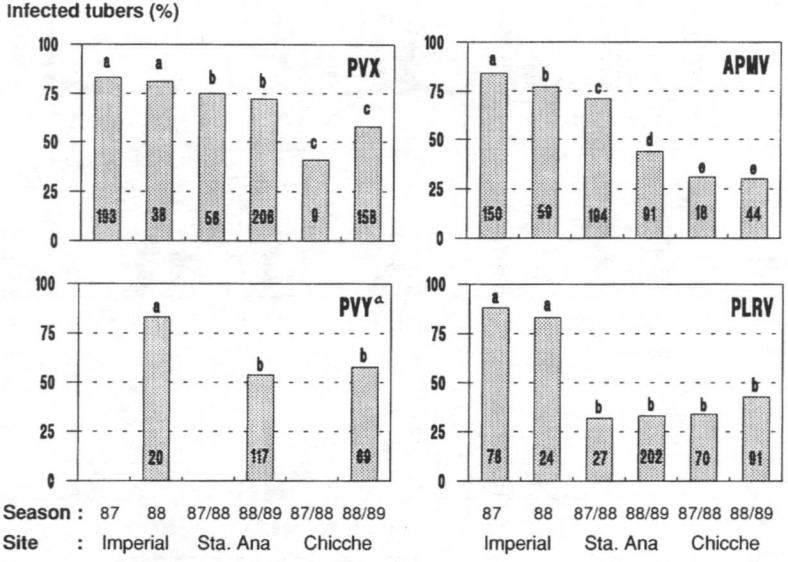

Fig. 1.3. Efficiency of autoinfection (% infected tubers) of plants secondarily-infected with PVX, APMV, PVY and PLRV in three agroecological zones of Peru. The cultivar Yungay *(Solanum tuberosum ssp. tuberosum x Solanum tuberosum ssp. andigena)* was used. Numbers in columns represent the number of analysed plants. Three randomly chosen tubers per plant were tested with ELISA. Values of columns with the same letter are not significantly different in a comparison with Fisher's exact test ($P<=0.05$).

[a] Seed tubers were co-infected with PVX.

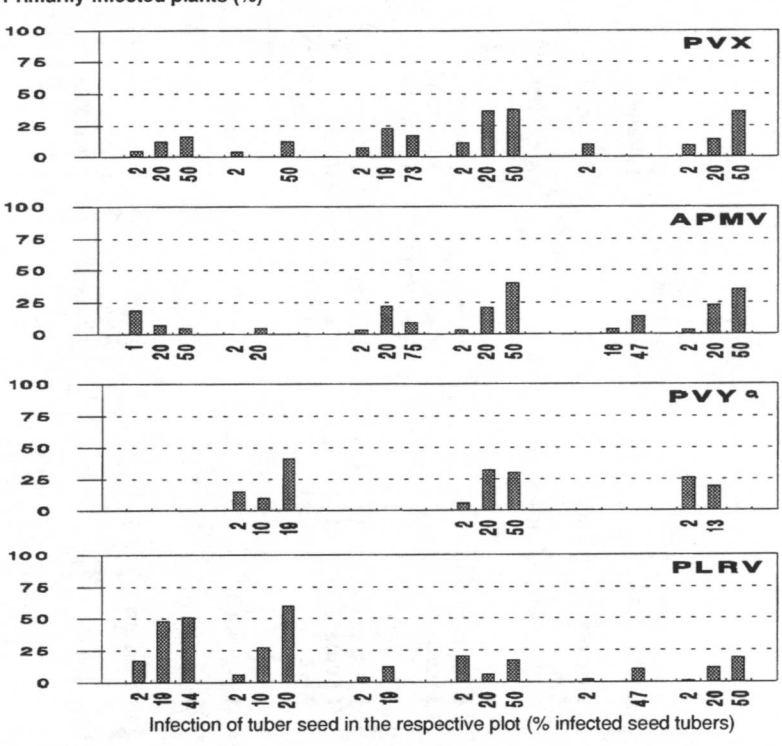

Fig. 1.4. Primary infection of plants in experimental plots with determined seed infection (% infected tubers) with PVX, APMV, PVY and PLRV respectively in three agroecological zones of Peru. The cultivar Yungay *(Solanum tuberosum ssp. tuberosum x Solanum tuberosum ssp. andigena)* was used. Seed infection in the respective plot is indicated below each column. Virus infection of a plant was detected with ELISA (three tubers per plant). Each of the presented percentages is based on a number of detected primarily-infected plants which was corrected for infected plants of which three healthy tubers had been sampled.

[a] Seed tubers were co-infected with PVX.

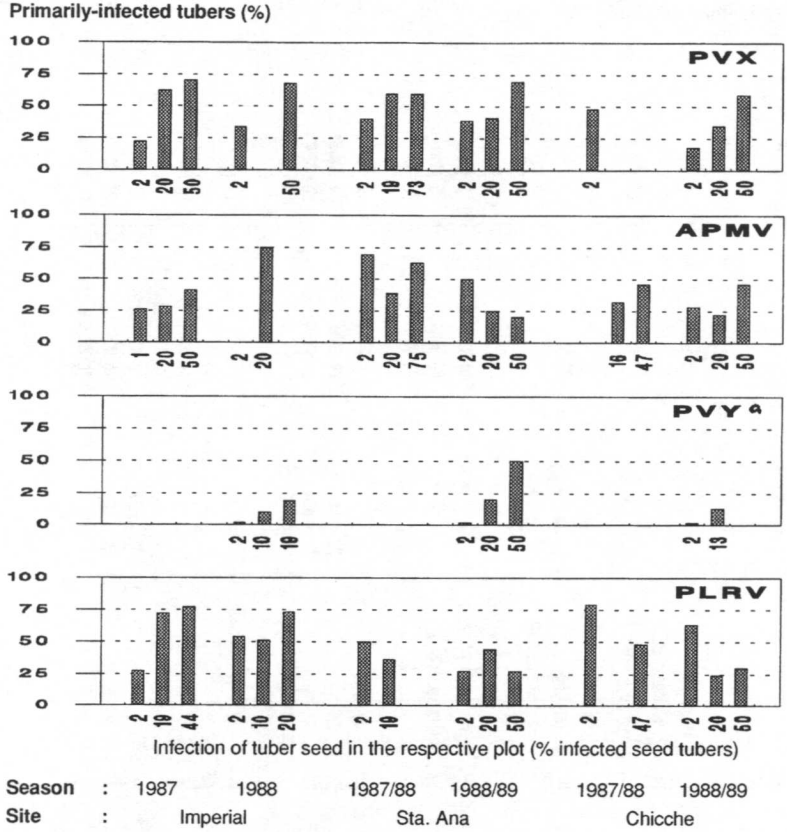

Fig. 1.5. Tuber infection of primarily-infected plants in experimental plots with determined seed infection (% infected tubers) with PVX, APMV, PVY and PLRV respectively in three agroecological zones of Peru. The cultivar Yungay *(Solanum tuberosum ssp. tuberosum x Solanum tuberosum ssp. andigena)* was used. Seed infection in the respective plot is indicated below each column. Virus infection of a tuber was detected with ELISA (three tubers per plant). Each of the presented percentages is based on a number of detected primarily-infected plants which was corrected for infected plants of which three healthy tubers had been sampled.

[a] Seed tubers were co-infected with PVX.

PVY. Virus incidence in the tuber harvest of 5 plots of a total of 8 was higher than in the respective seed lot. Three of them were at 112 m.a.s.l. with a more than 100% increase of infection, whereas the other two were at 4000 m.a.s.l. (Fig. 1.2., Table 1.4.). In two plots incidence remained the same, and in one plot a reduction was observed. Autoinfection was clearly lower in the highlands (Fig. 1.3.). No consistent tendency was detected for the relation between seed infection and harvest infection or primary infection of plants across the three agroecozones (Fig. 1.4.). AT all experimental sites, larger numbers of infected seed tubers increases the proportion of infected tubers of primarily-infected plants (Fig. 1.5.).

PLRV. Virus incidence in the tuber harvest of 7 plots of a total of 17 was higher than in the respective seed lot. All but one of these plots were at 112 m.a.s.l., yielding doubled to tripled seed infection in the tuber harvest (Fig. 1.2., Table 1.4.). In three plots incidence remained the same, and in the remaining seven which all had an moderate or high seed infection, virus incidence decreased. Autoinfection was over 80% on the coast, whereas in the highlands it was always lower than 43% (Fig. 1.3.). In all but one experiment (3280 m.a.s.l. in 1988/89), increasing seed infection yielded more primary infections (Fig. 1.4.). At 112 m.a.s.l., more primary infection yielded an equal or higher percentage of primarily-infected tubers, whereas at higher elevations this was not true (Fig. 1.4.).

Aphid population. In Imperial in 1987, trap size was not the same as in the other seasons and sites (Table 1.5.). Total catches of traps of different sizes are not directly comparable, and estimates of the proportion of each species differ with differently sized traps (18). In some experiments, data could not be obtained at the beginning of a season (Table 1.5., Fig. 1.6., 1.7.). However, the comparison of data obtained with traps of the same size (Imperial 1988 and Sta. Ana 1987/88 and 1988/89) demonstrates that total catches of alatae were higher at 112 m.a.s.l. than at 3280 m.a.s.l. (Table 1.5.). *Myzus persicae* was not the most frequent species, neither at 112 m.a.s.l. nor at 3280 m.a.s.l.. The total and relative presence of this species was lower at 112 m.a.s.l. than at 3280 m.a.s.l. Most of the alatae captured at 112 m.a.s.l. were specimens of *Macrosiphum euphorbiae*, whereas at 3280 m.a.s.l. *Brevicoryne brassicae* (1987/88) or *Brachycaudus rumexicolens* (1988/89) were the predominant species (Table 1.5.). Only at Imperial (112 m.a.s.l.) in 1988 could a clear mass flight of alatae be observed (Fig. 1.6., 1.7.). Data obtained at 3280 m.a.s.l. with traps of the same size suggest that the numbers of trapped aphids and the fluctuation of the population may vary considerably from season to season (Table 1.5., Fig. 1.6., 1.7.). Population size was clearly higher at 3280 m.a.s.l. in 1987/88. The presence of vectors in Chicche (4000 m.a.s.l.) was confirmed by aphid counts on leaves in 1987/88: an average of 0.4 winged and 2.3 wingless aphids per 100 leaves was determined 103 days after 50% emergence, and 4.6 and 49.0 respectively 31 days later. The winged aphids were specimens of *M. persicae*, *Myzus ornatus*, and one of *Rhopalosiphum padi*. *M. persicae* and *M. ornatus* transmit PVY as well as PLRV; *R. padi* is known to be a vector of PVY only.

TABLE 1.5: Trap and count data of winged aphids (averages/trap) for yellow water traps in two agroecological zones of Peru and two seasons.

Site	m.a.s.l.	Season	General data				
			Traps				
			Duration weeks[a]	No.	Size (cm)	Area (cm^2)	Total weeks of monitoring[b]
Imperial	112	1987	15	2	6x30x42	1260	13
		1988	15	1	10x60x60	3600	12
Sta. Ana	3280	1987/88	18	4	10x60x60	3600	18
		1988/89	18	3	10x60x60	3600	15
			Counts				
			Alatae[c]	Species	GPA[c,e]	Most frequent species[c]	TVP PVY[c,f]	TVP PLRV[c,f]
Imperial	112	1987	(62)[d]	19	(5)[d]	*M. euphorbiae*: (33)[d]	(9)[d]	(21)[d]
		1988	375	18	7	*M. euphorbiae*: 325	40	170
Sta. Ana	3280	1987/88	314	32	57	*B. brassicae*: 72	101	64
		1988/89	37	20	9	*B. rumexicolens*: 4	17	12

[a] Weeks counted from 50% emergence of plants in the respective experiment to 20 days before 100% senescence.
[b] Difference to growing season weeks is due to lack of data at the beginning of the respective experiment.
[c] Back-transformed averages of log transformed weekly counts per trap (Log(x+1)).
[d] Numbers of Imperial 1987 are not directly comparable to other sites and seasons because of different trap size.
[e] Green peach aphid: *Myzus persicae*.
[f] Trapped vector pressure (TVP): [catches of a species]*[relative efficiency factor of this species], totalled over all species.

Total counts of alatae or totals of TVP (Table 1.5.) did not correlate with primary infection of plants (Fig. 1.4.) in plots with a similar seed infection. None of the computed simple regressions between untransformed or transformed *Pi*(k) or *hi*(k) of a particular infection level and any of the computed aphid indices was significant (regressions with up to 8 data points, depending on the infection level). Aphid indices did not reduce significance of the regression between seed infection and *Pi*(k) or *hi*(k) for PVY. However, in the case of PLRV, significance and r^2 were improved by aphid indices. The highest significance of a multiple regression was obtained by the correlation of untransformed *Pi*(k) and TVP indices (P=0.001, $r^2_{ad.}$=0.489, regression d.f.=2, total d.f.=19).

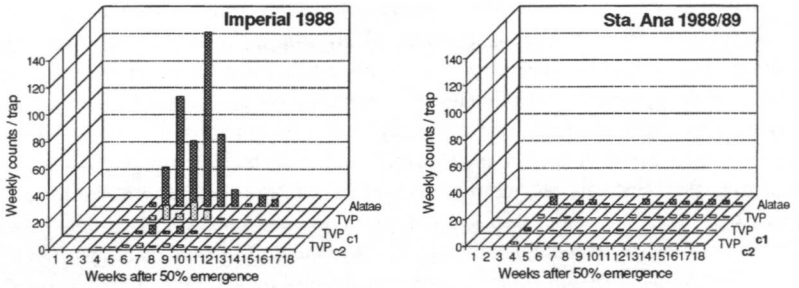

Fig. 1.6. Aphid catches in yellow water traps (counts/trap) of one season in two agroecological zones of Peru, and trapped vector pressures (TVP) for PVY^N, corrected for plant susceptibility to virus infection at determined ages (TVP_{c1}), and for aphid attraction by the yellow trap colour (TVP_{c2}). Values are back-transformed averages of weekly counts/trap which have been transformed by Log(x+1). One trap was used in Imperial 1988, and three in Sta. Ana 1988/89 (all 10x60x60 cm).

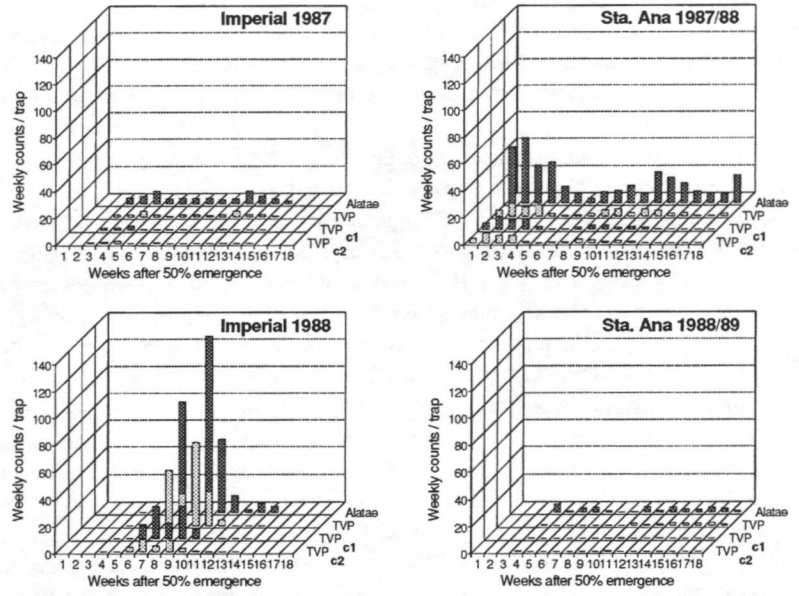

Fig. 1.7. Aphid catches in yellow water traps (counts/trap) of two season in two agroecological zones of Peru, and trapped vector pressures (TVP) for PLRV, corrected for plant susceptibility to virus infection at determined ages (TVP_{c1}), and for aphid attraction by yellow trap colour (TVP_{c2}). Values are back-transformed averages of weekly counts/trap which have been transformed by Log(x+1). Two traps were used in Imperial 1987 (6x30x42 cm), one in Imperial 1988, four in Sta. Ana 1987/88, and three in Sta. Ana 1988/89 (all 10x60x60 cm).

Spatial spread. The TDDCM was used to detect in a plot non-randomness of the spatial distribution of non-emerged plants, plants with no tuber harvest, plants with non-rotten tubers, plants with no tubers to be tested, and infected plants at harvest (discriminating positions). The first and last categories are agronomically relevant. The others were tested to avoid biased data and erroneous interpretation of results (see above: special data testing). The implemented model accumulates, for all possible two-dimensional distance classes, the frequency of pairs of discriminating positions in the respective field. The model then tests whether these frequencies deviate significantly from class frequencies representing random distribution of discriminating positions in the respective plot. These random number are obtained by 400 runs of random allocation of the observed amount of discriminating positions on all available plant positions (34). Class [r,p] represents a distance class of pairs of plants in positions which are separated by r rows and p plants along-the-row. The significance for classes [0,1] to [0,10], [1,0] to [1,5], and [2,0] to [2,2] were considered to be of particular interest. If observed frequencies in these classes were significant and formed an approximately compact cluster, it was concluded that discriminating positions occurred in the respective field in patchy spatial groups of the size of the cluster.

All experimental plots were tested for randomness of the spatial distribution of non-emerged plants. No clusters of distance classes with significantly high frequencies were found in the above-mentioned range. Plants with "no tuber harvest", "non-rotten tuber harvest" and "no tubers to be tested" were also found to be randomly distributed in all plots.

For the analysis of the spatial pattern of infected plants at harvest, only plots of low and moderate, but not high seed infection were tested. It is not reasonable to analyse plots with high seed infection because every second plant was secondarily-infected in these plots. This would yield significant frequencies for classes [0,2], [0,4], etc., and a high probability that classes [0,1], [0,3], etc. would have significant frequencies, because there are not enough healthy plants for conclusive mapping of a pathogen's spread. A summary of the plots with frequencies of distance classes which deviate significantly from randomness in the above-mentioned range is presented in Table 1.6.

Clusters of contact-transmitted viruses are different from those of aphid-transmitted viruses (Table 1.6.). The former produces clusters including mostly classes with along-the-row distances, and few significant frequencies of distance classes which include across-the-rows and diagonal distances (only 8 of 34 significant class frequencies). The largest cluster size was detected for APMV at 3280 m.a.s.l. (1988/89, 2% of seed infection) with 3 positions along-the-row. The most common significant class frequency represents spread to an adjacent plant in the same row. In contrast, half of the significant class frequencies for aphid-transmitted viruses included across-the-rows or diagonal distances (21 of 40). The largest clusters were determined for PLRV at 112 m.a.s.l. (Imperial 1987, 2% seed infection) and 3280 m.a.s.l. (Sta. Ana 1988/89, 2% of seed infection). The latter case is a good example of how well the class frequencies obtained

reflected the spatial distribution of infected plants at harvest: class frequencies indicate that infected plants are aggregated in the field in a cluster which covers up to 4 plants along-the-row and 1 adjacent row, which was in fact true (Fig. 1.8.). The spatial distribution of other plots is not presented here, as the case presented in Fig. 1.8. demonstrates well the potential of the methodology, and because all information on spatial patterns of infected plants can be retrieved from Table 1.6.

TABLE 1.6: Two-dimensional distance class model analysis of the spatial pattern of infected plants in experimental plots of degeneration studies: summarised distance classes with frequencies of infected plant pairs which are higher than expected by assuming random distribution[a].

Plot	Site	Elevation (masl)	Season	tuber seed infection	Distance classes with higher frequencies[b]
PVX	Imperial	112	1987	20	[0,1], [0,5], [0,10]
	Sta. Ana	3280	1987/88	2	[0,1]-[0,2], [0,10], [1,0]-[1,2]
				21	[0,1]
			1988/89	2	[0,1] - [0,2]
				20	[0,1]
	Chicche	4000	1988/89	2	[0,1]
APMV	Imperial	112	1987	2	[1,1]
				19	[0,5], [0,10], [1,1]
			1988	20	[0,5], [0,10], [2,1]
	Sta. Ana	3280	1987/88	2	[0,1]
				20	[0,5], [0,10], [2,1]
			1988/89	2	[0,1]-[0,3]
				20	[0,5], [0,10], [2,1]
	Chicche	4000	1987/88	20	[1,4]
			1988/89	2	[0,1]
				20	[0,5]
PVY	Imperial	112	1988	2	[0,2]
				20	[0,7]
	Sta. Ana	3280	1988/89	2	[1,4]
				20	[0,4]-[0,5], [0,10], [1,2]-[1,3]
PLRV	Imperial	112	1987	2	[0,1], [0,3]-[0,4], [0,7], [0,10], [1,3,[1,4], [2,0], [2,2]
				18	[0,1], [0,5]
			1988	2	[1,1]
				10	[1,5]
				20	[1,0], [1,2]
	Sta. Ana	3280	1987/88	2	[1,1], [1,5]
				16	[0,5], [1,3], [2,0]
			1988/89	2	[0,1]-[0,4], [1,1]-[1,4]
				20	[0,5], [1,1], [1,4], [2,1]

[a] In a distance class [r,p], r represents the plant separation in distance units between rows, and p the separation in distance units between plants within a row.

[b] Only distance classes within the range [0,1] to [0,10], [1,0] to [1,5] and [2,0] to [2,2] are presented.

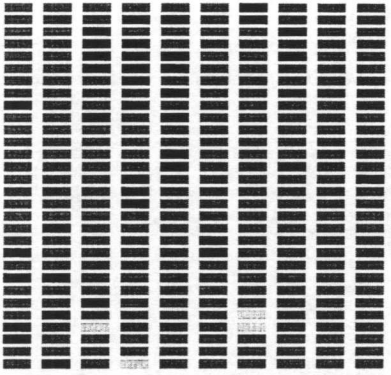

Fig. 1.8. Spatial pattern of infected plants at harvest in a plot, with 2% of PLRV infected seed tubers, in Sta. Ana 1988/89 (3250 m.a.s.l.). Grey, black and light grey rectangles represent healthy, infected and missing plants respectively. Infection was detected by the analysis of 3 tubers per plant with ELISA. The modern cultivar Yungay was used (*Solanum tuberosum ssp. tuberosum x Solanum tuberosum ssp. andigena*). Plot size was 10 x 10 m.

TABLE 1.7: Tuber loss of plants grown from a healthy seed tuber, between harvest and tuber check with ELISA[a].

Plot	Site	Elevation (masl)	Seasons	Seed infection (%)	Tuber loss (%)	
					tuber production	
					healthy	infected
PVX	Imperial	112	1988	2	30.9	4.2
	Sta. Ana	3280	1987/88	73	11.3	2.4
	Chicche	4000	1987/88	2	33.1	9.6
				15	30.1	10.8
				51	19.2	0.0
APMV	Imperial	112	1988	50	26.3	9.1
	Sta. Ana	3280	1987/88	20	5.2	0.0
	Chicche	4000	1987/88	2	35.2	0.0
				15	9.9	0.0
				47	18.7	4.3
PLRV	Imperial	112	1987	19	13.0	0.3
			1988	2	30.1	4.2
	Sta. Ana	3280	1987/88	14	1.7	2.1

[a] Four tubers per plant were harvested. The presented cases are those for which the comparison of tuber loss between harvest and ELISA test was significant (Fisher's exact test, $P<=0.05$).

Additional results. Special data testing, for the elimination of plots with biased data from further analysis (see above), yielded results which are related to problems, upon which this research was not focused. However, given the interesting information which these results provide, they are noted here.

The difference between the frequencies of lost positions of secondarily-infected plants, and of plants with a healthy seed tuber between harvest and ELISA test, was in most cases not significant. In only three cases, loss of plant positions for secondarily-infected plants was higher (Imperial 1987, PLRV, 20% of seed infection; Imperial 1988, APMV, 20% of seed infection and PVY, 50% of seed infection).

Wherever the difference was significant between the tuber loss of plants of which none of the tested tubers proved to be infected, and of primarily-infected plants, the loss was higher for plants of the first group (Table 1.7.). This suggests that healthy tubers were more susceptible to the causes for tuber loss (e.g. *Macrophomina sp.* infection, see above) than were primarily-infected ones.

Discussion

First, a theoretical model is developed which allows for an explanation of how harvest infection may be higher, equal or lower than the respective seed infection in a plot. This facilitates subsequent discussion of particular results obtained for each virus. The biological principles which underlie seed degeneration and which are highlighted by the present study are then discussed. The methodology for the determination of the efficiency of autoinfection and primary infection and the analysis of spatial patterns is analysed. Finally, practical suggestions for seed production in Peru are deduced from the results.

A theoretical model for the interpretation of obtained results. Understanding of the determined harvest infections in particular plots is improved if it is known how much each of the decisive variables for harvest infection contributed to *hi*(k).

Phenological variables (emergence, tuber-producing plants and plants with non-rotten tuber harvest) were not different in the majority of the cases for plants with secondarily-infected and healthy seed tubers. It is concluded that the interaction of these variables with virus infection of the seed tubers play no decisive role for degeneration of a seed lot which is saved during consecutive generations for seed. Three variables remain which can have a major impact on the rate of degeneration: efficiency of autoinfection (*tsi*), primary infection of plants (*Pi*), and infection of the tuber harvest of primarily-infected plants (*tpi*).

No importance for seed degeneration has been attributed so far to the efficiency of autoinfection, i.e. the percentage of tubers which are produced by secondarily-infected plants and which are infected. The principal investigations in potato virus epidemiology

until today have been made in developed countries which are situated mainly in zones with homogeneous temperate climate conditions. It is generally assumed that the efficiency of autoinfection for the studied viruses is constant and approximately 100% in these countries.

Andean cultivars are grown under a wide range of agroecological conditions. As host-pathogen interactions are strongly related to climatic conditions, this may offer a first explanation for the observed variability of the efficiency of autoinfection in Peru. If the efficiency of autoinfection is not 100%, harvest infection can never reach 100%, even if the same seed is carried forward many growing seasons and if all the plants in a field are infected at one particular time. The lower the efficiency of autoinfection, the higher its significance becomes for limiting virus infection of the tuber harvest. This mechanism does not restrict significantly the increase of virus-infected tubers in the case of improved seed because few secondary infections are present. It becomes increasingly decisive for harvest infection however, if such seed is multiplied during consecutive seasons and harvest infection, which is the seed infection for the following season, increases. A low efficiency of autoinfection challenges, therefore, parallel to an increase of seed infection, the impact of primary infection on harvest infection. At some point, there may be an equilibrium between infected tubers produced by plants with a healthy mother tuber and healthy tubers produced by plants with an infected mother tuber.

If such a theoretical model for seed degeneration is realistic, a reduction between seed and harvest infection may occur only if seed infection is exceptionally high, i.e. if a low efficiency of autoinfection overcompensates for high primary infection. In fact, a reduction of virus infection was found only in plots with a moderate or high, but not with a low seed infection (Fig. 1.2.). An autoinfection of less than 100% may also explain the virus incidence reported for farmers' seed in Peru (7). Despite multiplication during numerous growing seasons without input of pathogen-free seed, virus incidence was determined to be less than 100%. The efficiency of autoinfection possibly contributes also to the positive experience which farmers make with exchanging seed from sites at high elevations. Such reflections suggest that farmers' seed is most probably close to an equilibrium between both above-mentioned mechanisms.

A similar phenomenon is well-documented for the increased incidence of a fungal pathogen during a single growing season. An epidemic may run out of spores because it is limited by the progeny/parent ratio of a lesion. This was called the effect of "dwindling inoculum" (52), which accounts for the failure of fungal disease to increase up to 100%. In the fungal case the progeny/parent ratio determines the level of impact of a single spore infection on disease dispersal, and how quickly the declining availability of healthy leaf tissue limits the spread of the pathogen. In the potato virus case described here, the ratio of infected daughter tubers per infected mother (parent) tuber determines the level of impact a source plant (similar to the fungal lesion) may have on

the increase of harvest infection in a tuber lot which serves as seed during consecutive growing seasons.

How strongly are primary infection of plants and tubers theoretically related to the presence and amount of secondarily-infected plants in a field? Secondary infections serve as a source for the in-field spread of the virus. Primary infection of plants and tubers are mostly expected to be higher in plots with high seed infection compared with plots with low seed infection. This is because the probability of a healthy plant becoming infected in a field with a random spatial distribution of secondarily-infected plants is an average parameter which is homogeneously dispersed over the field for each particular spatial distance of a healthy plant from a secondarily-infected source plant. More opportunities for infection with these given probabilities exist in a field with high seed infection compared with a field where virus spread depends on rare events, because few source plants are available. Primary infection of plants is therefore expected to be lower in the latter case. Secondly, spontaneous unforeseen events in discrete parts of a particular field, such as an aphid settling or an animal walking through the field, have a higher probability of yielding primary infections in a plot where more virus source plants are randomly distributed. If at a particular time the probability for primary infection in one plot is higher compared with that in another, tuber infection of primarily-infected plants will be higher in the first case because of the plant's increasing resistance to infection with time (mature plant resistance; 3).

The discussion of degeneration data for particular viruses focuses on how much each of the three variables - efficiency of autoinfection, primary infection of plants and primary infection of tubers - have influenced degeneration by the respective virus.

PVX. Seed degeneration with contact-transmitted PVX was in contrast to the other viruses, as fast at 3280 m.a.s.l. as at 112 m.a.s.l. (Fig. 1.2.). Only at 4000 m.a.s.l. did degeneration tend to slow down. This is explained first by an efficiency of autoinfection which is still high at 3280 m.a.s.l. and therefore does not drastically limit degeneration at this elevation, and secondly, by a higher primary infection of plants at 3280 m.a.s.l. than at 112 m.a.s.l. in plots of similar seed infection. This suggests that plant susceptibility to the virus and/or virus infectivity is higher under conditions at 3280 m.a.s.l. than at the other elevations and that host-pathogen interaction is affected by climatic conditions. Such a conclusion is supported by the evaluation of symptoms of infected plants: symptoms reflect the extent of the disorder that a pathogen produces in its host. More plants which were secondarily-infected with PVX showed symptoms in the highlands, compared with those in the coastal zone (Table 1.8.) indicating that the pathogen established more easily in its host in the highlands. PVX is the most widespread potato virus in the highlands of the Andes (7, 55). The above observations may be explained by a higher infectivity of the virus and a higher susceptibility of the plant under highland conditions. In plots of high seed infection the primary infection of tubers was higher compared with that in plots with low seed infection (Fig. 1.5.) which coincides well with the above theoretical reflections on the relation of seed infection with the

probability of virus transmission. This, as well as the spatial pattern analysis of infected plants at harvest, confirms that PVX spreads mainly within the field from secondarily-infected foci to one to two plants within the same row. This is biologically meaningful since the virus is spread with viruliferous sap of infected plants to wounds of healthy plants which are caused by field management practices, wind injuries etc. Infection is most probable therefore for plants neighbouring an infected plant.

TABLE 1.8: Symptom expression of plants which were secondarily-infected with PVX, APMV, PVY and PLRV respectively in three agroecological zones of Peru (% plants with symptoms, cultivar Yungay [a,b]).

Plot	Main symptom	Site	Elevation (masl)	Season	No. of evaluated plants[c]	Plants with symptoms (%)[d]
PVX	Mosaic	Imperial	112	1987	208	0 a
				1988	206	4 a
		Sta. Ana	3280	1987/88	242	17 b
		Chicche	4000	1987/88	64	66 c
APMV	Severe mosaic, mottle	Imperial	112	1987	196	92 a
				1988	195	63 c
		Sta. Ana	3280	1987/88	251	79 b
		Chicche	4000	1987/88	120	38 d
PVY[e]	Severe mosaic, rugosity	Imperial	112	1987	151	91 a
				1988	124	93 a
		Sta. Ana	3280	1987/88	33	64 b
		Chicche	4000	1987/88	167	31 c
PLRV	Leafroll[f]	Imperial	112	1987	154	99 a
				1988	56	80 b
		Sta. Ana	3280	1987/88	28	75 c
		Chicche	4000	1987/88	132	2 d

[a] *Solanum tuberosum ssp. tuberosum x Solanum tuberosum ssp. andigena.*
[b] Health state of seed tubers was verified by ELISA.
[c] Symptom evaluation 6-10 weeks after planting. Visual evaluation was done by the same person 4 to 7 weeks after planting.
[d] Numbers which belong to the same virus and which are followed by the same letter are not significantly different in a Chi-square test ($P<=0.05$).
[e] Seed tubers were co-infected with PVX.
[f] Leafroll is a complex of symptoms which is typical for PLRV (leaf-rolling, especially of lower leaves, yellowing, reduction of the angle between leaf axis and stem, crinkel).

APMV. This virus is also contact-transmitted. No evidence exists for transmission by beetles under field conditions (28, 30) as reported for other comoviruses. APMV is, like PVX, a virus with which farmers' seed is heavily infected (7, 55). More plants tend to become primarily-infected in the highlands compared with the coastal zone, demonstrated by plots of approximately 20% seed infection in both sites (Fig. 1.4.). Compared with PVX there are many more plots with a decrease in tuber infection between plant-

ing and harvest (Fig. 1.2.). This is explained by a combination of fewer primarily-infected plants in plots of similar seed infection, especially at 112 m.a.s.l. (Fig. 1.4.), and a lower efficiency of autoinfection in all trials except for 1987 in Imperial (Fig. 1.3.). The host-pathogen interactions with APMV and PVX appear to respond differently to variation in climatic conditions: at 3280 m.a.s.l., the 1987/88 season was hotter, and had higher efficiency of autoinfection than 1988/89 (Table 1, Fig. 1.9.). However the efficiency of autoinfection with PVX was the same in both seasons. Thus the variation in temperature between seasons was critical for the multiplication and for tuber infection with APMV, but not for PVX.

PVY. The coast is clearly the zone of fastest degeneration with this virus, which is aphid-transmitted in a non-persistent manner. The lack of correlation between alatae counts or any aphid index and primary infection of plants was unexpected. The high population in 1988 at 112 m.a.s.l. in Imperial (Fig. 1.6.), compared with the experiment at 3280 m.a.s.l., did not yield proportionally more primary infections of plants (Fig. 1.4.). This may be explained in part by the different spectrum of species present: TVP indices for both seasons are not as different as untransformed total counts (Fig. 1.4., Table 1.5.). Further explanation is offered by postulating an alteration of the transmission ability of species which are present at both sites due to climatic conditions (e.g. a low efficiency at high temperatures in the coast compared with a high efficiency at cooler temperatures at 3280 m.a.s.l.; see "aphid data" below). Additionally, conditions at the coast may selectively favour the spread of viruses of the PVY^O group, which is the predominant strain there (24, 25), but which was present in only up to 10% of the utilised seed tubers.

Viruses which are transmitted non-persistently produce a patchy distribution pattern of infected plants around infectious foci (75), because their massive spread depends mainly on the colonising population of apterous aphids. Spatial pattern analysis of infected plants at harvest yielded few distance class frequencies which differed significantly from randomness. This means that few patches of PVY-infected plants were detected by the TDDCM. Five of the eight significant distance classes obtained belong to an experiment at 3280 m.a.s.l. (1988/89), including the only two clusters of significant distance classes which cover more than one class (Table 1.6.). This suggests that in the latter case an apterous population was present which was effectively transmitting PVY from infected to adjacent healthy plants. In the other cases however, in-field spread occurred not clustered but mostly at random, most probably by alatae that probed but did not colonise. An absent or ineffective virus-transmitting population of apterous aphids would explain also a non-patchy and low virus spread. Personal observations (unpublished data) indicated, that an apterous population was able to build up in experimental plots despite the occasional application of insecticides according to farmers' common practice. This strengthens the hypothesis that the alatae population in Imperial was transmitting PVY^N inefficiently and that REF values are altered by climatic conditions.

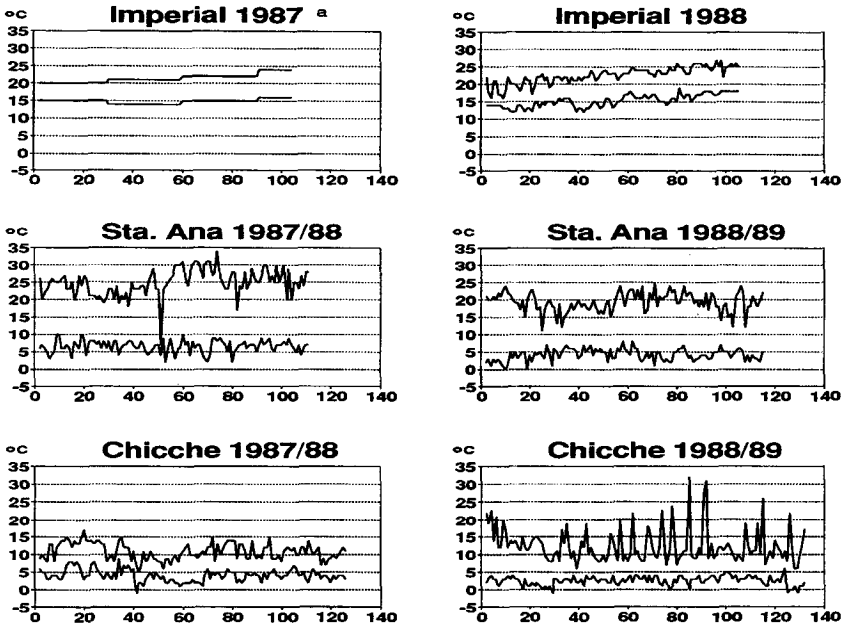

Fig. 1.9: Minimum and maximum temperatures during experiments for the quantification of seed potato degeneration by PVX, APMV, PVY and PLRV in three agroecological zones in Peru.

[a] For Imperial 1987, only monthly average data are available.

PLRV. The greatest differences between harvest and seed infection were obtained with PLRV. This virus spread very rapidly at the coast. Highest efficiency of autoinfection, primary infection of plants and primary infection of tubers were also determined with PLRV. A good correlation between primary infection of plants and seed infection with TVP indices demonstrates the importance of alatae for this virus and the approximate correctness of the chosen REF values. Alteration of an aphid's effectiveness in virus transmission and of the susceptibility of a plant to infection seems to be less

important for the spread of PLRV compared with PVY. PLRV is persistently transmitted. Infectious aphids which carry the virus over long distances may produce a randomly distributed primary spread of PLRV within a field (72). However, PLRV and PVY often spread similarly in small plots (75). The experimental plots of this study were too small and/or had too many secondary infections to detect random primary virus spread into the field. Results of the spatial pattern analysis of infected plants at harvest reflect PLRV spread from source plants with clustered patterns to adjacent plants along-the-row, across-the-row and also diagonally. In other cases, plants with two and more distance units apart from the source plant were infected with preference (Table 1.6., example of Fig. 1.8.). This suggests that the spread from infected source plants in the same field is important. The colonising aphid population, as well as winged feeding aphids which jump from plant to plant inside this field, may be responsible for this pattern of spread.

The relative importance of decisive variables for harvest infection. It has been shown above that the phenological variables play not a decisive role for degeneration. In accordance with above developed theoretical model for the interpretation of obtained results, the efficiency of autoinfection was increasingly decisive for harvest infection with increasing seed infection and decreasing primary infection (Fig. 1.2.). The decrease of infection between planting and harvest with aphid-transmitted viruses at 3250 and 4000 m.a.s.l. is spectacular and is explained on the first glance by the low efficiency of autoinfection, as outlined above. It is evident however, that the low or absent primary infection of plants is the principal reason for the low or negative rates of virus infection at high altitudes, and this is related to a low or inefficiently virus-transmitting aphid population (see below). This argument for multiplying improved seed at higher altitudes is strongly confirmed by the presented study.

Climatic variables and seed potato degeneration. Aphid population has been considered in the past as the most important variable for seed potato degeneration by viruses. This study however, elucidates the crucial importance of climatic variables acting mainly by altering the pathogen's multiplication rates, the host's susceptibility to an infection, and the vector's transmission efficiency for the pathogen. Among climatic variables, temperature is supposed to be the most important. Other variables such as humidity, precipitation, and radiation may have indirect importance, because plant growth and aphid population respond to them but not the above-mentioned mechanisms related to the viral pathogen. Temperature may be effective at any time and at many levels of the interaction between pathogen, host and vector, even at the molecular level which determines this interaction. The exposure of the virus to the other variables is possible only at the moment of mechanical insertion of the pathogen into its host. Radiation may have some direct impact on virus-plant interaction for some minutes after the virus transfer into the host, if the transmission is to superficial plant cells (PVX, APMV, PVY). It has been reported for tobacco mosaic virus (TMV) that virus localisation and local acquired resistance interact with ultraviolet light but systemic acquired

resistance does not (15). The latter is relevant for the present study as it is focused on the number of systemically infected tubers in a field.

Autoinfection with potato viruses: About terms and importance. "Autoinfection" is an expression used by plant pathologists in relation to fungal pathogens, describing infection in which the donor host individual is the same as the recipient host individual (63). This definition was exemplified by coffee leaf rust, caused by *Hemileia vastatrix*. This pathogen is distributed by spores and produces lesions after successful spore infection. Plant viruses are systemic cell parasites and do not produce propagules. Autoinfection is a less evident mechanism in this case. It may be of practical interest in only two cases: if the marketable part of a plant is affected and economically damaged by the pathogen, or if the autoinfection relates to an economically important viral pathogen which is carried forward to successive plant generations through botanical or vegetative seed.

Because the expression "autoinfection" has not been used in the past in plant virology, the question arose as to how to define this mechanism. So far, except for potato mop-top virus (19), no references document that some daughter tubers of a secondarily-infected potato mother plant may be healthy. A recent case of a plant virus pathosystem including incomplete autoinfection of secondarily-infected plants that of African Cassava Mosaic Virus (ACMV), based on extensive epidemiological studies in the Ivory Coast (21): a percentage of secondarily-infected plants may give rise to ACMV-free stems. Here, the limited infection of the vegetative progeny of a secondarily-infected plant was named "reversion" (21). In first reports on the potato virus epidemiology presented here, the phenomenon was called "autoliberation" (5). The term "autoliberation" suggests an active response of the plant to infection, that limits virus spread within a plant. Relating the observed phenomenon to an active plant response to infection is entirely hypothetical; The expression "autoinfection" is more neutral in this respect. If "autoinfection" is used for the systemic infection of tubers of secondarily-infected plants, it would include neither the mechanical insertion of the pathogen into its host, nor host-pathogen interactions in the early stages of infection. Autoinfection with fungal pathogens includes these components. For viral pathogens, only the second is of importance in relation to the mechanism of host-pathogen interaction since the first is passive i.e. it depends entirely on wounds produced mechanically by external factors such as vectors or field management practices.

The data presented suggest that autoinfection is close to 100% under conditions with an moderate average temperature and little daily fluctuation (Fig. 1.3., 1. 9., Table 1.1.). Temperatures at 3280 m.a.s.l. are characterised by almost daily fluctuation of more than 15°C and an average temperature of 10 to 15°C or higher. Average temperature at 4000 m.a.s.l. is lower, and the daily fluctuation is less - 5 to 10°C. Only on certain days, during 1 to 2 hours, the daily maximum may rise to high temperatures as at 3280 m.a.s.l. (Chicche 1988/89, Fig. 1.9.). This combination of average temperature and daily fluctuation at 3280 and 4000 m.a.s.l. does not seem to be favourable for the

multiplication and/or translocation of viruses in a secondarily-infected plant, yielding a lower efficiency of autoinfection than at 112 m.a.s.l. Developed countries are situated mainly in temperate climate zones, with temperature conditions similar to those at the experimental site at 112 m.a.s.l. in Peru, and which favour a high efficiency of autoinfection. The genotypes which are grown in these countries, and which are very different from those grown in the Andes, may respond differently in relation to the interaction of climate and autoinfection. But the prevalent temperature conditions also make it most probable that the efficiency of autoinfection has little importance for potato production in terms of being a "dilutor" of infection in temperate climates. Developed countries with such conditions, however, were the main sources of phytopathological research in this century, which may have inhibited an earlier elucidation of the importance of tuber autoinfection by viruses for potato production. This study demonstrates that the efficiency of autoinfection has considerable importance for explaining variable degeneration rates in Peru, and perhaps in other countries where potatoes of similar genotypes are grown under comparable conditions.

Autoinfection with potato viruses: an attempt to explain its biological principles. The biological processes which underlie autoinfection in plant virology have not been investigated so far. The high maximum temperature and radiation in experimental sites suggested that the tubers might undergo a kind of heat treatment which inactivates viral particles. However, measurement of soil temperature during the growing season 1987/88, at noon at 10 cm depth yielded 20°C in Imperial (112 m.a.s.l.), 13°C in Chicche (4000 m.a.s.l.), and 9°C during 1988/89 in Sta. Ana (3280 m.a.s.l.), invalidates this hypothesis. Differences between the efficiency of autoinfection may be better explained by an alteration of virus multiplication and/or spread within a secondarily-infected plant due to changing temperature conditions. Such response is suggested by observed symptom expression of secondarily-infected plants: Symptom severity reflects virus concentration in a plant (11). The lower the daily average temperature, and the greater the difference between the daily minimum and maximum (Table 1.1., Fig. 1.7.), the lower was the symptom expression in the respective plots (Table 1.8.). Such symptom expression correlated well with a lower autoinfection (APMV and PLRV in Fig. 1.2., Table 1.8.).

There are no references on the quantitative relationship between potato virus multiplication and translocation, temperature and other environmental parameters at the physiological or molecular level. But the regulation of the viral infection process, replication, and translocation are now being investigated intensively using molecular techniques for numerous viral plant pathogens. Nucleotide sequences and organisational diagrams of potyviruses have been published (61, 66). The protein which regulates cell-to-cell movement, and which is a product of the viral genome, has been identified for potyviruses (20). Sequence similarity has been found among such proteins of tobamo-, tobra-, caulimo-, como-, nepo- and potyviruses (e.g. 10). A temperature mutant of TMV, temperature sensitive in this transport function protein, suggests that the viral translocation in a plant may be influenced by temperature. Reports (31) of more practi-

cally-oriented research for resistance screening of potato clones conclude that virus concentrations in plants with PLRV, PVY and PVX infection depend on the temperature at which in-vitro plantlets are cultured. Potato genotypes were discriminated better at 26/22°C than at 18/15°C. Such data support the hypothesis that virus translocation within a plant may be restricted at sub-optimal temperatures for transport regulation. Based on these observations, the most probable expression for a temperature sensitive efficiency of autoinfection may be:

1) that the multiplication rates of virus particles are less than those of the plant cells under certain conditions, which results in a dilution of the pathogen and an increase in virus-free cells, and,

2) that translocation of the virus in the host is inhibited or restricted due to a reduced presence of factors (proteins and maybe others) which are essential for successful movement.

Primary infection. The results obtained suggest that primary infection of plants and tubers respond strongly to temperature conditions. Several mechanisms involved in primary infection must be studied in relation to this: predisposition of the plant to infection, introduction of the pathogen into its host (infection process), and the expression of viral gene products in the host cell leading to virus multiplication and translocation. That these components of the viral propagation cycle may be closely related to their response to differences in temperature has been demonstrated in part by the correlation between autoinfection and symptom expression (see above). The correlation between symptom expression and primary infection with PVY (Table 1.8., Fig. 1.4.) gives further evidence for this. The following references demonstrate that all these mechanisms are altered by changing temperature conditions: a strong relation between temperature and systemic and necrotic symptom expression; virus translocation in the host; and virus multiplication were documented for primary infection with PVX, including strong differential effects, if particular resistance genes interact with particular PVX strains (1). Symptom expression as well as virus concentration after primary infection of potato plants with PVY^N or PVY^O were recorded as high at 22/17 and 26/21°C, but low at 14/9 and 1/12°C, respectively (11). Primary infection of tubers after successful infection of plants with PVY^N and PVY^O was reported to be scarce under low temperature conditions (10-15°C) after infection (79). The suggested explanation for the degeneration data mentioned above may therefore be correct, even if the quantitative relationships of the interaction between temperature and particular components of the viral transmission and propagation process differ for each virus strain-plant genotype combination.

Aphid data. Since certification systems were developed in industrialised countries, mass flight initiation of aphids was an important criterion for determination of the date for haulm destruction (56). The pattern of fluctuation of aphid populations, in a particular experimental site but distinct seasons, did not match (Fig. 1.7.). The difference between populations in two seasons but with similar degeneration suggests that mass

flight may not necessarily be of principal importance for the understanding of primary infection under Peruvian conditions (even if the capacity of the prevalent aphid population in transmitting virus certainly is). The lack of correlation between primary infection of plants with PVY and alatae counts or TVP indices supports this hypothesis. PVY is non-persistently transmitted by many aphid species. The relevance of winged vector species that probe but do not colonise, and the colonising population for harvest infection may be confused easily in the case of PVY.

Several problems need to be discussed to clarify the meaning of the results obtained: the trapping method, and the interaction of plant and aphid with the environment in relation to their characteristics respectively as a virus host and a virus vector. In particular, plant susceptibility, the virus concentration in its host, the relative virus transmission efficiency (REF) of an aphid species, and the build-up of a colonising apterous population, need to be considered.

Trapping method: YWT catches provided a satisfactory criterion for the fixation of the haulm destruction date in the Netherlands, as long as PLRV was the most important potato virus and *M. persicae* its principal vector (43). PVY^N was invading Europe in the 1950's which called for an improvement of risk assessment for the spread of this virus as it is transmitted by several aphid species in a non-persistent manner. This led to the first reported proposal for using REF values to calculate vector pressure (VP) for potato viruses (43). REF values were calculated based on suction trap data, avoiding the problem of selective attraction of different species by the yellow colour. The spread of a non-persistently transmitted virus has also been related to the colonising aphid population, which was assessed by counts on leaves (54, 60). In England, PVY transmission in the field was monitored by trapping aphids on vertical nets, and placing them after immediate removal on test plants to measure whether they were viruliferous (41). Similar approaches have been made for viruses which are persistently transmitted, although not for PLRV. A vector pressure, called infectivity index, was calculated for barley yellow dwarf virus (BYDV) transmission (57). Alatae are caught live for this purpose with a suction trap and placed on test plants to assess infectivity of each potential species. Even if such methods are biologically more meaningful, they are difficult to implement in a country with limited infrastructure, scarce financial resources, and few trained specialists. In contrast, YWTs are easy to handle. YWT data had been successfully correlated to virus infection in tuber harvest (67). This was the principal reason for using this method in this study. The correction of counts with species-specific attraction factors (Af) did not improve regression fits. In an attempt to estimate the precision of chosen Af values (22), these were compared with the calculated ratio between YWT data and aphid data which had been obtained by using a non-selective trap type (fishing-line trap; 53) in Sta. Ana (3280 m.a.s.l.). Further comparisons were made with recently published data from Canada, including data from green, white, or yellow colour traps, leaf counts and suction traps (9). The chosen Af values were sufficiently precise according to such comparisons.

Plant susceptibility and virus multiplication in the host cell: it is difficult to differentiate experimentally between a plant's predisposition to an infection (susceptibility) and the behaviour of a virus after the pathogen's insertion into a host cell under different temperature conditions. The question is how the activity of host cell components which are essential for virus survival (t-RNAs, ribosomes etc.) respond to different temperatures before the virus is degraded by host cell enzymes or other factors. The resistance of different potato genotypes against PVX, PVM, PVY and PLRV infection, expressed as the percent of infected plants after inoculation, is altered by different temperature conditions (31). It is hard to say, however, how much the plant contributes to such differentiation compared to a possible regulation of the transcription of the virus genome by viral genes which are temperature sensitive. Results of other descriptive experiments focus more on virus behaviour after a successful infection: each PVY strain responds differently to temperature changes with respect to virus multiplication (11). This affects virus transmission by the vector which is related to the pathogen concentration in the plant at the time of virus acquisition by the aphid (12).

Relative efficiency factors: the REF of a particular species represents the efficiency of virus transmission by this species, in comparison with others. This variable may be altered by temperature, by changing the aphids behaviour or the transmission efficiency of a single specimen (59, 70, 71, 73, 77). According to these reports, aphids of several species acquire and transmit virus best at temperatures of 20 to 25°C, although there are exceptions of virus-vector combinations with better transmission at 10°C (70). Differences among clones of an aphid species may also contribute to the variability of virus transmission rates (e.g. *M. persicae;* 70).

It is concluded that the methodology used, was the one which could yield the most information with the available resources. However, further quantitative research on the relation between host plant-virus interaction and climatic variables would improve understanding of the potato-virus pathosystem. The estimation of the respective variables with modelling techniques, by changing them iteratively until they fit existing experimental results, would be another approach which is less space- and resource-consuming.

Determination of the efficiency of autoinfection and of primary infection. The calculated efficiency of autoinfection *tsi*(k) was based on a pooled tuber number of plants of which 3 tubers had been tested. The pooled tuber number consisted therefore of a composed sample of dependent sub-samples, which questions the correctness of pooling test results. However, *tsi*(k) is theoretically equal for every secondarily-infected plant, small variations excluded, which may occur through microclimatic conditions and somaclonal variation between plants. The true percentage of *tsi*(k) is therefore estimated correctly by considering each sampled tuber as an independent sample and calculating with the pooled tuber numbers.

Two factors must be considered with respect to the precision of results for primary infections:

1) Detected primarily-infected plants of a particular plot and season *k*, *Pi*(k), were corrected for infected plants, of which 3 healthy tubers had been tested. This was based on the assumption that the frequencies of plants, of which 1, 2, and 3 infected tubers (out of 3 tested tubers) follow a binomial distribution. This would be true only if each primarily-infected plant produced tubers of which the same percentage *tpi*(k) is infected. In contrast to *tsi*(k) however, *tpi*(k) depends on mature plant resistance of the plant at the time of infection. Primary infections of plants do not necessarily happen simultaneously. The above assumption reflects therefore a rough approximation to reality.

2) The lower *tpi*(k) is, the more samples (primarily-infected plants) are necessary for an accurate determination of this percentage, because the probability of sampling three healthy tubers increases. For example, 20% of primarily-infected plants escape detection if corrected *tpi*(k) equals 0.42, and if 3 tubers per plant are tested. The lower primary infection of tubers is, the more the precision suffers. Results must be validated under this perspective. However, they are suitable for detecting tendencies, which is considered to be most important for the comprehension of the mechanisms which govern degeneration.

Spatial aspects. The distribution of secondarily-infected plants in a farmer's field is random. If infected seed tubers are allocated at random to available plant positions in a particular field, generated outputs can include patterns of clustered secondary infections, although with low probability. Secondarily-infected plants in experimental plots had an intentional, regular distribution. This regular distribution is reflected by the significant frequencies of respective distance classes in plots of 20% seed infection ([0,5], [0,10] etc.; Table 1.6.). Consequently, these distance classes can not be included in the analysis and detection of significant non-random spread between plants. A regular distribution of secondarily-infected plants in an experimental plot however, had been chosen

1) to avoid occasional clustering of secondarily-infected plants after random distribution, which would be undesirable because it might cause unrealistic effects on the performance of the spatial spread of primarily-infected plants in one particular small plot, not being representative for virus spread in the respective zone;

2) to standardise the spatial pattern of secondarily-infected plants which facilitates the comparison of results from different agroecozones; and

3) to standardise for all healthy plants, with the same distance to the next secondarily-infected plant, the probability that the secondary infection serves as source plant for the infection of a healthy plant.

The TDDCM proved to be a suitable tool to document statistically the different characteristics of spatial virus spread between contact and aphid-transmitted viruses. It proved suitable for testing spatial patterns in plant-lattices, i. e. to diagnose whether

plants are distributed according to a hypothesis (i. e. randomness) and to detect particular patterns of distribution (e. g. clusters of 5 plants along-the-row and 3 plants across-the-row). A mayor advantage of the method is that it is two-dimensional and allows for missing data. It was observed that the method produced, in particular plot-cases (few primarily-infected plants), significant frequencies in distance classes for very high across-the-rows and along-the-row distances. Further research is needed to determine the characteristics of the patterns that produce such outputs, and up to which percentage of primarily-infected plants a cluster detection is possible with this method. It is conditional that discriminating plant positions are effectively identified. In this particular study it therefore made sense to study spatial patterns of infected plants with the TDDCM only in plots with a high percentage of infected tubers of primarily-infected plants, *tpi*(k).

From the start of studies of spatial patterns of plant pathogens, calculation and comparison of disease gradients around infectious foci was a commonly used method (36, 37, 78, 80). Other methods utilised for analytical description of the spatial pattern of diseased plants in small plots represented by a (row number x plant number)-lattice, have been doublet analysis (27), the ordinary run test (32), and a complex distance-orientation class method (58). Compared to the latter three methods, the TDDCM is preferred because it is simple, it considers the spread to more than only adjacent plants, and it is tolerant of missing data. Gradient calculation was not reasonable for the present study, for two reasons:

1) Gradients determine pathogen incidence over a certain distance from an infectious focus (secondarily-infected plant). Experimental plots and infectious foci were too small to express virus incidence as a percentage of infected plants in a quadrant or concentric field segment, in a determined distance from an infectious focus. To base percentages on more observations, incidence in experimental plots could be expressed as the percentage of infected plants of the pooled number of plants in a particular distance from any secondarily-infected plant in the plot. In plots of low seed infection, however, this percentage would have a low precision because only up to 6 secondary infections served as infectious foci.

2) In plots of moderate seed infection, every fifth plant was secondarily-infected, making it difficult to assign a particular primarily-infected plant to a particular infectious focus.

The significance of this study for seed potato production in Peru. This study documents clearly that production zones in the highlands are the most appropriate for multiplication of high quality seed potato. Aphid-transmitted PVY and PLRV spread very rapidly to healthy plants in plots in the coastal zone. Degeneration with these severe viruses is too fast in this zone for seed multiplication. Despite the faster spread of PVX and APMV in the highlands compared with the coastal zone, fewer problems are created because these viruses cause much less yield reduction than do PVY and PLRV (33, 65). This study has demonstrated the importance that the efficiency of

autoinfection with the viruses studied has for seed degeneration in Peruvian production zones. It accelerates degeneration on the coast and slows it down in the highlands. This gives further justification for choosing the highlands as a multiplication zone.

This study is probably the most extensive investigation on seed potato degeneration by viruses in Peru so far but highlights tendencies of degeneration in different agroecozones rather than providing an accurate quantitative forecast of seed degeneration in the respective zones. The data presented reflect degeneration in two seasons only. Degeneration may change from year-to-year as a result of changing climate, aphid population, management practices and cultivated genotypes. A simulation model for seed degeneration, which incorporates the findings of this research and which responds to changes in the parameters mentioned above, would be most helpful for biologically meaningful and agronomically valuable forecasting of degeneration in different agroecozones of Peru.

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II. Development of EPIVIT, a simulation model for contact- and aphid-transmitted potato viruses

Abstract

A model for the simulation of the percentage of virus-infected tubers in the harvest of a potato field (harvest infection) would be useful for seed production specialists in developed countries as well as for seed programme managers in the developing world. In the first case, the model would be needed as a tool for the determination of the haulm destruction date in plots for seed potato production. In the second case, it would be needed for the estimation of the long term trend of harvest infection in distinct agroecozones if one tuber lot serves as a source for seed tuber selection during consecutive growing seasons. A model (EPIVIT) is presented for the simulation of harvest infection with a contact- or an aphid-transmitted virus. Its state variables are the efficiency of autoinfection, primary infection of plants and tuber infection of primarily-infected plants. Input variables are daily minimum and maximum temperature and data referring to aphid species presence and the fluctuation of their winged population above the respective field. The model version for contact-transmitted viruses is based on a single plant approach, which simulates the spread of the epidemic from infectious to healthy plants. The version for aphid-transmitted viruses is based on a population approach simulating primary infection by means of the negative binomial distribution. The code of both versions includes stochastic elements. Rate parameters for the simulation of the physiological age of the crop, the susceptibility of a plant to an infection and the state variables are temperature sensitive. Coarse and fine sensitivity analysis of the model are presented. EPIVIT was implemented for IBM PCs and compatibles. The programme is menu and mouse or keyboard driven. It displays graphically the increase of the percentage of primarily-infected plants in the simulated plot during a season or the increase of harvest infection in consecutive growing seasons during which the same tuber lot serves as source for seed selection. For contact-transmitted viruses the spatial pattern of infected plants in the simulated plot is displayed at the end of a season. Numerical outputs are presented by model versions for both virus types.

Introduction

The potato crop is infected by numerous viruses which differ in characteristics such as architecture, physico-chemical properties and mode of transmission. The transmission criterion is of practical value as it facilitates conceptual approaches for the management of virus diseases. Those viruses which are the most important in terms of global spread and yield reduction are contact- or aphid-transmitted. It is generally understood i) that the percentage of virus-infected tubers in a seed tuber lot (virus incidence) increases if the same tuber lot serves as source for seed tuber selection during consecutive seasons (polyethic epidemic), and ii) that this phenomenon is responsible for a successively increasing loss of the yield potential of the seed tubers.

In developed countries, a formal seed system supplies the farmers with seed tubers of guaranteed quality. Specialists recommend haulm destruction in plots for seed tuber production at specific dates, in order to avoid exposure of the crop to high aphid popu-

lations. In the developing world, however, for numerous reasons formal seed systems have not had the same impact as in developed countries. Field data on virus incidence and yield reduction caused by viruses are scarce. Farmers in this part of the world seldom have access to official seed programmes which produce and distribute high quality seed tubers.

A predictive model for harvest infection (percentage of infected tubers in the harvest of a plot) would be beneficial for potato production in both the developed and the developing world. It would be of particular interest to seed production specialists in developed countries as a potential tool for precise determination of the haulm destruction date. Additionally it would be useful for seed programme managers and for seed production specialists in developing countries. Zones which are suitable for the multiplication of high quality seed tubers could be more easily demarcated. Since many of the farmers in some of these countries live in zones where virus degeneration of seed potatoes is very slow (4), such a model would allow estimation of the number of successive generations for which a high quality seed tuber lot may be multiplied with traditional crop management practices.

Potato viruses have received little attention from modellers in the last decades. Only one simulation model for potato viruses has been published so far (36). It is thought to improve the understanding of the complex interactions between virus, vectors, host, and environment for PVY^O in Sweden. It calculates the percentage of PVY^O infected tubers in the harvest of a field. The state variable is the number of plants which act as a virus source in an average potato field within a region. It is computed with a difference equation, using a modified logistic model for polycyclic epidemics with one day as a time step. The input variable is the number of winged aphids that are caught in yellow water traps. Parameters include the efficiency of a species in transmitting virus, the susceptibility of a plant to an infection according to its respective mature plant resistance, and the latent period. The model assumes that the percentage of tubers which are infected among those produced by primarily- and secondarily-infected plants is 100%. The forecast which this model makes has been reported to be accurate enough for use by seed potato growers in Sweden (36).

Extensive quantitative epidemiological studies in different agroecozones of Peru (4) point to biological mechanisms additional to those mentioned in the above model which need to be considered for understanding potato virus pathosystems in contrasting environments and for their effective management. Under determined conditions some of the daughter tubers of secondarily-infected plants may be healthy. This phenomenon was attributed to a temperature response of the tuber infection of secondarily-infected plants which is assumed to be 100% under temperate climate conditions. The expression "efficiency of autoinfection" has been introduced for this mechanism (4). The studies mentioned above also suggest that temperature conditions affect an aphid's ability to acquire and transmit the virus. Experiments under controlled conditions (6, 37, 38, 46) indicate that changing temperatures alter host susceptibility to an infection as well as virus replication and translocation. This confirms that temperature is an important variable of the potato virus pathosystem.

A model for the spread of virus diseases of potatoes which is adaptable to different agroecological conditions would contribute to the improvement of the potato crop in developed and developing countries, and possibly also to that of other vegetatively propagated crops with similar virus pathosystems. This publication reports the development of a model which was stimulated by a potato improvement programme in Peru

(11). Its objective was a biologically significant model which explains, as far as possible, the spread of virus disease in contrasting agroecozones. The model should estimate harvest infection which is regarded for the work presented as a measure of degeneration of a particular tuber lot, and it should respond to changes in temperature conditions and plant genotype, and be suitable for use in forecasting polyethic epidemics of the most important potato viruses in the Andes. It was to be implemented on a PC in a way which should facilitate its use regardless of the model's level of complexity, as an educative and explanatory predictor of seed degeneration by viruses for potato specialists. Due to its flexibility in parameter fixation for the computation of the model's state variables and the response of rates to temperature conditions it has the potential to be adapted to growing zones other than the Andes, and to contact- and aphid-transmitted viruses of other vegetatively-propagated tuber and root crops such as cassava, sweet potato etc. Consequently, the model was named EPIVIT (**epi**demics of **vi**ruses of **t**uber and root crops, or in Spanish, in homage to the country which gave impetus to its development: **epi**demía de **vi**rus de cultivos de **t**ubérculos y raízes).

Modelling terminology and symbols are used as defined elsewhere (12, 31). In order to ensure internal consistency of the model and to guarantee the dimensionality of the model concept, dimensions and units are presented with equations and variable and parameter listings as suggested by Zadoks and Schein (47). Dimensions and units are placed in square brackets. Considered dimensions are [Ti] for time, [DT] for developmental time (measured in heat units such as degree-days), [T] for temperature, [N] for numbers and [1] for dimensionless ratios, proportions etc. Integer numbers are represented with symbols starting with a capital letter, whereas symbols which represent a percentage are written in lower case. In order to clearly separate variable and constant symbols from the text these are written in italic letters except in the formulae presented.

Model development

Simulation philosophy and system boundary. Mathematical modelling of crop disease has three broad objectives: description, prediction and explanation (24). The model to be developed needed to be descriptive, but explanatory as well, mimicking biological mechanisms which are decisive for harvest infection. If this model explains a part of the system studied, it should have good predictive value (15) for forecasting the virus infection in the tuber harvest of a potato field. Analysis of the respective system is necessary as a first step for the development of an explanatory model. Analysis of an epidemic or some part of it means resolution into a set of hypotheses concerning the nature and behaviour of the epidemic's components (15).

It has been argued that the extent to which an epidemic is resolved should be conditional upon minimising experimental error (43), but that this should in turn always be judged relative to the objective of the investigation (15). According to the objectives of the model presented the minimal resolution of the potato virus pathosystem had to consider four essential components: the inoculum (seed infection or the percentage of infected tubers in the seed tuber lot), efficiency of autoinfection (the percentage of tubers which are infected among those produced by secondarily-infected plants), the percentage of primarily-infected plants, and the percentage of tubers which are infected

among those produced by primarily-infected plants. These components needed to be simulated and synthesised by the model according to the rules derived from previous studies of the biological mechanisms which underlay the epidemic. The research project which facilitated the development of this model (4) provides much incidence and spatial data for the four variables, but only at planting and harvest and not at other times during a season. The model can be validated with such data only by relating the model's outputs with real data at two times in the season: at planting and at harvest. However, the existing knowledge of the principal mechanisms involved in virus transmission and the additional insights into the potato-virus-pathosystem obtained by the study mentioned above provide enough theoretical understanding of the respective pathosystems for the construction of the model.

The model is intended to be used for simulations in a small to medium (approximately 100 m^2) average potato field of a determined zone. The expression "average" refers to crop management, cultivar, vectors, climate, diseases and pests in the simulated field which are considered to be representative for the respective zone. The model is explanatory for polyethic epidemics of contact- and aphid-transmitted viruses. It is dynamic and mechanistic with modules that link continuous differential equations with time-discrete difference-equations. There was some argument for also incorporating probabilistic elements into EPIVIT: deterministic models are suitable in contrast to stochastic models for modelling trends of populations with a small random fluctuation. Degeneration experiments may require experimental designs demanding extensive serological testing and spatial monitoring in order to yield conclusive results (4). Conventional factorial designs with repetitions cannot be realised with such complicated plot designs, which may increase the fluctuation of a treatment result around the biological trend. External factors and spontaneous, unpredictable events such as the immigration of a winged aphid population carrying virus despite a very low virus incidence in the respective zone, or an animal which walks through the field, are not registered by the experimenter. However they are responsible for what has been called environmental stochasticity (30) and what would be considered as random fluctuation of the treatment result as long as external factors which cause population fluctuations are not correlated to the respective independent variables (e. g. time). Stochastic elements were therefore incorporated into EPIVIT in addition to the deterministics, i. e. elements which allow for the description of both the trend and the fluctuations (30).

Basic assumptions. Agroecological conditions within a field are homogeneous. A plant ages on a physiological, but not calendar, time scale. Physiological age depends on temperature conditions. The period from emergence to senescence is considered to be the part of a growing season which is relevant to virus spread in a field. It is assumed that degeneration does not increase greatly during tuber storage. The growing period is initiated for computations at 50 % emergence in the respective field and stops at 100% senescence (34). The crop does not suffer water stress. No virus is carried into the simulated field from outside sources. It was further assumed that no differences exist between the efficiency of autoinfection of individual plants within the same field. After the successful transfer of a viral particle to cells of a previously healthy plant (virus transmission), the latter becomes latently infected during a certain period of time (latent period) during which the virus replicates. The virus titre is too low, however, for the plant to act as a virus source plant. After this latent period, the plant becomes infectious and the systemic infection of the tubers with virus particles begins. Latently infected plants do not produce infected tubers. Parameters of the simulation of the efficiency of

autoinfection relate to virus multiplication and translocation within a plant. EPIVIT assumes these parameters also to be valid for the tuber infection of primarily-infected plants, pretending that the same biological mechanisms are relevant in both cases. Those assumptions which are specific for the computation of a particular variable or a particular virus type are given in the respective section below.

Temperature sensitive growth rates and biological development. Temperature has been proposed to be the principal climatic variable for potato virus-host plant interaction (4). Many rates which are used by the proposed model are temperature sensitive. The response of a growth process to changes in temperature conditions can be characterised by the temperature range, within which growth occurs, and a function, which determines the growth rate at any temperature within this range, relative to the maximum rate. A function which is most variable in shape and which has been used to relate temperature to the development of pathogenic fungi is the beta function (2, 5, 19, 20; equations 1 and 2). It's plot is bell-shaped with cardinal temperatures T_{min} and T_{max} (see an example in Fig. 2.1A.). The parameters m and n are host - pathogen specific, and a is a scaling factor.

$$b(T) = a*T_n^n*(1-T_n)^m \quad [1] \quad [-] \quad \text{eq. 1}$$

$$T_n = (T-T_{min})/(T_{max}-T_{min}) \quad [1] \quad [-] \quad \text{eq. 2}$$

EPIVIT uses this function to relate rates with temperature. In a discontinuous version, b(T) increases according to equation 5, starting with b(T)=0 at T_{min} and with (T_{max}-dr) as cardinal temperature at the upper end of the relevant temperature range. Once the function gets to 1.0, b(T) is held at this value during a given delay range of temperature degrees (dr) before it declines again, according to equation 5, with a lower cardinal temperature of (T_{min}+dr) until it falls to 0 at T_{max} (see an example in Fig. 2.1B.).

The calculation of degree-days (or heat sums) has been used extensively for the calculation of developmental heat as a model for relating recurring phenomena of insect and plant development to environmental changes with time. This method is also used in the study presented. Degree-days are computed by integration of the area under the sine curve through historical minima and maxima of daily temperature above a determined threshold.

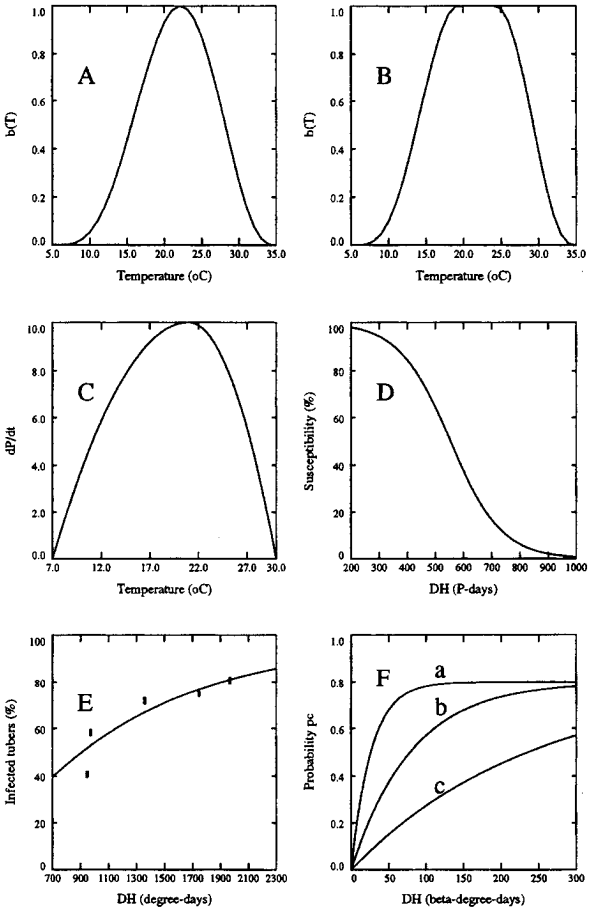

Fig. 2.1. Plots for the illustration of some selected models which were essential for EPIVIT's development: **A)** An example of a beta function with temperature T as independent variable (parameters: m=3, n=4; cardinal temperatures of 5 and 35°C); **B)** The modified, discontinuous version of the beta function (same parameter values and temperature range as A); delay range of 5°C); **C)** Function for the simulation of the rate of advancement of the physiological crop age with T reported by Sands et al. (1979); **D)** Model for the logistic decrease of a plant's susceptibility to a virus infection with accumulated developmental heat (DH) related to physiological crop age (DH_0= 98, r= -1.0998E-2); **E)** Fit of the monomolecular function through data on the efficiency of autoinfection (tsi) with PVX plotted against DH in degree-days accumulated above 0°C in the respective agroecozones (Fig. 1.3. and 1.9.); model: tsi=1-((1-0.145)*exp(-9.13E-4*degree-days)); fit (with values transformed to the linear model version): r^2= 0.941; **F)** Selected multiple infection transformations relating DH (in beta-degree-days) to the probability of infection with a contact-transmitted virus (pc) for a healthy plant situated adjacent to a virus source plant; model: $pc=Suc*(1-\exp(-r_{mi}*DH/Su_c))$, Su_c=0.8; r_{mi} is with case a: 3.0; case b: 1.0; case c: 0.33.

EPIVIT's fundamentals. EPIVIT defines the state of the pathosystem at the end of a season with the efficiency of autoinfection (*tsi*), the percentage of primarily-infected plants (*Pi*), and the percentage of infected tubers of primarily-infected plants (*tpi*). The model does not use a rate representing the change of harvest infection (*hi*) per season (*k*), but computes harvest infection at crop senescence by mechanistically connecting these state variables mentioned above. The number of infected seed tubers in the simulated field (*Si*) is the number of seed tubers used (*Ns*) multiplied by harvest infection of the last season (hi(k-1); equation 3). The number of emerged infected seed tubers (*SiE*) is the product of *Si* with the respective emergence (*esi*; equation 4). The number of plants which do not act as a virus source until harvest (*HeE*, including latently infected plants) is the difference between the product of the number of healthy seed tubers with the respective emergence (*eh*), and primarily-infected plants (*Pi*) which become infectious, i. e. a virus source plant for further spread until the end of a season (equation 5). EPIVIT simulates the value of the state variables at crop senescence of a particular season according to hypotheses on the underlying biological principles (see below). The efficiency of autoinfection (*tsi*) and the percentage of infected tubers of primarily-infected plants (*tpi*) are averaged respectively over all secondarily and infectious primarily-infected plants (%). The output variable *(hi)* is computed at crop senescence according to equation 6, the numerator representing the simulated number of infected tubers which were produced in the respective field and the denominator representing the simulated total number of tubers produced. *NSi*, *NPi* and NHe are constants for the number of tubers which are produced, respectively, by secondarily-infected, primarily-infected, and healthy plants.

$$Si(k) = hi(k-1)*Ns(k) \quad [N] \quad [plants] \quad \text{eq. 3}$$

$$SiE(k) = Si(k)*esi(k) \quad [N] \quad [plants] \quad \text{eq. 4}$$

$$HeE(k) = ((100-Si(k))*eh(k)) - Pi(k) \quad [N] \quad [plants] \quad \text{eq. 5}$$

$$ih(k)=(SiE(k)*tsi(k)*NSi+Pi(k)*tpi(k)*NPi)/(SiE(k)*NSi+Pi(k)*NPi+HeE(k)*NH) \quad [1] \quad [\%] \quad \text{eq. 6}$$

EPIVIT is driven by daily minimum and maximum temperatures and weekly aphid catches of an appropriate insect trap which allows for the monitoring of the vector species present and the estimation of their respective specimen numbers for a particular week. The model actualises daily independent variables for the simulation of the state variables. Primary infection of plants is computed weekly from weekly aphid input data. The remoteness of a selected production zone and/or the limited availability of trained technicians may make daily recordings of aphid data difficult in developing countries. Data collection is most probably possible however, every third or more days, which determines weekly input of aphid data into the model.

EPIVIT's input, output and auxiliary variables, and the model's constants are listed in Tables 2.1. and 2.2. All parameters for the simulation of state variables and essential auxiliary variables and indices used are summarised in Table 2.3.

TABLE 2.1: List of EPIVIT's input, output and auxiliary variables.

Variables			
Input variable		Dimension [a]	Unit
T_{min}, T_{max}	Historical minimum and maximum of daily temperature	[T]	[°C]
Tc	Weekly aphid trap catches	[N]	[aphid]
Output variable			
hi	Percentage of infected tubers in the harvest	[1]	[%]
Auxiliary variables			
bd	Beta-degree (rate of advancement of DH with t)	[DT/Ti]	[bdd/hour] [b]
DH	Developmental heat sum	[DT]	[bdd]
esi	Emergence of secondarily-infected seed tubers	[1]	[%]
eh	Emergence of healthy seed tubers	[1]	[%]
Hf	No. of virus-free plants	[N]	[plant]
He	No. of non-infectious plants (includes latently infected plants).	[N]	[plant]
HeE	No. of emerged He	[N]	[plant]
i	Week	[Ti]	[week]
In	Simulated, performed inoculations in the field	[N]	[inoc.]
Inp	Simulated , performed inoculations per plant	[1]	[inoc./plant]
k	Season	[Ti]	[season]
Lpw	Latent period (measured in weeks)	[Ti]	[week]
Ne	No. of planted tubers in the simulated field	[N]	[tuber]
Ns	No. of emerged tubers in the simulated field	[N]	[tuber]
pa	Probability of a landing aphid being viruliferous	[1]	[%]
pc	Probability of infection with a contact-transmitted virus	[1]	[%]
REF_{sp}	Relative efficiency factor of aphid species sp (T sensitive)	[1]	[-]
pi	Percentage of primarily-infected plants which are infectious	[1]	[-]
PiP	No. of primarily-infected plants	[N]	[plant]
r_p	Rate of advancement of physiological age with t	[DT/Ti]	[P-day/hour]
Si	Secondarily-infected plants or seed tubers	[N]	[plant]
SiE	No. of emerged Si	[N]	[plant]
Sp	No. of aphid species	[N]	[species]
Vp	Simulated vector pressure	[N]	[Vpu] [c]
t	Season time	[Ti]	[day]
T	Temperature	[T]	[°C]
Ta	Average daily temperature at which T is at its maximum	[T]	[°C]
Tv_{sp}	Simulated viruliferous aphids of species sp	[N]	[aphid]

[a] Dimensions are: DT: developmental heat; N: numbers; T: temperature; Ti: time; 1: dimensionless (ratios, proportions etc.).

[b] bdd: beta-degree-days.

[c] Vpu: Vector pressure units.

TABLE 2.2: List of indices and constants used by EPIVIT.

Indices		Constants	
a	Refers to DH [a] related to tsi	kp	Scaling factor for r_p
at	Refers to DH related to tsi	NHe	No. of daughter tubers of a He [b]
b	Refers to DH related to Pi [c]	NPi	No. of daughter tubers of a Pi [c]
br	between planted rows	NSi	No. of daughter tubers of a Si [d]
c	Constitutive	C	Randomisation constant
Lp	Relates to the latent period		
mr	Relates to mature plant resistance		
pi	Relates to primary infection		
p	Relates to physiological age		
sp	Aphid species		
wr	within a planted row		
z	Aphid specimen of a single plant		

[a] Developmental heat.
[b] Non-infectious plants.
[c] Primarily-infected, infectious plants.
[d] Secondarily-infected plants.

Physiological time. Several models for potato crop growth and development have been published (10, 21, 23, 29, 34). Some are more complex than others. Because growth is temperature-dependent (28), all calculate a temperature-related physiological age of the crop or determined organs instead of using a calendar scale, in order to obtain a realistic scale for growth. The models compute physiological time by using a temperature-dependent function for the relation between the rate at which physiological time advances relative to real time, and the instantaneous temperature. Some use compound theoretical non-linear response functions, each of which is valid for a determined temperature range (23, 34); others use compound linear equations for determined ranges (29, 10) or experimental data directly which are entered into the model as an array of data points accessed by look-up and integration functions (21). For the present model, the direct use of empirical data was discarded in order to maintain the flexibility of the model and its potential to be applied to different conditions. Two approaches for the simulation of the crop's physiological age were compared in order to choose the more accurate, which was to be integrated into EPIVIT. Both approaches accumulate physiological age (P-time) until season time t, relative to real time t at a rate r_p which depends on temperature T (equation 7 and 8).

$$r_p(t) = \frac{dP}{dt} = kp * f(T(t)) \qquad [DT/Ti] \quad [P\text{-days/hour}] \qquad \text{eq. 7}$$

$$P\text{-time}(t) = \int_0^t p_r(T(t))\, dt \qquad [DT] \quad [P\text{-days}] \qquad \text{eq. 8}$$

TABLE 2.3: The parameters of EPIVIT's state variables and of its essential auxiliary variables.

Variables and parameters	Description	Dimension [a]	Unit
Efficiency of autoinfection (tsi) **and primary tuber infection** (tpi)		[1]	[plant^{-1}]
m_a, n_a, dr_a,	Beta function parameters	[1]	[-]
$T_{min;a}$, $T_{min;a}$		[T]	[°C]
TH	Trigger developmental heat	[DT]	[bdd_a]
r_a,	Rate parameter and y-axis intercept of the monomolecular function representing tsi=f(DH)	[DT^{-1}]	[bdd_a^{-1}]
tsi_0		[1]	[%]
Primary infection of plants (Pi)		[N]	[P_{Lp}-days]
General			
Lp	Latent period	[DT]	[P-days]
m_{Lp}, n_{Lp},dr_{Lp}	Beta-function parameters	[1]	[-]
Contact-transmitted viruses:		-	-
m_b, n_b,	Beta function parameters	[1]	[-]
$T_{min:b}$, $T_{max;b}$		[T]	[°C]
C_{wr}, C_{br}	Plant age at canopy closure within and between rows	[DT]	[P-days]
r_{mi}	Rate parameter of the multiple infection transformation	[DT]	[bdd_b^{-1}]
Aphid-transmitted viruses:			
k_{pi}	Parameter of the negative binomial distribution	[1]	[-]
h_1, h_2, h_3	Average daytime when aphid activity starts, ends, and temperature reaches its maximum	[Ti]	[-hour
$REFc_{sp}$	Constitutive relative efficiency factor for species sp ranging from 0 to 1.0 (not temperature sensitive)	[1]	[-]
m_{sp}, n_{sp},	Beta function parameters related to the temperature	[1]	[-]
$T_{min:sp}$, $T_{min;sp}$	sensitive REF_{sp} ranging from 0 to 1.0	[T]	[°C]
Af_{sp}	Relative attraction factor for species sp ranging from 0 to 1.0	[1]	[-]
q	Scaling parameter for REF and Af	[1]	[-]
M	Number of aphid moves in a field before leaving	[N]	[moves]
Physiological time (P-time)		[DT]	[P-days]
m_p, n_p, dr_p,	Beta function parameters for the calculation of the rate	[1]	[-]
$T_{min:p}$, $T_{max;p}$	of physiological time advancement with time, $r_p(t)$	[T]	[°C]
Susceptibility of plants to an infection (Su_{mr})		[1]	[-]
Su_c	Constitutive susceptibility index ranging from 0 to 1.0	[1]	[-]
P_{max}	Physiological age at 100% senescence of the crop	[DT]	[P-days]
Mri	Physiological age at the initialisation of mature plant resistance	[DT]	[P-days]
r_{mr}	Rate parameter of the logistic function for mature plant resistance	[DT^{-1}]	[P-days^{-1}]
Qualitative parameter			
Spatial pattern	Seed tubers are distributed onto the (row x plants/row)-plot lattice at random, uniformly, or according to a historical field design	-	-

[a] Dimensions are: DT: developmental heat; N: number; T: temperature; Ti: time; 1: dimensionless (ratios, proportions etc.).

The constant *kp* is a scale factor and was set to 10 (34). The difference of the computation of degree-days is in the non-linear relationship between development and temperature represented by f(*T*). The first approach uses two continuous functions with adjacent upper and lower cardinal temperatures respectively for f(*T*), and was taken from the literature (34). The other method uses the beta function as a theoretical function, representing physico-chemical principles of the response of a growth rate to temperature. Both approaches require historical daily temperature data and were applied to a reported data set (Fig. 1.9.). These data cover the time from 50% emergence to senescence, and refer to a modern potato cultivar which was planted in different agroecological zones of Peru. The zones represent a wide range of climatic conditions. One set of physiological ages was calculated for the seasons included in these data with the first method (34) by using the reported cardinal temperatures of 7 and 21°C, and 21 and 30°C respectively, for the two compound continuous functions (Fig. 2.1C.). Several sets of physiological ages were then calculated by using the beta function, by a stepwise change of the parameters and cardinal temperatures of this function. The covered range of values for the beta parameters was 0.5 to 3.0 for *m* and *n* with a step size of 0.5, delay range (*dr*) of 0, 5, and 10°C, and cardinal temperatures of 0, 2, 4 and 6°C for the minimum, and 30 and 35°C for the maximum. It was assumed that the physiological age at senescence of a plant is a genotype inherent constant which is not variable, and which needs to be reached until senescence of the plant in any environment. The method which yielded the smallest difference between the physiological ages computed for a crop data set related with all sites and seasons of the above mentioned study (Table 1.1.) was chosen for use in EPIVIT.

Host response to virus infection. EPIVIT includes elements which relate to the plant's response to the viral pathogen. This is essential for the simulation of all state variables. It is assumed that the plant response of composed by a constitutive susceptibility index (Su_C) ranging from 0 to 1.0 which is given by the genotype of the respective cultivar, and a variable susceptibility factor, Su_{mr}. The latter reflects mature plant resistance and refers to the establishment of the pathogen in the host and subsequent systemic translocation in the plant's organs towards the tubers (35). The model considers mature plant resistance to be a function of the physiological, but not of the calendar, age of the crop. It responds therefore to changes in temperature conditions and ranges also from 0 to 1.0. Su_{mr}, which is the difference of mature plant resistance from 1.0, and decreases from 1.0 to 0 during plant ageing. Decrease is initialised at a physiological age of *Mri* and ends at the physiological age P_{max} which a cultivar accumulates from 50% emergence until senescence. It is logistic as suggested by the array of data points which was used by Sigvald (36) for the same purpose. The steepness of the decrease is determined by the rate parameter r_{mr} of the corresponding logistic function (see an example in Fig. 2.1D.).

The latent period. During this period the virus is supposed to establish and multiply in the infected and some neighbouring host cells accumulating up to a considerable concentration. The plant then becomes infectious, i. e. it may act as a source for virus spread to healthy plants. Systemic spread inside the plant starts at this point. The latent period (*Lp*) is needed for the simulation of primary infection of plants. EPIVIT assumes that the *Lp* is biologically significant measured in units of developmental heat which are computed similarly as for the physiological age. The developmental heat to be attributed to *Lp* is therefore computed according to equations 7 and 8. The parameters m_{Lp}, n_{Lp} and dr_{Lp} of the respective beta function may be equal to or different from those used

for the computation of the rate parameter r_p for the calculation of physiological age (equation 7). The model multiplies the amount of developmental heat accumulated in this way by the susceptibility Su_{mr} before the product (in P_{Lp}-days) is assigned to *Lp*.

The efficiency of autoinfection. Recent studies (4) suggested a strong temperature-dependence of the efficiency of autoinfection (*tsi*). Since the quantitative relationship between *tsi* and temperature is unknown, a model was developed based on a tentative analytical hypothesis for this mechanism. Two basic concepts may explain this phenomenon, both of which are highly temperature sensitive and relate to temperature-sensitive production rates for substances which contribute to the pathogen's multiplication and translocation. First, virus-free cells may arise in the meristem of the sprouting tuber when the conditions represent a comparative disadvantage for virus multiplication compared with cell production. This may passively inhibit the virus from becoming systemic in the sprout and growing stem. Secondly, environmental conditions may not favour cell-to-cell transport and translocation of water, photosynthesis and respiration products, essential minerals and others. Evidence exists for temperature-sensitive viral genes which regulate the transport of the pathogen in the host plant. Such findings have been discussed elsewhere in relation to a tentative explanation of the efficiency of autoinfection (4).

In a preliminary attempt to determine the relation between the efficiency of autoinfection and temperature, accumulated degree-days were calculated with temperatures > 0°C for the published data set mentioned above (4; Fig. 1.3. and 1.9.). This set includes the historical weather data of the considered agroecozones and seasons, phenological data of one potato cultivar and the measured efficiencies of autoinfection of potato virus X (PVX), Andean potato mottle virus (APMV), the PVY^N-strain of potato virus Y, and potato leafroll virus (PLRV). Total degree-days between 50% emergence and senescence were plotted against the respective reported efficiencies of autoinfection (*tsi*). This plot for PVX suggested a monomolecular relation between these variables (Fig. 2.1E.). In order to improve the biological meaning of this model and the significance correlations, EPIVIT transforms temperatures with a beta function to obtain the rate bd_a (index $_a$ relates to the efficiency of autoinfection) at which developmental heat (measured in beta-degree-day units; bdd_a) is accumulated (equation 9). The model multiplies the rate bd_a with the age-specific susceptibility $Su_{mr}(t)$ before it integrates with time t to obtain the developmental heat DH_a (equation 10) which serves as independent variable for the calculation of the efficiency of autoinfection (*tsi*) as explained below.

$$bd_a(t) = T(t) * b(T(t))_a \qquad [DT/Ti] \quad [bdd_a/hour] \qquad \text{eq. 9}$$

$$DH_a(t) = \int_0^t db_a(t) * Su_{mr}(t)\, dt \qquad [DT] \quad [bdd_a] \qquad \text{eq. 10}$$

The beta function serves as a model for the biological weighting of actual temperatures in relation to their significance for virus multiplication and translocation inside the plant. Preliminary testing of this method with the data set mentioned above yielded significant correlations with PVY and PLRV only after the incorporation of a further parameter into the model. This was called trigger developmental heat (*TH*) measured in beta-degree-days bdd_{at} which is explained as follows. A simulation run starts with the

accumulation of developmental heat (measured in beta-degree-days) with the rate bd_{at}. An amount of *TH* beta-degree-days triggers the accumulation of developmental heat DH_a with the rate bd_a (equation 11). If the temperature falls below or rises above developmental minimum and maximum temperatures (e. g. 0 and 40°C), the model sets the amount of beta-degree-days bdd_{at} (accumulated for obtaining the *TH*) back to zero. A number of *TH* beta-degree-days are required again for triggering further heat accumulation for DH_a. The model uses the same parameter values for computing db_a related to DH_a and db_{at} related to *TH*.

$$DH_a(t) = \int_0^t db_a(t) * Su_{mr}(t)dt \text{ with } DH_{at}(t)=TH \text{ and } T_{min;a}<=T(t)<=T_{max;a}$$

[DT] [bdd_a] eq. 11

$$tsi(t_{max}) = 1-((1-tsi_0)*\exp(-r_a*DH_a(t_{max})))$$

[N^{-1}] [$Plant^{-1}$] eq. 12

An explanation of the biological significance of *TH* is hypothetical so far. Trying to understand how the viral genome is transcribed in the host cell and what is essential for the translocation of the pathogen in the plant may provide a first tentative explanation: Proteins which are encoded by the viral genome may be essential for the transportation of the pathogen inside the host plant. The sequence which encodes for such proteins can be regulated such that it is transcribed effectively only if a determined quantity of products of formerly transcribed genes is present. It is proposed that *TH* accounts for the developmental heat which is necessary for the production of these substances which are essential for virus translocation.

Equation 12 presents the analytical model (monomolecular) according to which EPIVIT computes the efficiency of autoinfection at 100% senescence ($tsi(t_{max})$) by using DH_a as the independent variable.

Primary infection of plants. The mode of transmission of a virus greatly influences the spatial pattern and sequence of spread and hence the overall dynamics of disease progress (41). The transmission of contact-transmitted viruses depends on the direct introduction of a viral particle or at least intact viral RNA into a wounded but living host cell. This transfer is most probably directly from plant-to-plant (e. g. potexviruses; 6) or by carriage on farm implements, clothing, animals or man walking into a planted plot (42). Plants which are adjacent to infectious ones have the highest probability of being infected. This is reflected in a potato plot by patchy clusters of infected plants mostly oriented alongside the row (4), parallel to the orientation of field management practices. The pattern of the spatial spread of aphid-transmitted viruses cannot be predicted easily because they depend on selectively transmitting winged and apterous insect species. Each vector species is submitted to complex interactions with climatic conditions, field management practices and competition with other organisms of the ecosystem. Consequently, EPIVIT uses different approaches for modelling the spread of contact- and aphid-transmitted viruses.

Contact-transmitted viruses: For contact-transmitted viruses EPIVIT characterises each plant by its spatial position in the field and simulates the virus spread from infectious to adjacent healthy plants. A successful infection is the result of the interaction between plant and virus to which the plant contributes the host substrate with nucleic and amino acids, the apparatus of transcription and expression of viral genes, transcrip-

tion products of the plant genome directly related to the pathogen interaction and other molecules. The components of this apparatus respond to climatic conditions of which temperature may be the most important variable. It interacts directly with the above components, whereas other climatic variables such as humidity, precipitation and radiation mainly interact indirectly. It has been documented for PLRV though not for contact-transmitted PVX and APMV that susceptibility of a potato plant to infection is altered by changing temperature conditions (38). Such a phenomenon may be explained in part by temperature-sensitive susceptibility Su_{mr} (related to mature plant resistance) in the model outlined above for the explanation of a plant's susceptibility to an infection. EPIVIT, however, relates mature plant resistance only to the biological mechanisms that operate after a successful insertion of a virus into a host cell. Further components of the infection process need to be considered for contact-transmitted viruses which are related to the success of this insertion. The ease with which a cuticle and epidermal cell may be wounded and the conditions for events during the early stages of the virus insertion may be important for a successful infection. No reference was found which elucidates the principles and the quantitative relationship between temperature conditions and these biological parameters of primary infection which EPIVIT computes weekly (see above). In an attempt to relate such mechanisms to temperature weekly, degree-days were thought to provide explanatory help in this respect also because they reflect the fluctuation of temperature within a given time range in a biologically significant way.

$$bd_b(t) = T(t)*b(T(t))_b \qquad [DT/Ti] \quad [bdd_b/hour] \quad \text{eq. 13}$$

$$DH_b(t) = \int_{tc}^{t} (Su_{mr}(t)*db_b(t)) \qquad [DT] \quad [bdd_b] \quad \text{eq. 14}$$

$$pc_z(i) = Su_c*(1-exp(-r_{mi} * DH_b(z,i)/Su_c)) \qquad [1] \quad [\%] \quad \text{eq. 15}$$

$$Pi(i) = Pi(i-1)+PiP(i-Lpw) = Pi(i-1)+f(pc_z(i-Lpw),Hf_z(i-Lpw)) \text{ for } z=1 \text{ to } Hf(i-Lpw)$$

[N] [Plants] eq. 16

$$PiP(i-Lpw) = \sum_{z=1}^{Hf(i-Lpw)} Hf_z(i-Lpw) \quad \text{with } C_z=\text{random}(C) <= C*pc_z(i-Lpw)$$

[N] [Plants] eq. 17

EPIVIT relates weekly beta transformed degrees (bd_b; equation 13) to the probability of virus spread from an infectious to an adjacent healthy plant. Beta-degrees are multiplied to Su_{mr} before integration and accumulation for obtaining beta-degree-days (bdd_b). The model assumes that virus spread to adjacent plants may only occur if plants are touching each other, which is possible after a certain amount of growth of the plants. Integration of bd_b is computed therefore following the time when the plant canopy closes (*tc*; equation 14). The model uses the constants C_{wr} and C_{br} for the physiological ages at which a canopy closes respectively within a row and between rows (Table 2.2.).

EPIVIT uses Gregory's multiple infection transformation (18) to calculate probabilities of infection. A plant *z*, which is healthy and adjacent to an infectious plant during

week i, becomes infected with a probability of $pc(z,i)$. Equation 15 is the multiple infection transformation for the calculation of such a probability, Su_c being the constitutive susceptibility, r_{mi} a rate parameter of the multiple infection transformation, and $DH_b(z,i)$ the developmental heat measured in beta-degree-days which have been accumulated until week i for the respective plant z since C_{wr} (adjacent infectious plant in the same row) and since C_{br} (adjacent infectious plant across the row). Su_c acts as asymptote of the function (for an example see Fig. 2.1F.).

Once the plants touch each other, the model simulates weekly primary infection and records the spatial position of a plant and its health state (healthy, latently infected, and infectious). The model computes the total number of primarily-infected plants which are infectious (*Pi*) in the respective plot with a difference equation (equation 16). To the number *Pi* of the previous week, it adds the plants which became infectious during week i. The newly infected plants $PiP(\text{i-Lp}_w)$ are the plants which had been infected one latent period (*Lp*) earlier. The letter w in *Lpw* indicates that *Lp* is transformed to week units. $PiP(\text{i-Lpw})$ is calculated as a function of all probabilities pc_z which refer to the probabilities with which an individual virus-free plant (Hf_z) at the time (i-Lpw) may become infected. It is the accumulated number of individual HF_z plants (*Hf*) of the week (i-Lp_w) for which a random number C_z within the range 1 to C (randomisation constant) is less than or equal to C, multiplied by the plant specific probability of infection $pc_z(\text{i-Lpw})$ (equation 17).

The version of EPIVIT presented simulates the spread of contact-transmitted viruses only to plants which are directly adjacent to infectious plants within the respective row and across the row. The model assumes that primarily-infected plants which become re-infected do not contribute significantly to the variability of harvest infection.

Aphid-transmitted viruses: For aphid-transmitted viruses EPIVIT is based, as for contact-transmitted viruses, on a difference equation (equation 18). Symbols for the same variable are equal in EPIVIT for contact and for aphid-transmitted viruses. The number of plants which are primarily-infected during a particular week (i-Lpw), however, is a function of other parameters such as the parameter (k_{pi}) of the binomial distribution; the latent period (*Lp*); the trap catches (*Tc*) and relative efficiency factors (REF) for virus transmission of particular aphid species; the attraction factors (*Af*) representing the relative attraction of an aphid species by the respective trap colour; the plant's susceptibility determined by mature plant resistance (Su_{mr}); and M, which is a parameter related to aphid behaviour (see below). The constitutive susceptibility is not incorporated into the model in the present version.

$$Pi(i) = Pi(i\text{-}1) + PiP(\text{i-Lpw}) = Pi(i\text{-}1) + f(k_{pi}, Lp, Tc, REF, Af, Su_{mr}, M) \quad [N] \quad [\text{plants}] \quad \text{eq. 18}$$

The model is based upon a population approach, in contrast to EPIVIT for contact-transmitted viruses. Aphid behaviour in a particular potato field (settling behaviour, flight patterns, walking distances, reproduction rates etc.) is responsible for the spread pattern of aphid-transmitted viruses, but data which document this behaviour are most often lacking for data sets available on virus incidence in the harvest of determined field plots. It has been reported however, that dispersal patterns of aphid-transmitted viruses in small fields tend to be clumped (4, 40). A clumped pattern is one among three hypothetical classifications which are used by biologists to characterise the spatial pattern of individuals in populations: random, clustered, and uniform (25, 26) which is the same

as regular (7). Clustered or clumped means that every plant in a field does not have an equal probability of being infected (7). The negative binomial distribution is commonly used by biologists as a statistical frequency distribution to represent clustered patterns (25). The number of infections which occur per plant in a field with a clustered pathogen distribution has been deduced from the negative binomial distribution (44). These findings have been incorporated into a reported model for the simulation of the impact of soybean mosaic virus (which is a potyvirus) on yield and on the level of botanical soybean seed transmission (33). EPIVIT for aphid-transmitted viruses uses this model with slight modifications. The model assumes that immigrating aphids into the simulated field do not carry virus and that the moves of alatae within the field are responsible for the virus spread from plant-to-plant. The apterous population is not explicitly considered by EPIVIT.

The negative binomial distribution is used to estimate the number of plants *PiP*(i) which become primarily-infected during week i (equation 19). *Hf*(i) represents again the number of virus-free plants at the end of week i. It is multiplied by an expression within which k_{pi} is the parameter of the negative binomial distribution and *Inp*(i) the average number of inoculations per plant during week i.

Hf(i) is obtained by the difference of the number of emerged seed tubers (*Ne*) with the number of secondarily-infected emerged seed tubers (*SiE*(i)), infectious primarily-infected plants (*Pi*(i)), and latently-infected plants at the end of week i (*PiP*(i-Lpw+1); equation 20).

$PiP(i) = Hf(i)*(1-(k_{pi}/(k_{pi}+Inp(i)))^{k_{pi}}$	[N]	[plants]	eq. 19
$Hf(i) = Ne-SiE-Pi(i)-PiP(i-Lpw+1)$	[N]	[plants]	eq. 20
$Inp(i) = In(i)/Ne$	[1]	[inoc./plant]	eq. 21
$In(i) = Su_{mr}(i)*Vp(i)$	[N]	[inoc.]	eq. 22
$Vp(i) = q * \sum_{sp=1}^{Sp(i)} Tv_{sp}(i)*REF_{sp}(i)/Af_{sp}$	[N]	[Vpu]	eq. 23
$REF_{sp}(i)=REFc_{sp}*b(Ta(i))$	[1]	[-]	eq. 24
$Tv_{sp}(i)= \sum_{z=1}^{Tc_{sp}(i)} Tc_{sp,z}$ with C_z=random(C) <= C*pa(i)	[N]	[aphids]	eq. 25
$pa(i)=((SiE+Pi(i)) / Ne)*(M/(M+1))$	[1]	[%]	eq. 26

Inp(i) is estimated by *In*(i) which is the total number of inoculations in the field during week i, divided by *Ne* (equation 21). The estimation of *In*(i) is obtained by multiplying a simulated vector pressure index *Vp*(i) (measured in vector pressure units Vpu) with the plant's susceptibility (Su_{mr}), which is determined by mature plant resistance at the end of the respective week (equation 22). Equation 23 explains the calculation of *Vp*(i) relating the simulated number of viruliferous trapped specimens $Tv_{sp}(i)$ of Sp aphid species with the respective relative transmission efficiencies $REF_{sp}(i)$ and their constant factors for attraction Af_{sp} by the respective trap colour. *Vp*(i) represents the simulated number of landing aphids which are viruliferous and accumulated during the

respective week. Since both REF_{sp}(i) and Af_{sp} are relative parameters they need to be calibrated by q to yield an integer number of inoculations *In*(i) (equation 23). A REF_{sp} responds to changes in temperature conditions by multiplication of a constant constitutive $REFc_{sp}$ of the respective species with a beta function value b(*Ta*(i)) (equation 24). *Ta*(i) is the average temperature in week i of daily temperature means ((minimum+maximum)/2) between a determined morning time (h_1) and an afternoon time (h_2), reflecting average temperature conditions during main times of daily activity of aphids. Tv_{sp}(i) is obtained stochastically as an accumulated number of Tc_{sp}(i) trapped specimens of species sp during week i for which a random number C_z within the range 1 to *C* (randomisation constant) is less than or equal to *C*, multiplied by the probability *pa*(i) (equation 25). This last variable represents the probability that a aphid landing in week i is viruliferous. For non-persistently transmitted viruses, *pa*(i) reflects the direct flights of winged aphids from source plants within the field to healthy plants because specimens lose the virus particles after some probing. In the case of persistently-transmitted viruses, *pa*(i) refers to the probability that an aphid feeds at least once on a viruliferous plant, meanwhile moving within the respective field during an average presence time which is the same for all aphids. This probability is estimated as the proportion of source plants (emerged secondarily-infected and infectious primarily-infected plants) among all plants in the field, multiplied by the term (*M*/(*M*+1)) (equation 26). *M* represents the average number of moves an aphid makes within a field before leaving (33).

Primary infection of tubers. The percentage of tubers which are infected among those produced by infectious primarily-infected plants during season *k* (*tpi*(k)) is calculated as an average value according to equation 27. EPIVIT needs to total the *tpi* of each individual primarily-infected plant at 100% senescence (t_{max}) for this purpose. Equation 12 provides the necessary functional relationship between beta-degree-days which are accumulated since the week of infection and *tpi* of individual plants. Parameters for computing the *tpi* of individual plants and the efficiency of autoinfection (*tsi*) are the same.

$$tpi(t_{max}) = \frac{\sum_{z=1}^{Pi(t_{max})} tpi_z(t_{max})}{Pi(t_{max})} \qquad [N^{-1}] \quad [plant^{-1}] \quad \text{eq. 27}$$

Each plant considered for *tpi* calculation is identified by its attributes related to the spatial position in the field for contact-transmitted viruses, whereas such plants are indexed only with the respective week by EPIVIT's version for aphid-transmitted viruses.

Computations. Temperature is the independent variable for computing beta-degrees and the rate at which physiological age increases (equations 7, 9, and 13). The daily temperature cycle is approximated by the sine wave through historical daily minimum and maximum temperatures. This practice (1) has gained broad acceptance for the calculation of degree-days in entomology and has also been recommended for epidemiological research with plant pathogens (13). Physiological age and beta-degree-days are

obtained by integration of the physiological time and beta-degrees respectively with time using the trapezoidal numerical integration method and a time step of 1 hour.

EPIVIT for aphid-transmitted viruses needs to know the temperatures at average daily hours when aphid activity initiates and stops (h_1 and h_2; Table 2.2.). Since the sine wave has no specific relation to a particular time of day, EPIVIT requires additionally the input of the average daily hour at which the temperature rises to its maximum (h_3).

The model determines, at the beginning of a season, the number of emerged healthy and secondarily-infected plants. It computes the physiological age starting at 50% emergence. This is essential for the determination of Su_{mr}, C_{wr}, and C_{br}, and the computations for the accumulation of latent periods which correspond to primarily-infected plants. The beta-degree-days are accumulated simultaneously for the computation of the efficiency of autoinfection at harvest. Since many equations of EPIVIT contain delayed arguments such as $PiP(\text{i-Lp}_w)$ in equation 16, the model uses the fixed boxcar train approach (16) for its calculations. The totality of emerged plants is divided into three boxcars of which one contains the healthy plants, one the latently infected plants, and one the infectious primarily-infected plants. Their contents are actualised weekly. For each virus-free plant, the box holds one compartment which is associated with the accumulated amount of beta-degree-days, bdd_b, for that plant and uses $pc(z,i)$ for the calculation of the respective probability. For aphid-transmitted viruses this box has only one compartment holding the number of totally virus-free plants. Plants which become latently infected with a contact-transmitted virus are assigned to individual compartments of EPIVIT's second box. Each compartment is associated with the accumulated fraction of the latent period of the respective plant. For aphid-transmitted viruses one such compartment holds the number of the whole group of plants which became newly infected in the respective week. The box for infectious primarily-infected plants is structured in the same way as the one for latent infections, but individual plants or weekly plant groups are associated with the accumulated fraction of infected tubers of the respective primarily-infected plants. At the end of a season the model relates the plant numbers in the boxes, the number of emerged secondarily-infected plants, the simulated efficiency of autoinfection, and the average percentage of infected tubers of primarily-infected plants according to equations 6, 12, 16 or 18, and 27, in order to calculate harvest infection *hi*. If desired, seed infection of the next season is computed (equation 3).

Missing historical weather data for up to three days were treated as described elsewhere (9).

A random-number generator was incorporated into the model (45) for the random selection of spatial positions in a field (first selection: row; second selection: plant) to assign not emerged and eventually secondarily-infected plants in the simulated plot (see implementation), and for random runs according to equations 17 and 25.

Implementation. The source code was written in Pascal programming language and implemented for IBM-PCs and compatibles (Turbo-Pascal compiler, version 6.0, Borland International, 4585, Scotts Valley Drive, Scotts Valley, CA 95066). The program is menu-driven via keyboard or mouse. Parameters and input data can be changed interactively. Secondarily-infected seed tubers may be distributed according to a historic field design, at random, or uniformly (25, 26) onto the (row x plants/row)-lattice pattern of a field. Non-emerging seed tubers are randomly distributed onto all available positions.

A simulation run starts if desired with the reading of the spatial position of secondarily-infected and non-emerged seed tubers in a selected historic field. Weather and aphid data are then looked up. Two basically different options may be chosen in reference to the increase of infection with time: if the simulation for a single season is preferred the increase of the number of infectious primarily-infected plants during the season is displayed graphically. Multiple season simulations may be chosen however, assuming that the harvest of one season serves as seed source for the next season. In this case the increase of harvest infection during successive growing seasons is displayed. Results are also displayed numerically. A representation of the spatial pattern of non-emerged, healthy, latently infected, infectious primarily and secondarily-infected plants can be produced for contact-transmitted viruses at the end of a simulation run.

The number of tubers which is produced by plants of different health state, *NSi*, *NPi*, *NHe* is not treated as a variable in the present version but as a constant set to 20. It has been observed that the tuber number is not significantly affected by the plant's health state (4). The rate parameter of the logistic function for mature plant resistance, r_{mr}, is calculated by EPIVIT's actual implementation version to produce a symmetrical sigmoid decrease of Su_{mr} between initialisation of mature plant resistance at *Mri* and maximal physiological age P_{max}.

Results shown below were produced on an IBM PS/2 Model 70 386 personal computer. EPIVIT includes a printer interface. Graphics related to the model output were generated directly by EPIVIT except for those related to multiple season simulations. Outputs were printed on a Postscript laser printer (HP-LaserJet III Si).

Results

Physiological time. Physiological ages were computed with the phenological and historical temperature data of the data set reported which covers one cultivar, three agroecozones and two seasons (4). The smallest difference between these ages was obtained by using a beta function for the growth rate calculation (equation 7), with the parameters m=0.5, n=0.5, dr_p=5.0, $T_{min;p}$=0, and $T_{max;p}$=35 (Fig. 2.2.). The highest number of P-days was 1047, obtained for a season of 126 days (50% emergence until senescence). The greatest difference in P-days between different seasons and sites was 15. Of the 144 parameter combinations tested, all others yielded differences greater than 50 which corresponds to 5 - 6 days. With the other method tested (34) the greatest number of 892 P-days was obtained for a season of 104 days. The difference from the season with the lowest number of P-days was 624, which corresponds to 60 - 70 days. The particular shape of the beta function presented in Fig. 2.2. is different from the functions applied by other authors (21, 34) who used mostly skewed bell-shaped functions. The beta function obtained however, reflects the growth-temperature relationship of an Andean potato cultivar (*S. tuberosum ssp. tuberosum x S. tuberosum ssp. andigena*) which may be different from those characteristic for cultivars grown at higher latitudes (*S. tuberosum ssp. tuberosum x S. tuberosum ssp. andigena*). The beta function approach proved to be more flexible and was therefore incorporated into EPIVIT.

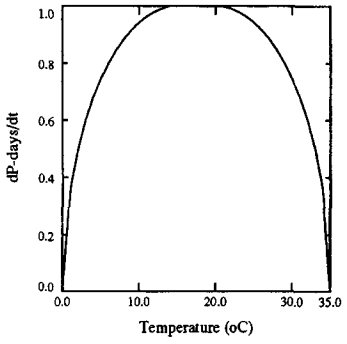

Fig. 2.2. The beta function with the best fit to emergence and senescence data of the modern potato cultivar Yungay in different agroecozones of Peru, representing the rate of advance of the physiological crop age at temperature T.

Implementation. A simulation run with EPIVIT for contact-transmitted viruses for a season with 104 days, from 50% emergence to senescence, requires 24 seconds on an IBM-PS/2 Model 70 386 PC (with mathematical co-processor). EPIVIT for aphid-transmitted viruses uses less computer time (18 seconds) because no spatial simulation is performed.

Demonstration runs. The output of the implemented model is illustrated by using a hypothetical parameters settings (footnotes d and f of Table 2.4.). The generated output is fictive and does not aim at representing a particular real situation, but at demonstrating the output that EPIVIT produces. Parameter values and input data sets do not relate to a particular season in a specific site but lie within roughly estimated boundaries for a temperate climate and a modern potato cultivar. The weather and aphid data are presented in Fig. 2.3. (aphid population 1). Aphid data are also hypothetical and represent yellow water trap catches which is supposedly the most frequently method used worldwide for studying aphid populations in potato fields. Parameter settings are not justified and verified in this section. The relative significance of their values compared to others may be estimated individually by considering the relative sensitivity of each parameter (see fine sensitivity analysis below).

Single season simulations: The average output of 10 runs of EPIVIT for contact-transmitted viruses is presented in Table 2.4. for a plot with seed tubers of which 20% are infected and distributed at random onto the (row x plants/row)-plot lattice. Means are reported as the model output is variable due to the stochastic model code (equation 17). The increase of the percentage of infectious primarily-infected plants in this plot is displayed for these 10 runs in Fig. 2.4A.

TABLE 2.4: EPIVIT's output (%) of demonstration runs for contact- and aphid-transmitted viruses (means of 10 runs; random spatial distribution of infected and non-emerged seed tubers).

Virus type and variable	Mean [a]	Standard deviation [b]
Contact-transmitted (20% seed infection) [c,d]		
Efficiency of autoinfection (tsi)	81.6	- [e]
Primary infection of plants (pi)	53.1	2.5
Tuber infection of primarily-infected plants (tpi)	76.6	1.7
Harvest infection (hi)	57.1	2.4
Aphid-transmitted (19% seed infection) [c,f]		
Efficiency of autoinfection (tsi)	83.3	- [e]
Primary infection of plants (pi)	32.5	7.3
Tuber infection of primarily-infected plants (tpi)	90.4	0.7
Harvest infection (hi)	46.0	7.0

[a] Back-transformed means of arc sine transformed percentages.

[b] Stochastic elements in EPIVIT's model code condition the variance of the model output.

[c] Emergence of infected and healthy seed tubers: 0.96. Temperature data of Imperial, Peru, 1988 (Fig. 2.3.).

[d] Plot of 300 plants in 10 rows. Selected parameters for runs for contact-transmitted viruses were as follows: For tsi: m_a=2, n_a=3, dr_a=5, $T_{min;a}$=5, $T_{max;a}$=35, TH=0, r_a=11.17E-4, tsi_0=44.84; for pi: L_p=70, mL_p=4.0, nL_p=5.0, drL_p=3.0, m_b=4.0, n_b=3.0, $T_{min;b}$=5, $T_{max;b}$=35, C_{wt}=100, C_{bt}=150, r_{mi}=0.2; for P-time: m_p=0.5, n_p=0.5, dr_p=5, $T_{min;p}$=0, $T_{max;p}$=35; for susceptibility: SU_c= 0.4, P_{max}=1030, Mri=200, r_{mr}=11.0E-3.

[e] EPIVIT's output for tsi has no variance since the code for its simulation does not include stochastic elements.

[f] Plot of 216 plants in 6 rows. Hypothetical aphid data (Fig. 2.3.). Selected parameters for runs for aphid-transmitted viruses were as follows: For tsi: m_a=1, n_a=4, dr_a=0, $T_{min;a}$=5, $T_{max;a}$=35, TH=20, r_a=29.88E-4, tsi_0=56.22; for pi: L_p=70, mL_p=3.0, nL_p=4.0, drL_p=3.0, q=10.0, k_{pi}=2.0, h_1=10, h_2=16.0, h_3=14.5; for all aphid species: m_{sp}=2.0, n_{sp}=4.5, $T_{min;sp}$=5, $T_{max;sp}$=35, M=20; for P-time: m_p=0.5, n_p=0.5, dr_p=5, $T_{min;p}$=0, $T_{max;p}$=35; for susceptibility: P_{max}=1030, Mri=200, r_{mr}=11.0E-3. $REFc_{sp}$ and Af_{sp} are presented in Table 1.2.

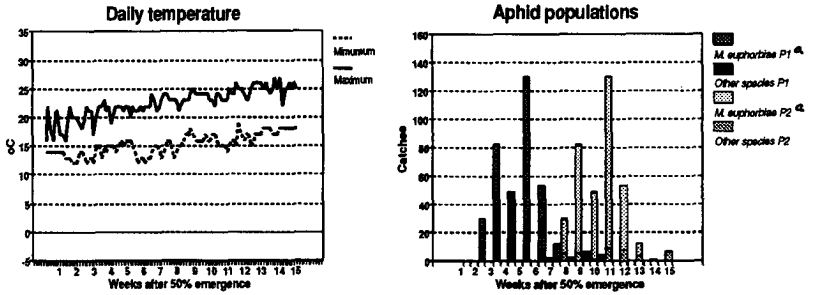

Fig. 2.3. Minimum and maximum daily temperature data of Imperial, Peru, 1988, and hypothetical yellow water trap catches of winged aphids (populations 1 and 2) used for sample runs and sensitivity analysis of EPIVIT.

[a] P1: Population 1; P2: Population 2.

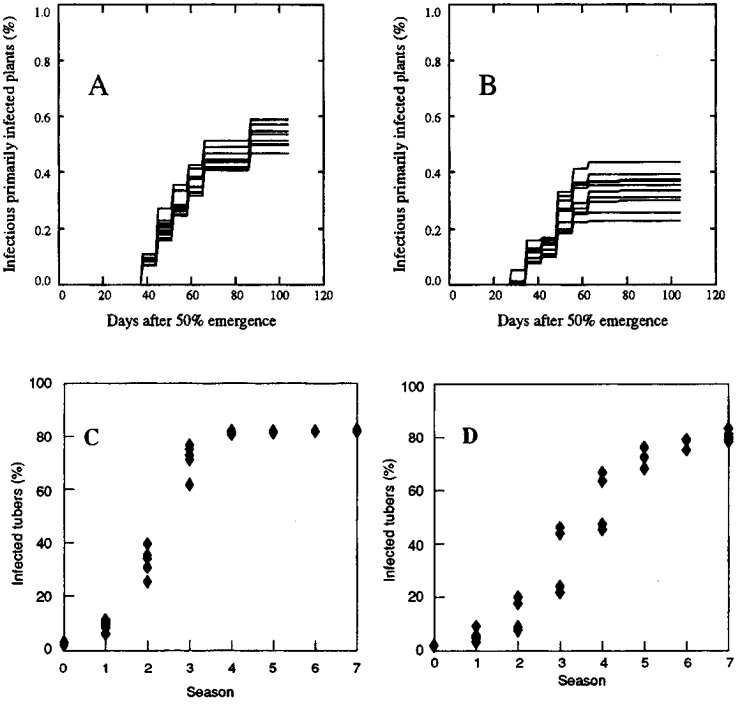

Fig. 2. 4. Graphical output of successive demonstration runs with EPIVIT for contact- and aphid-transmitted viruses with historical weather data of Imperial, Peru 1988, and hypothetical yellow water trap catches of winged aphids.
A) Ten single season simulations of the percentage of infectious primarily-infected plants in a field for contact-transmitted viruses. **B)** Ten single season simulations for aphid-transmitted viruses. **C)** Five Multiple season simulations for contact-transmitted viruses of the percentage of infected tubers in the harvest of a potato plot starting with 2% of infected seed tubers and without changing the seed in subsequent seasons. **D)** Five Multiple season simulations for aphid-transmitted viruses.
Values used for the model parameters and temperature and aphid data are presented respectively in Table 2.4. and Fig. 2.3.

Outputs of EPIVIT for aphid-transmitted viruses and the respective, used parameters values are also shown (Fig. 2.4B and Table 2.4.). Compared to the demonstration runs for contact-transmitted viruses the high variability of primary infection of plants (standard deviation of 7.3%) is noteworthy. It conditions the variability of harvest infection (standard deviation of 7.0%). The stochastic model code (equation 25) causes again the variability of the model output. The reasons for the difference in variability of outputs between EPIVIT for contact- and aphid-transmitted viruses are discussed below.

Multiple-season simulations: The model output of five simulation runs is displayed graphically for contact- and aphid-transmitted viruses (Fig. 2.4C. and 2.4D.), assuming that seed infection in the first season is 2% and that the seed tubers for the following seven seasons are selected from the harvest of the same plot. The same parameter values were used as for single season simulations.

Model evaluation. The evaluation of a model may be divided into verification and validation (39). To verify a model means to compare its structure and general behaviour with the real system and to test whether the model works in the intended way. Validation consists of comparing quantitatively a model's behaviour with the real system (39). The model was verified continuously during construction. It was conditional for the stepwise development of EPIVIT. A first view of the model's operation is presented above (demonstration runs). Sensitivity analysis may further contribute to the verification of whether the model behaves reasonably (see below). This means exploring the model by examining the effect of changes in model structure (coarse sensitivity analysis) and model parameters (fine sensitivity analysis) on its output variables (8). The first involves omitting or drastically changing processes to test their overall effect, and the second consists of small positive or negative changes of parameter values to study their effect on output. Relative sensitivity is used to quantify the changes of relevant model outputs to changes in parameter values. It is computed as the proportion between $\Delta v/v$ and $\Delta p/p$, Δv being the change in output variable v caused by a change Δp in parameter p. EPIVIT was analysed with both techniques (see below). Verification additionally involves parametrization (32) which has also been called calibration or tuning (8). It is the adjustment of parameters in order to make the model represent optimally the real system. Parametrization and a first validation of EPIVIT with experimental data will be presented elsewhere (3).

Coarse sensitivity analysis. Increasing seed infection from 2 to 50% greatly increases EPIVIT's output for infection by contact-transmitted viruses, i. e. primary infection of plants (*Pi*, or as percentage *pi*), tuber infection of primarily-infected plants (*tpi*) and harvest infection (*hi*; Table 2.5.). The efficiency of autoinfection (*tsi*) is not related biologically to seed infection nor to the model code. Output for *tsi* are therefore not affected by the manipulation of seed infection. Making *tsi* temperature insensitive and setting it to 100% does not affect the model's output as long as seed infection is low (2%) (Table 2.5). If seed infection is high (50%) however, *hi* is higher (90.5%) than for runs for the same seed infection but a temperature sensitive *tsi* (81.4%). Making *tpi* temperature insensitive and setting it to 100% shows the same overall effect as for the corresponding manipulation of *tsi*.

TABLE 2.5: The reaction of EPIVIT's output to large changes in the model structure or of input variable values (means of 10 runs; random spatial distribution of infected and non-emerged seed tubers).

Virus type and manipulated variables	Seed infection	Variables [a]			
	(%)	tsi (%)	pi (%)	tpi (%)	hi (%)
Contact-transmitted [b,c]					
Reference runs	2	81.6 [d]	12.3	73.6	10.7
Increased seed infection	50	81.6	49.1 *	82.8 *	81.4 *
Efficiency of autoinfection (tsi) of 100%	2	100.0 [e]	13.1	73.9	11.7
	50	100.0 [e]	48.4 *	83.0 *	90.5 *
Tuber infection of Pi (tpi) of 100%	2	81.6	11.4	100.0 [e]	13.0
	50	81.6	49.0 *	100.0 [e]	89.8 *
Aphid-transmitted [b,f]					
Reference runs ; aphid population 1	2	81.6 [d]	5.1	89.9	3.6
Increased seed infection; aphid population 1	19	81.6	32.8 *	90.4 *	45.9 *
aphid population 2	19	81.6	3.8 *	81.2 *	19.1 *

* An asterisk indicates a significant difference to the reference runs mean according to the LSD-test (P<=0.05).

[a] Back-transformed means of arc sine transformed percentages.

[b] Emergence of infected and healthy seed tubers: 0.98. Temperature data from Imperial, Peru, 1988 (Fig. 2.3.).

[c] Plot of 300 plants in 10 rows. The model parameters were set to the values which are presented in Table 2.4., except m_b=4.5, n_b=2.0,

[d] The efficiency of autoinfection (tsi) does not respond to changes of the manipulated variables neither biologically nor according to the model code.

[e] Manipulated variable.

[f] Plot of 216 plants in six rows. Hypothetical aphid data (Fig. 2.3.). The model parameters were set to the values which are indicated in Table 2.4. $REFc_{sp}$ and Af_{sp} are presented in Table 1.2.

The tendency of the response of EPIVIT's output for aphid-transmitted viruses to seed infection increasing from 2 to 19% is similar to that for contact-transmitted viruses (Table 2.5.). A later aphid immigration, simulated by exchanging aphid populations one and two (Fig. 2.3.) greatly reduces *pi*, *tpi* and *hi* (*hi* from 45.9% to 19.1% in a plot with 19% seed infection; Table 2.5.).

EPIVIT's response to these large changes in the model structure and input variables are biologically meaningful. In reality the probability of tuber infection of primarily-infected plants (*tpi*) needs to respond positively to an increase of seed infection for contact- and aphid-transmitted viruses (4). EPIVIT's outputs are compatible with such a response (Table 2.5.).

Model outputs (harvest infections) were compared for simulations with a temperature-sensitive and a constant efficiency of autoinfection (*tsi*) with *tsi*=100% in the latter case. The model was set to the mode for multiple season simulations assuming that the same tuber lot serves as a source for seed selection during consecutive seasons. One of the cases presented in Fig. 2.5. corresponds to a high seed infection of 98% (A) in the first season and another to a seed infection of 2% (B). The output of the first case demonstrates how *tsi* may contribute in the long term to a slight reduction of harvest infection from 98% to 82%. If *tsi* is held constantly at 100% (C) harvest infection gets to

100% at the end of the fifth season contrasting case B where the temperature-sensitive *tsi* limits harvest infection to a maximum of 82%.

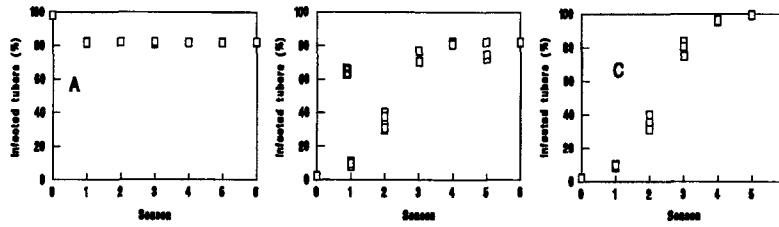

Fig. 2.5. Scatter plots simulating harvest infections (% infected tubers) produced with five successive demonstration runs of multiple season simulations for six consecutive seasons. **A)** Seed infection of 98%, temperature sensitive efficiency of autoinfection. **B)** Seed infection of 2%, temperature sensitive efficiency of autoinfection; **C)** Seed infection of 2%, efficiency of autoinfection fixed at 100%.
The values of the model's parameters used are listed in Table 2.4.; temperature data are presented in Fig. 2.3.

EPIVIT's output of runs on plots with low (2%) and moderate (approximately 20%) seed infection with a random spatial distribution of secondarily-infected seed tubers was compared with the outputs obtained with a uniform spatial distribution of infected seed tubers. Plots of 300 and 216 plants were simulated for contact- and aphid-transmitted viruses respectively (10 and six planted rows). Twenty successive runs were executed. No significant difference was determined between means of arc sine transformed percentages obtained for the state and output variables with random and regular distribution of infected seed tubers.

Fine sensitivity analysis. The purpose of the fine sensitivity analysis is to obtain information on the reaction of the model output to changes of particular parameters. If a small change in a parameter leads to a large change in model output then its value must be known precisely (8). It is desirable that only one parameter is changed at a time to test its effect on model output, especially if a model has many parameters (8). As EPIVIT had not been validated so far, reference parameter settings were not available. Testing all possible levels of parameter values with every possible combination of values of the other parameters would quickly lead to an exponential increase of computing time with a parallel decrease in the probability of obtaining meaningful results. It was decided to depart from a realistic initial value for each parameter and to change them within the limits of experimental precision and practical relevance. The latent period for example was set initially to 70, which corresponds approximately to 1 week in a site with temperature fluctuations between 13 and 23 °C. This should be a first realistic though rough estimate for the latent period with a modern potato cultivar in Peru (personal observation). Changes were made to 80 (one additional day) and 140 (one

additional week), relating to time steps of relevance for the resolution of the time scale in practical experimentation.

Applying this procedure yields percent changes (relative sensitivity) in the values of the numerous parameters which are directly comparable as they are based on distinct percent changes of the parameter values (columns "change" in Tables 2.6. and 2.7.). This procedure was preferred because it provides results of more practical relevance.

The analysis was applied to plots with 2% and approximately 20% seed infection. It was expected that the model outputs would be more sensitive to parameter changes if EPIVIT is run on plots with low seed infection. Since the model code is probabilistic, random discrete events may yield higher percent changes if they are calculated on low initial variable values compared to cases of high initial variable values. This may be explained by an example. Fewer opportunities for virus transmission exist in plots with low seed infection compared to plots with high seed infection. An aphid population may consist mainly of individuals of *Macrosiphum euphorbiae* with a constitutive relative efficiency factor for virus transmission ($REFc_{sp}$) of 0.1 and few *Myzus persicae* with a $REFc_{sp}$ of 1.0. The expected percent change of output variables after changing the $REFc_{sp}$ of these aphid species may now be compared between plots of low and high seed infection. A random selection of an individual of *M. persicae* for being viruliferous (according to equation 25), compared to a selection of an individual of *M. euphorbiae*, is expected to yield a higher percent change of the output variable caused by the manipulation of $REFc_{sp}$ in plots with low seed infection (e.g. 2%), because the reference value of the output variable on which the percent change is calculated is expected to be lower in such a plot.

The infected seed tubers were randomly distributed for sensitivity runs onto the available positions in the (row x plants/row)-plot lattice for 300 plants in 10 rows (contact-transmitted viruses) and 216 plants in six rows (aphid-transmitted viruses).

Contact-transmitted viruses: It was generally observed that the efficiency of autoinfection (*tsi*) and tuber infection of primarily-infected plants (*tpi*) respond with a low percent change compared with the change in parameter values (Table 2.6.). Output values (v in the formula for the calculation of the relative sensitivity; see above "model evaluation") on which the percentages of change were calculated were always higher than 70% for both variables with the utilised parameter values of m_a, n_a, dr_a, $T_{min;a}$ and $T_{max;a}$ (data not presented). High relative sensitivities in such a situation would be obtained only by a large change of v as a result of the parameter value change. Such large changes were generally not observed (Table 2.6.). It was concluded that the accuracy of estimation of the respective parameters is sufficient for these state variables if parameter values lie within the range of changes used in this sensitivity analysis.

TABLE 2.6: Relative sensitivity of EPIVIT's state and output variables to changes in parameter values for contact-transmitted viruses (calculated on means of 10 runs per parameter combination [a]; infected seed tubers with random spatial distribution [b]).

Parameter [c]	Unit	Parameter Value		Change	Relative sensitivity: Seed infection 2 %				Seed infection 20 %			
					Variables				Variables			
		initial	new	(%)	Pi	tpi	tsi	hi	Pi	tpi	tsi	hi
Lp	PLp-days	70	80	+14	-1.16	+0.55	- [d]	-1.03	-0.21	+0.05	-	-0.09
			140	+100	-0.58	+0.08	-	-0.48	-0.13	-0.01	-	-0.07
mLp	-	4	3	-25	+0.29	-0.14	-	+0.15	+0.06	-0.01	-	+0.02
nLp	-	5	6	+20	-0.43	-0.02	-	-0.39	-0.01	-0.01	-	0.00 [e]
drLp	°C	3	0	-100	+1.00	+1.00	-	+0.85	+1.00	+1.00	-	+0.50
		3	6	+100	-0.26	+0.06	-	-0.11	0.00	+0.01	-	0.00
mb	-	4.5	3.5	-22	+0.81	+0.01	-	+0.78	-0.01	0.00	-	-0.01
nb	-	2.0	3.0	+50	-1.19	-0.01	-	-0.18	0.00	0.00	-	-0.01
Tmin;b	°C	5	3	-40	+0.19	-0.48	-	+0.08	0.00	-0.01	-	-0.01
Tmax;b	°C	35	30	-14	+1.19	+0.05	-	+1.06	+0.02	+0.08	-	+0.07
C_{WR} [f]	P-days	100	110	+10	-2.41	+0.72	-	-1.62	-0.33	+0.05	-	-0.15
			150	+50	-0.78	+0.06	-	-0.65	-0.09	-0.04	-	-0.06
			90	-10	+0.77	-0.21	-	+0.52	-0.13	-0.07	-	-0.07
			50	-50	+0.04	-0.17	-	-0.11	+0.01	-0.05	-	-0.01
Suc	-	0.4	0.8	+100	+0.17	+0.01	-	+0.15	+0.03	+0.01	-	+0.02
			0.2	-50	+0.79	0.00	-	+0.67	+0.09	+0.04	-	+0.12
Mri	P-days	200	190	-5	+6.60	-1.60	+0.04	+4.81	+0.48	-0.18	+0.04	+0.22
			130	-35	+0.66	-0.22	+0.04	+0.41	+0.05	-0.02	+0.04	+0.05
rmi	bdd_b^{-1}	0.5	1.0	+100	+0.09	-0.01	-	+0.07	+0.02	0.00	-	+0.01
			0.1	-80	+0.65	0.00	-	+0.55	+0.14	+0.06	-	+0.10
ma	-	2.0	1.0	-50	-	+0.08	+0.14	+0.20	-	+0.08	+0.14	+0.11
na	-	3.0	2.0	-33	-	+0.01	-0.08	+0.11	-	-0.01	-0.08	-0.05
dra	°C	5.0	3.0	-40	-	+0.03	+0.03	+0.18	-	+0.02	+0.03	+0.02
			7.0	+40	-	+0.02	+0.03	-0.21	-	+0.01	+0.03	+0.02
Tmin;a	°C	5	3	-40	-	-0.04	-0.03	+0.13	-	-0.01	-0.03	-0.03
Tmax;a	°C	35	30	-14	-	+0.04	-0.16	+0.26	-	+0.02	-0.16	-0.16
TH	bdd_a	3 [g]	5	+67	-	-0.01	0.00	+0.01	-	0.00	0.00	+0.01
			10	+233	-	+0.01	+0.01	-0.02	-	0.00	+0.01	0.00

[a] Back-transformed means of arc sine transformed percentages.

[b] Plot of 300 plants in 10 rows of 10m. Emergence of infected and healthy seed tubers: 0.96. Temperature data of Imperial, Peru 1988 (Fig. 2.3.).

[c] The model parameters were set to the values presented in Table 2.4., except m_b=4.5, n_b=2.0, and r_{mi}=0.5,

[d] Dashes indicate that the manipulated parameter has no relation to the respective variable according to EPIVIT's code.

[e] A value of 0.00 means that the relative sensitivity is <0.005 and >-0.005.

[f] C_{br} was changed proportionally from 150 to 165, 225, 135 and 75 respectively in the order as indicated from top to bottom in the Table.

[g] In order to allow the parameter change to be expressed as a percentage, TH was set to an initial value of three instead of zero as for the other model runs.

Outputs for primary infection of plants (*pi*) showed a high relative sensitivity to changes of some specific parameters. With 2% seed infection moderate changes of the variables *Mri*, C_{WR} (parallel with C_{br}), $T_{max;b}$, n_a, *Lp* and dr_a yielded a considerable percent change of pi (Table 2.6.). Large percent changes were obtained also with a large change of the constitutive susceptibility Su_c (relative sensitivity of 0.79 to a change of -50%; Table 2.6.). An observed high relative sensitivity of the model output for harvest

infection (*hi*) is conditioned by the high sensitivity of *pi*. The relative sensitivity for *hi* however is always lower compared to that for *pi*.

Increasing the absolute percent change of a parameter value results in a decrease of the relative sensitivity (e. g. an increase of C_{wr} of 10% and 50% yield a relative sensitivity of -2.41 and -0.78 respectively). This points to a non-linear relation of the percent change of the parameters with the percent change in the respective output variable.

The tendency of the model response to changes in parameter values is similar for model runs on a plot with 2 and 20% seed infection (same sign of the respective relative sensitivities with remarkable value, i. e. >|0.3|). In any case the relative sensitivity was lower in the plot with 20% seed infection. The signs of the relative sensitivities are biologically meaningful, even if in the plot with a low seed infection the sign was opposite to that expected in a few examples. Primary infection is expected to decrease if the timing of canopy closure between plants (C_{wr} and C_{br}) is extended, because there will be less season time available within which virus transmissions may occur. The sign of the model response for the increase of C_{wr} with 10% is negative in the plot with 2% seed infection (-2.41), indicating a model behaviour which is compatible with the above theoretical concept. For a decrease of C_{wr} an increase of *pi* would be expected yielding again a negative sign for the respective relative sensitivity. The value obtained however is positive (+0.77 for a decrease of the parameter value of 10%). Such incompatibilities with theoretical expectation were never obtained if the model was run on the plot with approximately 20% seed infection.

Aphid-transmitted viruses: EPIVIT for aphid-transmitted viruses reacts similarly to parameter changes as the version for contact-transmitted viruses. General observations coincide such as for example those related to the relative sensitivity of *tsi* and *tpi*, and the difference in response of the model to plots with low and high seed infection (Table 2.7.). In contrast to the version for contact-transmitted viruses however, there are more parameters with a high relative sensitivity which is best noted by comparing the relative sensitivities of both versions run on the plot with approximately 20% seed infection (Tables 2.6. and 2.7.). Most of these parameters correspond to aphid behaviour and characteristics related to their attraction by a trap colour and the aphid's capacity for virus transmission (Table 2.7.). The model output is sensitive to changes of the temperature range within which the relative efficiency is modelled ($T_{max;sp}$), but also to changes of Af_{sp}, h_1 (together with h_2 and h_3), n_{sp}, and m_{sp}. Other parameters with a considerable relative sensitivity are the calibration parameter q, or are related to the host plant (*Mri*). The model's reaction to these parameters is over-proportional (relative sensitivity > |1.0|) even if the model is run on a plot with 19% seed infection. The model's sensitivity to changes in q is linear: its relative sensitivity is approximately the same regardless of the amount of change in parameter value. Such information is useful for the later calibration of the model to real data because it facilitates estimation of the precision and range of parameter values to be tested.

TABLE 2.7: Relative sensitivity of EPIVIT's state and output variables to changes in parameter values for aphid-transmitted viruses (calculated on means of 20 runs per parameter combination [a]; infected seed tubers with random spatial distribution [b]).

Parameter [c]	Unit	Parameter Value		Change	Relative sensitivity: Seed infection 2 %				Seed infection 19 %			
					Variables				Variables			
		initial	new	(%)	Pi	tpi	tsi	hi	Pi	tpi	tsi	hi
Lp	P_{Lp}-Days	70	80	+14	+1.89	+0.01	- [d]	+1.33	+0.16	0.00	-	+0.13
			140	+100	+0.11	-0.02	-	+0.05	+0.13	+0.10	-	-0.09
mLp	-	3	2	-33	+0.37	-0.01	-	+0.24	+0.10	0.00 [e]	-	+0.06
nLp	-	4	5	+25	+0.07	-0.02	-	-0.01	-0.07	0.00	-	-0.05
drLp	°C	3	0	-100	+1.00	+1.00	-	+0.72	+1.00	+1.00	-	+0.61
		3	6	+100	+0.51	0.00	-	-+0.02	-0.03	0.00	-	-0.01
q	-	10	5	-50	+1.30	-0.01	-	+0.95	+0.95	0.00	-	+0.59
			1	-90	+1.10	+1.00	-	+0.79	+1.01	0.00	-	+0.63
			20	+100	+1.04	0.00	-	+0.74	+0.65	0.00	-	-0.80
kpi	$plant^{-1}$	2.0	0.2	-90	+0.09	+0.05	-	+0.07	-0.07	-0.03	-	+0.07
			0.02	-99	+0.38	0.00	-	+0.27	+0.62	0.00	-	+0.38
			20.0	+900	-0.03	-0.02	-	-0.03	0.00	0.00	-	-0.01
h1 [f]	-	10	9	-10	-1.07	-0.02	-	-0.68	-0.76	0.00	-	-0.52
			11	+10	+1.46	0.00	-	+1.01	-1.52	0.00	-	-0.91
m_{sp} [g]	-	2.0	3.0	+50	+1.55	-0.22	-	+1.09	-1.13	0.00	-	+0.71
n_{sp} [g]	-	4.5	5.5	+22	-2.55	0.00	-	-1.81	-1.37	0.00	-	-0.84
$T_{min;sp}$ [g]	°C	5	0	-100	-1.19	+0.03	-	-0.79	-0.45	0.00	-	-0.27
$T_{max;sp}$ [g]	°C	35	30	-14	-5.62	+0.10	-	-4.01	-3.88	0.00	-	-2.41
M	-	20	10	-50	+0.81	+0.01	-	+0.61	+0.74	0.00	-	+0.45
			2	-90	-0.08	0.00	-	-0.05	-0.01	0.00	-	0.00
REF_{sp} [h]	-	0.1	0.2	+100	+0.09	0.00	-	+0.05	+0.68	0.00	-	+0.41
			0.5	+400	+1.20	0.00	-	+0.85	+0.32	0.00	-	+0.20
Af_{sp} [h]	-	0.588	0.688	+17	-2.65	-0.03	-	-1.70	-1.83	+0.01	-	-1.11
			0.988	+68	-1.18	-0.29	-	-0.81	-0.75	0.00	-	-0.47
Mri	P-days	200	190	-5	+6.18	+0.08	+0.04	+4.60	+1.09	0.00	+0.04	+0.64
			130	-35	+1.24	0.00	+0.04	+0.89	+0.10	0.00	+0.04	+0.10
m_a	-	1.0	2.0	+100	-	+0.06	+0.11	+0.29	-	+0.06	+0.11	+0.02
n_a	-	4.0	5.0	+25	-	-0.25	-0.37	-0.33	-	-0.25	-0.37	-0.29
dr_a	°C	3.0 [i]	5.0	+67	-	-0.06	+0.05	+0.20	-	+0.03	+0.05	+0.02
$T_{min;a}$	°C	5	3	-40	-	-0.04	-0.10	-0.15	-	-0.04	-0.10	-0.29
$T_{max;a}$	°C	35	30	-14	-	-0.37	-0.65	-2.14	-	-0.36	-0.65	-0.35
TH	bdd_a	20	10	-50	-	+0.01	0.00	-0.20	-	0.00	0.00	+0.04
			0	-100	-	-0.01	-0.01	+0.05	-	-0.01	-0.01	-0.03
		20 [j]	10	-50	-	-0.01	-0.01	+0.38	-	0.00	-0.01	-0.13

[a] Back-transformed means of arc sine transformed percentages.

[b] Plot of 216 plants in six rows. Emergence of infected and healthy seed tubers: 0.96. Temperature data of Imperial, Peru, 1988 (Fig. 2.3.). Hypothetical aphid data (Fig. 2.3.).

[c] The model parameters were set to the values which are presented in Table 2.4.

[d] The manipulated parameter has no relation to the respective variable according to EPIVIT's code.

[e] A value of 0.00 indicates that the relative sensitivity is <0.005 and >-0.005.

[f] h_2 and h_3 were simultaneously changed from 16 and 14.5 to 15 and 13.5, and 17 and 15.5 respectively.

[g] These parameters were attributed to all species of the aphid population.

[h] The values correspond to the most important species of the applied population, *Macrosiphum euphorbiae*. REF_{csp} and Af_{sp} of a species were not allowed to become higher than 1.0 even after addition of the indicated difference.

[i] In order to allow the parameter change to be expressed as a percentage, dr_a was set to an initial value of three instead of zero as for the other model runs (footnote [c]).

[j] Results of further runs with m_a=2.0 and n_a=2.0 (instead of 0.1 and 4.0 respectively) to obtain more information on the relative sensitivity to changes of TH.

Discussion

Model structure. EPIVIT's structure is based on general knowledge of the mechanisms involved in potato virus epidemiology and the findings of recent studies of potato virus epidemics in different agroecozones of Peru (3). The coarse sensitivity analysis demonstrated a reaction of the model to changes in the model's structure which is compatible with this knowledge. Stochastic elements of the model determine the variability of its outputs. Other components however, may also have probabilistic distributions (such as the settling behaviour of aphids, for example) which may be related to significant fluctuations of the outputs of the real system. EPIVIT's validation with experimental data must prove whether the variability produced meets that of the real system or if other elements need to be incorporated into the model code.

Input variables. Temperature and aphid population are the only input variables of the model so that the degrees of freedom of the model are low. Other variables such as, for example, humidity and precipitation, may have theoretical importance for the output of the state variables, but only in an indirect way: humidity and precipitation directly affect aphid population and plant growth on which virus transmission, multiplication and translocation depend. EPIVIT uses trap catches as a parameter variable for the aphid population present above a field. It does not in its actual version simulate the population itself which would require additional input variables such as precipitation since this variable directly influences aphid population development and fluctuation. The potential for virus transmission of the caught population is not affected by precipitation. The virus multiplication inside a plant is also affected only in an indirect way by precipitation through the changing metabolism of the plant. It is therefore biologically reasonable to consider only temperature as the principal climatic variable for the modelled system.

Fine sensitivity analysis demonstrated that with EPIVIT for aphid-transmitted viruses a precise estimation of variables relating to aphid behaviour (h_1 and h_2) and virus transmission efficiency of different species is essential. It is to be proven whether the concept of relating the transmission efficiency with the weekly average of daily means between temperatures at h_1 and h_2 is correct. More detailed input data related to the aphid population may be suitable. Aphid activity, settling behaviour, and behaviour on the crop (alatae and apterous) in relation to virus spread is related to other variables such as wind (22), in certain cases precipitation (14), humidity and others. A conflict between precision and simplicity of the model arises however, and EPIVIT's validation may indicate whether the model suffices for the demands of the problems for which it was developed.

It should be emphasised that the model can be applied easily to aphid catch data other than those from yellow water traps. By setting the attraction factors Af_{sp} to 1.0 EPIVIT may handle data from traps and methods that are unbiased, such as from suction traps, nets, or leaf counts.

The simulation of state variables and essential auxiliary variables. The sensitivity analysis of the model indicates that the hypotheses which were necessary for the formulation of the simulation code for state variables allowed the development of a model which yields reasonable outputs. Again, later validation of EPIVIT will provide further information on the correctness of these hypotheses. A true proof for their suit-

ability however, can be obtained only by analytical experimentation of the real biological system.

Two hypotheses in particular must be verified in this respect: first the use of the multiple infection transformation for the relation of the probability of a plant becoming infected by a contact-transmitted virus using beta-beta-degrees as the independent variable, and second the application of the monomolecular model to the relation of the efficiency of autoinfection with accumulated beta-degrees. These hypotheses make sense either as practical evidence demonstrates (4) in the case of the efficiency of autoinfection, or as theoretical reflections on the relation between infection probabilities and temperature in a uniformly managed plot suggest.

The use of the negative binomial distribution for the simulation of primary infection by aphid-transmitted viruses may be controversial. This distribution has been widely used by biologists for modelling pest and disease populations which are clumped around initial foci, and which are not subject to additional input of the causal agent into the considered zone. Even if the system for which EPIVIT has been developed was limited to cases without virus input from outside a field, it may be argued that this limits the model to few cases. The negative binomial distribution however, approaches the Poisson distribution if the parameter k_{pi} is increased. The Poisson distribution is commonly used for modelling purposes under the assumption that within the region of interest events are randomly distributed in space which is an assumption to be made if virus input from outside the field needs to be modelled. With $k_{pi} >= 8$ the two distributions are not separable. The negative binomial distribution appears to provide more flexibility in this respect for the potential uses of EPIVIT. An erroneous application of the negative binomial distribution instead of the Poisson distribution yields large differences between the simulated outputs of these distributions only if $k_{pi} < 2$, and if the simulated respective proportion (in EPIVIT's case *pi*) is greater than 0.75 (27).

The model does not differentiate between persistently and non-persistently transmitted viruses. No reference has been found which compares the modelling of two such pathogens with practical and quantitative model experimentation. Some of EPIVIT's parameter variables may have distinct meaning for both cases, such as the probability *pa*(i) as explained above (see primary infection of plants: aphid-transmitted viruses). The key component behind the simulation of the primary infection with aphid-transmitted viruses is the use of the binomial distribution which is a theoretical concept. The assumptions related to this concept have been explained above and they are not incompatible *ex ante* with the two virus types. EPIVIT's application to real data will elucidate whether the model concept needs to be more specific to each of the two virus types, or whether specific parameter estimations yield outputs which are accurate enough and which satisfy the objectives of the model.

The simulation of the potato plant's physiological time is based on the assumption that plant age advances at temperature sensitive rates which increase and decrease in a bell-shaped way between cardinal temperatures. This is the approach used by various authors (21, 23, 29, 34). It has been argued recently (10) that this could produce lower ageing rates at high temperature compared to rates at low temperature (e. g. 36°C and 11°C). A linear relation to a rate increase up to the point of lethal temperatures has been proposed (10). Both approaches seem to be biologically unlikely. EPIVIT's code for the simulation of P-days provides enough flexibility for the adaptation of growth rates to particular situations in a biologically meaningful way. Care must be taken during model

validation and application however, that no cases arise such as the one which is mentioned above.

The use of a weekly output simulation was conditioned by the purpose of the model development as explained above. If appropriate, this time step may be easily changed to one day in EPIVIT's code.

The relative sensitivities and the variability of EPIVIT's outputs. The reasons for the higher relative sensitivities in plots with low seed infection compared to plots with high seed infection have been outlined above (fine sensitivity analysis). The expectations meet reality, which verifies the underlying concept. This suggests the use of data from plots with moderate to high seed infection for the calibration of the model's parameters.

The nature of the stochastic elements of EPIVIT for contact- and for aphid-transmitted viruses conditions the greater variability of outputs of the version for aphid-transmitted viruses, and the larger number of parameters with a high relative sensitivity. The probabilistic element of EPIVIT for contact-transmitted viruses is applied to individuals (healthy plants in a field) of uniform characteristics in relation to their importance for the epidemic. They are supposed to be equal in terms of susceptibility. This is not the case for the model version for aphid-transmitted viruses: it selects aphids among which there are species with a high relative efficiency for virus transmission and others with a low efficiency. This results in a higher variance of the model output. Again, model validation will provide more information on the compatibility of this concept with reality but it does not appear to be incorrect *per se*.

The high relative sensitivity of parameters related to aphid behaviour and of characteristics involved in their ability to transmit virus means that the model reacts strongly to changes in these parameters. Similar observations on the behaviour of these parameters in the real system have been deduced from field experiments in different agroecozones of Peru (4). This coincidence may not serve for further verification of the model but should be kept in mind for further consideration during EPIVIT's validation and parameter calibration. Harmony between the model and the real system in this respect would greatly increase the significance of the model as a mirror of reality.

Agreement with the objectives of EPIVIT's development and prospects for application. EPIVIT's development aimed at an explanatory, biologically significant simulation model which responds to changes in temperature conditions and plant genotype, and which is adaptable to changing agroecological conditions. EPIVIT satisfies these conditions. Genotype sensitive parameters are those for the simulation of the physiological time, susceptibility, and state variables. The model's parameters allow for high model flexibility and adaptability. The purpose of the model construction was to obtain a tool for forecasting epidemics of the most important viruses of the potato crop in the Andes. The purpose of the model implementation was to offer an educative and practical tool which can easily be used and understood by potato programme managers who have a basic understanding of potato virus epidemics. Validation and future model application still need to prove whether these goals have been meet. The attempt was made to overcome, as far as possible, a conflict which is inherent to some extent in the objectives of EPIVIT's development: the conflict of simplicity with flexibility and explanatory character of the model. The latter leads in the case of potato viruses to a considerable resolution of the pathosystem whereas simplicity would call for a low intricacy.

Two further aspects need to be discussed in relation to their compatibility with the objectives of EPIVIT's development: stochasticity and spread of the pathogen in space:

- According to their definition, stochastic elements should mimic random fluctuations of a treatment result around the treatment trend. If long-term forecasts are needed for harvest infection in a tuber lot which serves as a seed source during consecutive seasons, repetitive runs of the model are required. Several data sets of climatic variables may be used for this purpose. A long-term trend of seed degeneration which is representative for distinct agroecozones may be determined by averaging the model outputs of repetitive runs which cover, according to the model's concept, the entire range of possible outputs in the real system. The model may also be run repetitively with historical data from a single season. This points to EPIVIT's potential as a forecaster for primary infection during one season which is useful for seed production specialists in developed countries. The high variability and relative sensitivity of the model at low levels of seed infection may be a problem however, because of the need in these countries for prediction at low levels of seed infection. Randomly-distributed spontaneous events which by definition are not correlated to independent variables but rather by unconsidered system variables will always cause differences between the real system and model output. This calls for simulators that are applied as far as possible to one particular situation and mimic as many variables as possible. It is unreasonable to develop simulators which can only be applied to a single, very limited part of reality (i. e. one particular field). Precision of the model predictions will suffer increasingly with the level of generalisation of a model. However, because of its stochastic elements, EPIVIT provides the possibility, to predict the range within which an output of the real system may lie. This range is relevant for seed production specialists to determine haulm destruction dates and to demarcate zones of low risk of virus infection.

- EPIVIT makes possible approaches to model verification and validation which are innovative for quantitative plant disease epidemiology because of the incorporation of spatial components into the model code for contact-transmitted viruses. As mentioned above the simulated harvest infection is not affected by the spatial distribution of infected seed tubers (regular or at random) in small plots (300 plants). It needs still to be tested whether this indifference of the model's outputs is valid also for larger plots. The model code for contact-transmitted viruses is based on an individual plant approach as explained above. Several simple tools exist for the comparison of the spatial distribution of individuals in a demarcated field, e. g. the calculation and comparison of gradients and the analysis of distance class frequencies (17). Model experimentation may produce outputs which can be compared with theoretical spread patterns of individuals or patterns obtained by experimentation in the real system. The model validation by means of such techniques will provide further information on the correctness of EPIVIT's concept, and improve even more the comprehension of the interaction of the components of the potato virus pathosystem and their response to environmental changes.

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III. A first, exploratory validation of the simulation model EPIVIT and its application to seed potato degeneration data from Peru

Abstract

A first validation is presented of EPIVIT, a simulation model for the prediction of the percentage of potato tubers infected with a contact- or an aphid-transmitted virus in the harvest of a potato field (harvest infection). The model's parameters were calibrated by using harvest data of epidemiological experiments with PVX, APMV, PVY, and PLRV in contrasting agroecological zones of Peru. Some parameters were estimated empirically. Others were adjusted stepwise until a determined level of model prediction was obtained. The calibrated model estimated precisely ($P<0.05$) the efficiencies of autoinfection with PVX, APMV, PVY, and PLRV in diverse agroecozones. EPIVIT was not further calibrated for PVY due to the paucity of data provided by the historical data set for this virus. Additional parameter calibration is required for the simulation of the primary infection of plants with PVX and APMV because the model predictions with the selected parameter value sets did not meet the criteria of precision established. Suggestions are made, how to improve EPIVIT's precision in this respect. Simulations for PLRV were estimated to be sufficiently accurate. EPIVIT was applied to two diverse agroecozones for estimating the long-term average trend for harvest infection with PLRV. Harvest infections were simulated for a tuber lot of improved seed assumed to be multiplied during successive growing seasons in the same environment, with crop management practices which are representative for the respective agroecozone. The model validation proved that EPIVIT simulates the biological mechanisms which are relevant for harvest infection in Peru. The contribution of a temperature sensitive efficiency of autoinfection to the build-up of harvest infection was confirmed. The validation supported EPIVIT's assumptions on pathogen x host genotype x environment interactions and specified them.

Introduction

EPIVIT is a simulation model developed for simulating seed potato degeneration by a contact- or an aphid-transmitted virus (2), degeneration being understood as the increase of virus-infected tubers in a tuber lot which serves as source for seed selection during consecutive seasons. The model simulates the percentage of virus-infected tubers in the harvest of the simulated plot (harvest infection). It was expected to be useful for seed production specialists in developed countries as well as for potato programme managers in the developing world (2). The objective of EPIVIT's development was a simulation model which is biologically significant, explanatory and adaptable to different agroecological conditions, plant genotypes and viruses. This flexibility and explanatory character is reflected in the considerable number of parameters required for the simulation of the different components of the respective pathosystem. The model's input variables are daily minimum and maximum temperatures and weekly catches of winged aphids with an appropriate insect trap (e. g. yellow water trap). The state variables are the efficiency of autoinfection (an expression introduced only recently (2) for the percentage of infected tubers among those produced by a secondarily-infected

plant), primary infection of plants and the tuber infection of primarily-infected, infectious plants. The model uses temperature-sensitive growth rates for the computation of the advancement of the potato plant's physiological age and of other different types of developmental heat sums which are essential for the simulation of the state variables. The beta function serves for obtaining such temperature specific growth rates.

EPIVIT for contact-transmitted and for aphid-transmitted viruses includes stochastic elements. The variability of outputs is expected to reflect the experimental variance which is observed in the real system. Another innovative element of the model is the simulation of the spatial virus spread to individual plants in the simulated plot for contact-transmitted viruses.

The reaction of the model to changes in structure and parameter values was studied with sensitivity analysis (2). Validation is a further element of a model's evaluation. It comprises the quantitative comparison of its outputs with historical data. Independent data sets should be used for this purpose (7). In the case presented, however, this is not possible since no data for parameter estimation related to the considered plant genotype and viruses are available except those having been partly used for developing EPIVIT (2).

The complexity of application of a model to real data increases with the number of parameter variables the model uses as well as with the number of cases from the real system to which the model should be applied. Regarding the number of parameter variables which EPIVIT uses, the objectives of the study presented were to test, in a first attempt at model application to real data, with the simplest methodology possible, whether the model may reproduce degeneration data obtained in the real system (validation) and to verify whether the results obtained from this validation are consistent with the objectives to which EPIVIT was developed. Variables and symbols are explained as far as is essential for the understanding of the text (see Tables 2.1. and 2.3. for a full listing of variables and symbols). Variable symbols are written in italic letters in order to separate them clearly from the text.

Data and methods

Historical data set. The data set to which EPIVIT was applied relates to studies on the epidemiology of potato viruses realised during two growing seasons in three agroecological zones of Peru (3). The epidemiology of the contact-transmitted potato virus X (PVX) and Andean potato mottle virus (APMV), and the aphid-transmitted potato virus Y (PVY) and potato leafroll virus (PLRV), were studied with the modern potato cultivar Yungay (*S. tuberosum ssp. tuberosum x S. tuberosum ssp. andigena*) in plots with different seed infection. In plots with low (approximately 2%), intermediate (approximately 20%) or high (approximately 50%) seed infection, secondarily-infected tubers were distributed spatially in a regular way (6). The spatial pattern of infected plants at harvest was mapped. The data set provides the harvest infection, the efficiency of autoinfection, the primary infection of plants and the average tuber infection of such plants for each season, experimental site and plot. Daily temperature data and weekly aphid catch data of yellow water traps are also included.

Concept for the model's evaluation. According to EPIVIT's rationale the model's simulated variables may be compared with real data at the end of a season (harvest infection, efficiency of autoinfection, tuber infection of primarily-infected, infectious plants) or at any time during season (primary infection of plants). The historical data set described above provides data related to the end of a season. Therefore the present validation refers only to harvest data.

Validation requires prior parametrization. This is the adjustment of the model's parameters to values with which the model behaves as closely as possible to reality (7, 10). EPIVIT's output for the primary infection of plants is variable if the model is run repetitively with the same parameter value set. This is because of EPIVIT's probabilistic code (2). Consequently, the outputs for the tuber infection of primarily-infected plants and for harvest infection are also variable. In contrast to the other variables, EPIVIT's output for the efficiency of autoinfection has no variability since the model code is not probabilistic for this variable. These differences condition the parametrization and validation techniques for the respective variables described below.

The simulation of the percentage of infected tubers among those produced by primarily-infected, infectious plants requires the same parameters already needed for the simulation of the efficiency of autoinfection (*tsi*). It was assumed that the calibration of these parameter variables by the parametrization procedure for *tsi* yields values which are equally relevant for the infection of tubers of primarily-infected plants. Parametrization was performed therefore only for *tsi* and the primary infection of plants.

The graphical comparison of model output data with real system data without prior identification of the simulated data is one of the most frequently used methods for model validation (Turing test; 12). This is not possible in the case presented because the historical data are not continuous, but are results from individual experimental plots of which harvest infection does not provide the seed infection of the following season. Statistical comparisons were performed therefore between numerical model outputs and real system data (see validation techniques). If the validation proved that the model provides outputs that are precise enough, the model was applied further to the real system (forecasts etc.).

Parametrization techniques. For many of the parameters which EPIVIT uses, no data are available from the real world (e.g. beta function parameters related to temperature sensitivity of the latent period). Estimation of these parameters by parametrization was initiated with one particular set of parameter values, presented below. A regression was then fitted to the model outputs relating simulated data to data obtained from the real system. If the fit did not satisfy the conditions established below, parameters were changed stepwise, model outputs computed again, but with the new parameter value set, and subsequently fitted again to the real system data. This procedure was repeated until a satisfactory fit was found. The parameter values related to the resulting regression were assumed to be those which allow the model to best represent the real system.

Since EPIVIT uses many parameters the number of possible parameter value combinations to be used until a satisfactory fit is found may be very high. The question arose as to how to limit computing time for a first, exploratory model validation without losing information about the model's validity.

Some of the parameters had been estimated before and were not changed further during the parametrization procedure, e. g. simulation of the physiological age and the

maximum physiological age related to the cultivar Yungay (2). The value of other parameters among the remainder were estimated empirically. These were those parameter variables which are easy to assess in the real world and which could be estimated according to empirical personal observations made in situ (e.g. the physiological age at which the canopy closes between plants within a row (C_{wr}), or the hours (h_1, h_2 and h_3) related to the aphid activity) as well as to experimental evidence (e.g. the physiological age at which the logistic decrease of age resistance is initiated; Bertschinger, unpublished) eventually combined with conclusions retrieved from published data. These variables were not changed further during the parametrization process once they had been estimated.

Techniques which were applied for the parameter estimation were distinct according to the state variable to which they related:

Efficiency of autoinfection and tuber infection of primarily-infected plants: EPIVIT simulates the efficiency of autoinfection (*tsi*) according to the monomolecular model as a function of developmental heat measured in beta-degree-days (2). The calculation of the beta-degree-days requires a beta function with the parameters m_a, n_a, dr_a, $T_{min;a}$, and $T_{max;a}$ (2). The model accumulates beta-degree-days between 50% emergence and 100% crop senescence. Beta-degree-days were computed with different parameter value sets for all seasons which are part of the validation data set and which provide daily temperature data (5 seasons). These sets were obtained by stepwise changes in parameters and by building all possible combinations among obtained parameter values. The range of values covered for the beta parameters was 0.5 to 3.0 for m_a and n_a with a step size of 0.5, for the delay range (dr_a) 0, 5, and 10°C, for the cardinal temperatures 0, 2, and 5°C for the minimum $T_{min;a}$, and 30 and 35°C for the maximum $T_{min;a}$, and 0, 10 and 20 bdd_a for the trigger developmental heat *TH*. Every seasonal total of computed beta-degree-days was paired with the efficiency of autoinfection reported for the respective site and season which was transformed to the linear form of the monomolecular model ($\ln[1/(1-tsi)]$). Linear regressions were fitted through each obtained set of data pairs. The "best" regression was selected according to the criteria a) regression slope significant, b) the highest coefficient of correlation and c) unbiased residual of back-transformed function plot. The parameters which correspond to the "best" fit were selected to be those which allow for the best mimicking of the pathosystem related to the data set.

Primary infection of plants: Data referring to PVY were not included in the parametrization related to the primary infection of plants. The historical data set provides data for this virus of only 1 season per site which was insufficient for obtaining significant results and for testing EPIVIT's adaptability to different seasons and sites.

Among the parameter value sets tested the "best" was selected according to a procedure which is described below. Recognising EPIVIT's probabilistic code, five predictions were produced for contact-transmitted viruses by successive single season simulation runs with one particular parameter value set for each plot of the validation data set. For aphid-transmitted viruses, 10 repetitive runs were executed as the variability of this model version is greater (2). Simulated primary infections with aphid-transmitted viruses in plots with a low seed infection are even more variable compared with plots with a high seed infection (2). Regarding these model characteristics, outputs of 10 simulation runs may still be biased, i.e. not necessarily normally distributed. Consequently, plots with less than 10% seed infection with aphid-transmitted viruses were not included for parametrization.

With this procedure, each parameter set yields a series of repetitively predicted model outputs for primary infections of plants which correspond to particular seasons and experimental sites. Each simulated percentage of primary infection was paired with the corresponding value observed in the real system. Linear regressions were fitted through these data pair sets related to a particular parameter combination. A regression was to be found according to the criteria 1) significant slope, 2) slope as close as possible to 1.0 and associated with a coefficient of correlation which is as high as possible, and 3) y-axis intercept as close as possible to zero. This regression was expected to relate to those parameter values which allows the model to represent best the pathosystem of the historical data set (adapted from 7).

In accordance with the experimental design related to the historical data set used, the secondarily-infected plants were distributed regularly onto all available plant positions in the simulated plot. Non-emerged plants were selected at random independently from the plant's health state according to the findings of the respective study.

EPIVIT simulates a temperature-sensitive latent period *Lp* measured in P-days which is the unit of the developmental heat related to the physiological age of the crop. It was first assumed that the simulation of *Lp* requires the same parameter values of the respective beta function (m_{Lp}, n_{Lp}, dr_{Lp}, $T_{min;Lp}$, $T_{max;Lp}$) as with the computation of the physiological age. If these values did not provide the necessary accuracy m_{Lp}, n_{Lp} and dr_{Lp} were greatly changed to values with which a contrasting model output could be expected. They were then changed stepwise by adding or deducing 1.0 such that the bell-shape of the corresponding beta function changes significantly.

The parameter value set which was used first for repetitive parametrization runs is presented below. With sensitivity analysis (2) and preliminary model experimentation, first experience had been obtained in understanding how the model behaves. Subsequent adjustments were expected to yield better fits, according to this experience, and within a biologically meaningful range. Changes to the first parameter value set are commented on below (results) after the presentation of the significance of the regression fits related to the first parametrization runs. All tested parameter value sets are presented in Table 3.1.

First parameter sets of the parametrization for contact-transmitted viruses: Initially estimated parameter values which were not changed subsequently: the plant age at which the canopy closes within and between the planting rows (C_{wr} and C_{br}): 100 and 150 P-days after 50% emergence respectively; the plant age (*Mri*) at which the logistic increase of age resistance begins with the rate r_{mr}: 200 P-days, and 11.0E-3 P-day^{-1} respectively; the cardinal temperatures of the beta function for the simulation of the temperature-sensitive probability of infection *(pc)*: $T_{min;b}$=5°C and $T_{max;b}$=35°C.

Lp was set to 70 P-days (1 week if temperature fluctuates approximately between 13 and 23°C) in the parameter set selected for the first simulation runs; the constitutive susceptibility Su_c to 0.5 and the rate parameter of the multiple infection transformation (r_{mi}) for the probability *pc* to 1.0 (the corresponding plot for *pc* is presented in Fig. 3.1a.). The beta function parameters m_b and n_b related to the simulation of *pc* were changed stepwise each covering a range from 1.0 to 6.0 with a step size of 1.0.

TABLE 3.1: Parameter value sets used for the parametrization of variables which EPIVIT requires for the simulation of the primary infection of plants, and the variable set, which yielding the most accurate simulation [a,b].

Virus	Parameters [c]									
	Latent period (Lp) [d]				Primary infection (Pi) [e,f]					
	m_{Lp}	n_{Lp}	dr_{Lp}	Lp	m_b	n_b	$T_{min;b}$	$T_{max;b}$	Su_c	r_{mi}
PVX	0.5	0.5	5	70	1-6	1-6	5	35	0.5	1.0
	0.5	0.5	5	70	1-6	1-6	5	35	0.5	0.5
	0.5	0.5	5	70	1-6	1-6	5	35	0.5	0.25
	0.5	0.5	5	70	1-6	1-6	5	35	0.2	0.25
	4.0	5.0	5	70	1-6	1-6	5	35	0.5	0.25
	2.0	5.0	5	70	1-6	1-6	5	35	0.5	0.25
	3.0	5.0	5	70	1-6	1-6	5	35	0.5	0.25
	3.0	5.0	5	100	1-6	1-6	5	35	0.5	0.25
best set [g]	3.0	5.0	5	70	3	2	5	35	0.5	0.25
APMV	0.5	0.5	5	70	1-6	1-6	5	35	0.5	1.0
	4.0	4.0	5	70	1-6	1-6	5	35	0.5	0.25
	4.0	3.0	5	70	1-6	1-6	5	35	0.5	0.25
	4.0	3.0	5	140	1-6	1-6	5	35	0.5	0.25
	4.0	3.0	5	140	1-6	1-6	5	35	0.3	0.25
best set	4.0	3.0	5	140	5	4	5	35	0.3	0.25
	Latent period (Lp) [c]				Virus transmission efficiency (REF_{sp}) [e,h]						
	m_{Lp}	n_{Lp}	dr_{Lp}	Lp	m_{sp}	n_{sp}	$T_{min;sp}$	$T_{max;sp}$	q	M	k_{pi}
PLRV	0.5	0.5	5	70	1-6	1-6	5	35	8	20	0.2
	0.5	0.5	5	70	1-6	1-6	5	35	8	20	2.0
	0.5	0.5	5	70	1-6	1-6	5	35	8	2	2.0
	0.5	0.5	5	70	1-6	1-6	5	35	4	20	2.0
best set	0.5	0.5	5	70	3	4	5	35	4	20	2.0

[a] The model was run with input data presented in Fig. 1.7. and 1.9.

[b] The parameter values for the simulation of the physiological age were with all parameter combinations as follows: m_p=0.5, n_p=0.5, dr_p=5°C, $T_{min;p}$=0°C, $T_{max;p}$=35°C, P_{max}=1030 P-days (Fig. 2.2.).

[c] For detailled listing and explanation of parameter variables and symbols see chapter 2, Table 2.3.

[d] In all cases: $T_{min;Lp}$=0°C, $T_{max;Lp}$=35°C.

[e] In all cases: Mri=200 P-days, r_{mr}=11.0E-3 P-days^{-1}.

[f] In all cases: C_{wr}=100 P-days, C_{br}=150 P-days.

[g] See text ("parametrization techniques") for the selection criteria for the "best" set.

[h] In all cases: h_1=10 hours, h_2=16.0 hours, h_3=14.5 hours.

First parameter sets of the parametrization for aphid-transmitted viruses: Initially estimated parameter values which were not changed subsequently: the plant age (*Mri*) at which the logistic increase of age resistance begins with the rate r_{mr}: 200 P-days, and 11.0E-3 P-day^{-1} respectively; the cardinal temperatures of the beta function for the simulation of the temperature-sensitive relative efficiency factor REF_{sp} with which an individual of the aphid species *sp* transmits virus: $T_{min;sp}$=5°C and $T_{max;sp}$=35°C.

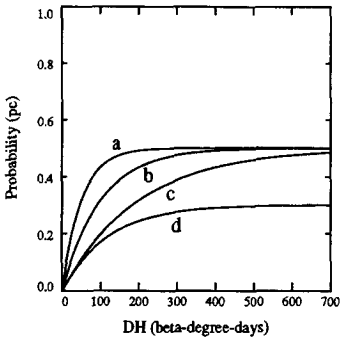

Fig. 3.1. Selected multiple infection transformations for the simulation of the probability of infection (pc) with a contact transmitted virus for a healthy plant which is situated adjacent to an infectious plant.
The probability $pc=Su_c*(1-\exp(-r_{mi}*DH/Su_c))$ is represented with
a) $Su_c=0.5$, $r_{mi}=1.0$; **b)** $Su_c=0.5$, $r_{mi}=0.5$;
c) $Su_c=0.5$, $r_{mi}=0.25$; **d)** $Su_c=0.3$, $r_{mi}=0.25$.

Lp was set to 70 P-days as for contact-transmitted viruses. The relative factors *Af* representing the attraction of different aphid species by the yellow trap colour (Table 1.2.) were transformed to cover a range from 0 to 1.0. The scaling factor q was then set to 8.0 (this scales the *Af* values to a range which assigns *Myzus persicae* the value 2.0); the average number of moves *(M)* which an aphid specimen makes before leaving the field to 20; and the parameter of the negative binomial distribution k_{pi} to 0.2 (representing a medium degree of clumping of the spatial virus spread around infectious foci). The parameters m_{sp} and n_{sp} of the beta function for the simulation of REF_{sp} were changed stepwise each covering a range from 1.0 to 6.0 with a step size of 1.0.

Validation techniques. These techniques should provide a quantitative measure of the accuracy of the model outputs for the state variables and harvest infection at the end of one season (single season simulations).

The 95% confidence limits of the binomial distribution were computed for the historical efficiency of autoinfection (n=number of secondarily-infected plants in the respective experimental site and season) and tested for whether or not the simulated data lay within these limits. Further, it was tested whether reported primary infections of plants lay within the 95% confidence interval of the mean for the respective model outputs (calculations for a normally distributed sample mean; 11). Means of arc sine-transformed percentages were tested.

Model application. If the parametrization and validation procedures had proven that the model yields outputs which are precise, enough EPIVIT was then used to predict seed degeneration. The model should estimate the trend of the change of harvest infection in different agroecozones of Peru if a farmer acquires a tuber lot of improved seed (2% of the tubers infected with the respective virus) and multiplies this lot by traditional crop management by continuously selecting seed tubers for the following season from the harvest of the present season.

The parametrization described above was performed with model runs on plots with a regular spatial distribution of secondarily-infected plants. Sensitivity analysis had demonstrated (2) that distribution of secondarily-infected seed, being either randomly or regularly among the available positions in a small plot (300 plants), does not affect the

model output for harvest infection. Conclusions drawn from the parametrization described above are therefore valid also for small plots with a random spatial distribution of secondarily-infected plants. Infected seed tubers and non-emerged plants were distributed at random for the model application because this is the most probable distribution in a farmer's field.

The parameter value set was applied which yielded the most accurate outputs according to the parametrization procedure. The historical data set provides temperature as well as aphid catch data of two seasons in sites at 112 and 3280 m.a.s.l. Aphid trap size was different in one particular season (Imperial 1987) from the trap size used in the other seasons (Table 1.5.). Catches may therefore not be compared directly. The relation between trap area and catches is linear on a square root scale for area and catches (8). Assuming that the linear regression with square root-transformed areas and catches has a y-axis intercept of zero (zero catches with zero trap area), catches of one trap (T1) can be transformed to catches with another trap (T2) by multiplying them with the proportion $area_{T2}/area_{T1}$. Trap catches of Imperial 1987 were therefore multiplied with 3600/1260=2.857 before using them as input variables for EPIVIT.

With each temperature and aphid data set, five multiple season simulations were performed. Outputs corresponding to one zone were joint in one single scatter plot.

Results

Daily temperature data of five seasons of the historical data set were available (3). Since EPIVIT requires the input of daily minimum and maximum temperatures parametrization and validation data refer to these five seasons.

Efficiency of autoinfection. The regression fits of reported efficiencies of autoinfection to the beta-degree-days accumulated until harvest (Fig. 3.2.) had coefficients of correlation between 0.887 (PLRV) and 0.996 (APMV). The beta function obtained for PVY is based on only three observations (three sites with one season each) and may therefore be considered as less reliable than those functions obtained for the other viruses. The results for this virus are presented nevertheless even if they may be considered as being preliminary.

All efficiencies of autoinfection predicted by the calibrated model are included in the 95% confidence limits of reported data (Fig. 3.2.) except in one case (PLRV at 4000 m.a.s.l. in 1988/89). The use of the discontinuous version of the beta function (Fig. 2.1.) for the calculation of beta-degree-days was beneficial except for PVY (Fig. 3.3.) where the conventional version provided good results (delay ranges of 5°C for PVX and APMV and of 10°C for PLRV). To obtain satisfactory fits for the aphid-transmitted viruses PVY and PLRV, a trigger developmental heat (*TH*) of 20 beta-degree-days was necessary whereas for PVX and APMV the accumulation of beta-degree-days did not need to be initialised by *TH* (Fig. 3.3.).

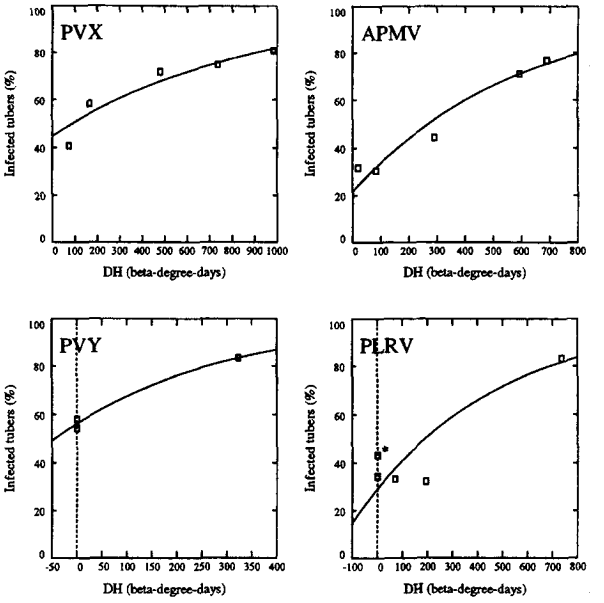

Fig. 3.2. Monomolecular functions fitted through data pairs of reported efficiencies of autoinfection and beta-degree-days which were accumulated in the respective site and season.

Historical data of the efficiencies of autoinfection are presented in Fig. 1.3. The beta functions which were used for the calculation of beta-degree-days are presented in Fig. 3.2. The monomolecular model is $tsi(t_{max}) = 1-((1-tsi_0)\cdot exp(-r_a \cdot DH_a(t_{max})))$ with t_{max}=date at 100% crop senescence. The parameters of the monomolecular fits are: for PVX: tsi_0=44.84, r_a=11.17E-4 $bdda^{-1}$; for APMV: tsi_0=21.40, r_a=16.87E-4 $bdda^{-1}$; for PVY: tsi_0=56.21, r_a=29.88E-4 $bdda^{-1}$; for PLRV: tsi_0=29.11, r_a=18.44E-4 $bdda^{-1}$. The coefficient of determination (r^2) obtained with the linearised monomolecular model is for PVX: 0.931; for APMV: 0.966; for PVY: 0.994; for PLRV: 0.887.

* The confidence limits (P<=0.05) of the binomial distribution for the indicated historical data do not include the simulated data at the respective DH.

Primary infection of plants, primary infection of tubers and harvest infection. Since primary infection of plants is conditional for the model output for tuber infection of primarily infected plants and for harvest infection, first priority is given to the presentation of results related to the primary infection of plants. Only if model outputs for this state variable were precise enough results are presented also for the tuber infection of primarily infected plants and for harvest infection.

The adjustment procedure of parameters for the primary infection of plants was stopped if the slope of the fit was <1.05 and >0.95 with a significance level not lower than 0.01, and if the coefficient of correlation of the fit was >0.7. The accuracy of the predictions for the primary infection of plants provided by EPIVIT's simulation with the actual parameter value set was then determined according to validation techniques described above.

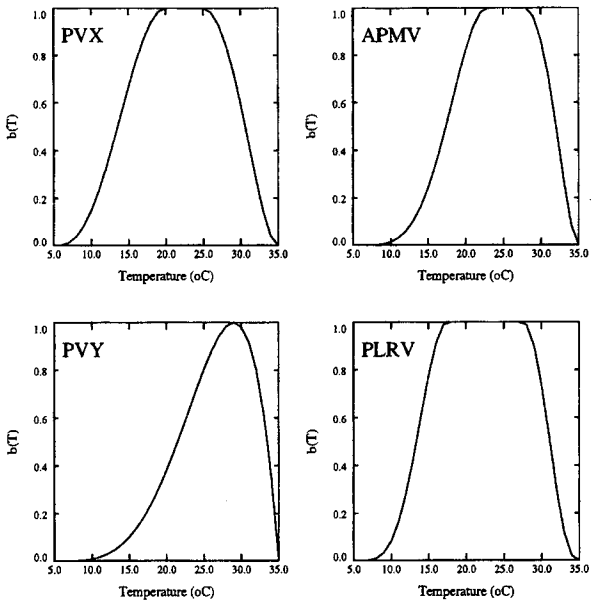

Fig. 3.3. Plot of beta functions used for the calculation of beta-degree-days, corresponding to the most accurate fit through data pairs of efficiencies of autoinfection with PVX, APMV, PVY and PLRV[a] with the seasonal beta-degree-days of the respective season and site.

The parameter values of the beta functions are for PVX: m_a= 2, n_a=3, dr_a=5, TH=0; APMV: m_a= 2, n_a=5, dr_a=5, TH=0; PVY: m_a= 1, n_a=4, dr_a=0, TH=20; PLRV: m_a= 3, n_a=5, dr_a=10, TH=20.

[a] The historical data are presented in Fig. 1.3. (efficiencies of autoinfection) and Fig. 1.9. (temperature data).

Contact-transmitted viruses: PVX: The fit obtained with the outputs of the simulation runs with the first parameter value set did not fulfil the above conditions. The sequence of parameter values sets which was subsequently tested is presented in Table 3.1. The multiple infection transformations which were applied with these sets are visualised in Fig. 3.1a. to 3.1c. The best fit for PVX was obtained with the set listed last for this virus in Table 3.1. (latent period of 100 P-days, a constitutive susceptibility of 0.5 and a rate of the multiple infection transformation of 0.25). The beta function corresponding to this best fit, used for the accumulation of beta-degree-days and the computation of the probability of infection *pc*, is slightly inclined to the left with a most efficient heat accumulation at 17°C (m_b=3 and n_b=2; Fig 3.4.). The slope of the respective regression fit is 1.008 with an error probability less than 0.0005, its y-axis intercept is 0.06 (i.e. on average, the model linearly overestimated the primary infection of plants

by 6%) and its coefficient of correlation 0.705. Simulated primary infections of plants deviated significantly from those observed in 9 of 12 plots considered (Table 3.2.). It was concluded that the model was not yet sufficiently fine-tuned for application to the simulation of primary and harvest infection in different agroecozones.

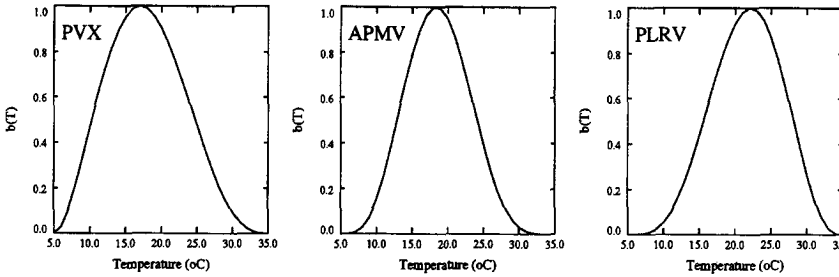

Fig. 3.4. Plot of beta functions for the calculation of beta-degree-days required for the simulation of the infection probability (pc) with contact-transmitted viruses, or for the calculation of the efficiency with which an aphid species transmits virus (REF_{sp}).

These particular functions yielded the most accurate estimation of the primary infection of plants among the tested parameter value sets (Table 3.1.) for EPIVIT's parametrization. The parameter values of the beta functions are: PVX) $T_{min;b}$=5, $T_{max;b}$=35, m_b= 3, n_b=2; APMV) $T_{min;b}$=5, $T_{max;b}$=35, m_b= 5, n_b=4; PLRV) $T_{min;sp}$=5, $T_{max;sp}$=35, m_{sp}= 3, n_{sp}=4.

APMV: All sets of parameter values tested for APMV are presented in Table 3.1. The multiple infection transformations which were considered with these sets are visualised in Fig. 3.1a., 3.1c. and 3.1d. The best fit for APMV was obtained with the set listed last for this virus in Table 3.1. (latent period of 140 P-days, a constitutive susceptibility of 0.3 and a rate of the multiple infection transformation of 0.25). Beta-degree-days were computed with m_b=5 and n_b=4 (Fig. 3.4.), the plot of the corresponding beta function being slightly inclined to the left with a most efficient heat accumulation at 18°C. The slope of the fit associated with these parameter values is 1.021 with an error probability less than 0.0005, its y-axis intercept is 0.06 and its coefficient of correlation 0.706. Ten plots were considered for this virus (Table 3.2.), and zero primary infection was simulated in four of them (at 3280 and 400 m.a.s.l.). No statistical comparison was made for this prediction because it has no variance and therefore, confidence limits can not be computed. Tuber infection of primarily-infected plants varies between plants (in contrast to the efficiency of autoinfection). This prohibits from applying the binomial distribution to these data. Only six plots remain for the comparison of model output and real data. In three plots, simulations deviated significantly from observed primary infections. The same conclusions were drawn as for PVX.

TABLE 3.2: Comparison of historical primary infections with PVX and APMV in experimental plots in Peru with outputs produced by the EPIVIT model[a].

Plot	Site	Elevation (masl)	Season	Seed infection (%)	Primary infection (%) observed	simulated [b,c]
PVX	Imperial	112	1988	2	4 a	8 b
				50	12 a	46 b
	Sta. Ana	3280	1987/88	2	7 a	6 a
				19	23 a	55 b
				73	17 a	21 b
			1988/89	2	11 a	2 b
				20	37 a	41 b
				50	38 a	46 b
	Chicche	4000	1987/88	2	10 *	0 *
			1988/89	2	9 *	0 *
				20	14 a	3 b
				50	36 a	17 b
APMV	Imperial	112	1988	2	0 a	1 a
				20	5 a	33 b
	Sta. Ana	3280	1987/88	2	3 *	0 *
				20	22 a	22 a
				75	9 a	15 b
			1988/89	2	3 *	0 *
				20	21 a	28 b
				50	40 a	41 a
	Chicche	4000	1987/88	20.0	4 *	0 *
				50.0	14 *	0 *

[a] The following data and parameter values were used: Temperature data: Fig. 1.9.; historical data for primary infection: Fig. 1.4.; parameter values for tuber infection of primarily infected plants (same as for the efficiency of autoinfection): Fig. 3.2. and 3.3.; parameter values for the primary infection of plants: "best" set in Table 3.1.; EPIVIT was run five times on each experimental plot with 300 plants using a random distribution of secondarily-infected tubers and of non-emerged plants onto the available plant positions of the plot lattice.

[b] Simulated percentages are back-transformed means of arc sine-transformed model outputs of five repetitive model runs on each experimental plot.

[c] Percentages with the same letter in the same line indicate that the 95% confidence interval of the mean of arc sine-transformed simulated outputs includes the observed percentage.

* A computation of the confidence limits is not possible because the model output of all five runs yielded zero primary infection.

Aphid-transmitted viruses: Only results for PLRV are presented (see explanation above): In contrast to simulations with EPIVIT for contact-transmitted viruses, reasonably good fits were obtained by using the same parameters for the simulation of the latent period as for the physiological time. The sequence of parameter value sets which was tested is presented in Table 3.1. The best fit for PLRV was obtained with the parameter value set listed last for this virus in Table 3.1. (latent period of 70 P-days, a calibration factor q of 4.0, an average number of within-field aphid moves (M) of 20, and a parameter of the negative binomial distribution (k_{pi}) of 2.0). The respective beta function associated with this value set being used for the computation of the temperature-sensitive virus transmission efficiency by aphids is slightly inclined to the right (Fig. 3.4.) with an optimum transmission factor at 22°C (m_{sp}=3 and n_{sp}=4). The slope of the "best" fit is 1.009 with an error probability less than 0.0005, its y-axis intercept is -0.07 and its coefficient of correlation 0.943. EPIVIT made accurate predictions in five

plots of eight plots considered (Table 3.3.) among which in one plot a zero primary infection of plants was simulated (2% seed infection at 3280 m.a.s.l.) The accuracy of model predictions was decided to be good enough for a further application of EPIVIT for aphid-transmitted viruses to PLRV data with the selected parameter value set.

TABLE 3.3: Comparison of historical primary infections of plants and tubers and of harvest infections with PLRV in experimental plots in Peru with outputs produced by the EPIVIT model[a].

Site	Elevation	Season	Seed infection	Primary infection (%) [b,c]		Primary tuber infection (%)		Harvest infection (%)	
	(masl)		(%)	observed	simulated	observed	simulated	observed	simulated
Imperial	114	1988	2	6 a	8 a	54 a	70 b	7 a	7 a
			10	27 a	33 a	52 a	71 b	27 a	32 a
			20	60 a	68 a	73 a	73 a	70 a	67 a
Sta. Ana	3280	1987/88	2	4 a	1 a	50 a	45 a [d]	3 a	1 a
			19	12 a	21 a	36 a	49 b	9 a	20 b
		1988/89	2	20 *	0 *	27 *	0 *	6 *	1 *
			20	6 a	1 b	44 a	42 a [e]	9 a	8 a
			50	17 a	1 b	27 a	42 b [d]	21 a	19 b

[a] The following data and parameter values were used: Temperature data: Fig. 1.9.; historical data for primary infection: Fig. 1.4.; parameter values for tuber infection of primarily infected plants: Fig. 3.2. and 3.3.; parameter values for the primary infection of plants: "best" set in Table 3.1.; EPIVIT was run 10 times on each experimental plot with 300 plants using a random distribution of secondarily-infected tubers and of non-emerged plants onto the available plant positions of the plot lattice.

[b] Simulated percentages are back-transformed means of arc sine-transformed model outputs of 10 repetitive model runs on each experimental plot.

[c] Percentages with the same letter in the same line indicate that the 95% confidence interval of the mean of arc sine-transformed simulated outputs includes the observed percentage.

[d] Primary infections occurred in only three multiple season runs.

[e] Primary infections occurred in only two multiple season runs.

* A computation of the confidence limits is not possible because the model output of all 10 runs yielded zero primary infection.

Model application. Improved seed with 2% seed infection which is multiplied during successive seasons in the same site degenerates according to the simulations for two seasons at two sites (Imperial at 112 m.a.s.l. and Sta. Ana at 3280 m.a.s.l.) much faster at 112 m.a.s.l. than at 3280 m.a.s.l. (Fig. 3.5.). Model outputs cover a wide range due to the model's probabilistic code but do not overlap between zones.

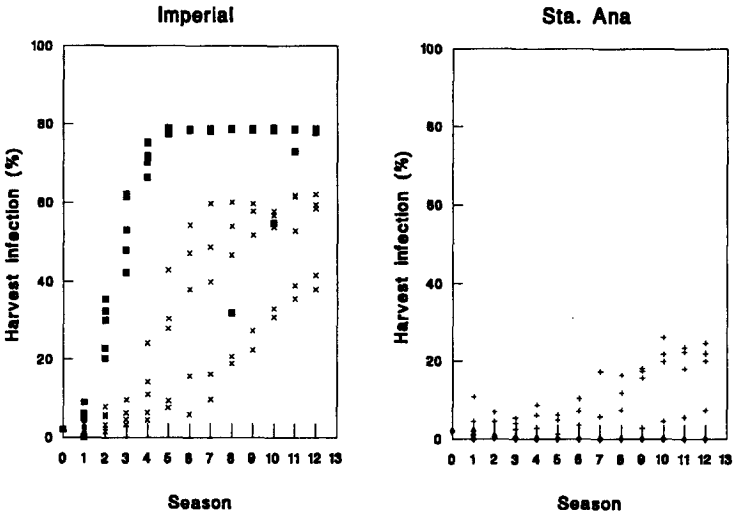

Fig. 3.5. Scatter plot of harvest infections produced by multiple season simulations with EPIVIT which was calibrated for PLRV and applied to temperature data of Imperial, Peru 1987 and 1988 (112 m.a.s.l.) and of Sta. Ana, Peru 1987/88 and 1988/89 (3280 m.a.s.l.).

Input data are presented in Fig. 1.7. (weekly aphid catches) and 1.9. (daily temperature). Aphid data were averaged per trap (back-transformed means of catches transformed by Log(x+1)). The used parameter value set is presented in Table 3.1. ("best" set"). Secondarily-infected and non-emerged plants were distributed at random on the available plant positions in a plot of 300 plants.

Five multiple season simulations for 12 successive seasons were performed with each aphid and temperature data set. Data corresponding to one site were joint in the same plot.

×: Imperial 1987, ■: Imperial 1988, +:Sta. Ana 1987/88, ◆: Sta. Ana 1988/89.

Discussion

Efficiency of autoinfection. The beta function can be interpreted biologically as the relative rate at which the respective virus is translocated towards the tubers at specific temperatures. These rates need to be verified ultimately by analytical experiments but they are compatible with published data related to tuber infections or virus concentrations in potato plants at specific temperature conditions (5, 14). The probable biological meaning of the trigger developmental heat has been discussed tentatively elsewhere (2).

The positive y-axis intercepts of the monomolecular fit can be interpreted such that virus is translocated also if zero beta-degree-days are accumulated. This also needs to be verified by experimental evidence. It is biologically significant, however, that some particles are translocated even under suboptimal conditions for virus multiplication and

transport. As long as the plant is alive and the synthesis of nucleic acids and proteins in the plant's cells is not null, it is expected that products of the viral genome are also produced. The simulated mechanism represents in this case a contribution to the final efficiency of autoinfection by virus particles which are translocated additionally to those translocated even under suboptimal temperature conditions.

The high accuracy of the model output calls for analytical research on this pathogen x host genotype x environment interaction in order to prove the assumptions which the model makes. However, it strongly supports the assumptions which have been made interpreting the reported efficiencies of autoinfection (3) and developing the model (2) from the underlying biological principles of this mechanism.

Furthermore, additional data for other cultivars would increase the relevance and importance of the findings presented. They could be obtained easily with limited additional input by planting secondarily-infected tubers of a selected cultivar in discriminating environments and applying the same methodology as described above.

Primary infection of plants. Additional parameter calibration is necessary for the model application to PVX and APMV data even if the trend of the results obtained seems to be correct: the beta functions determined for the computation of beta-degree-days (used for the estimation of the probability of a healthy plant becoming infected) exhibit highest values at 17°C for PVX and at 18°C for APMV (Fig. 3.4.). This means that simulations for PVX and APMV at cool temperatures yield higher probabilities of infection than at high temperatures. The experimental data used for the model validation point to an infectivity of PVX which is higher at the cool temperature site at 3280 m.a.s.l. compared with the moderate temperature site at 112 m.a.s.l. (Fig. 1.4., for site specific temperature data see Table 1.1. and Fig. 1.9.).

The regression fits between the observed and the simulated primary infections of plants must have a coefficient of correlation which is at least 0.9. As the parametrization for PVX and APMV demonstrated, a coefficient of 0.7 relate to fits with a deviation of the scatter plot around the regression line which is too high for an accurate model prediction even if the regression's slope is estimated as 1.0 with a high significance.

Contact-transmitted viruses: EPIVIT provides a high level of flexibility according to its model code. Better predictions can be approached most probably by a further systematic fine-tuning of the parameters. An accurate experimental estimation of the parameter variables which exhibit the highest relative sensitivity (Table 2.6.) would also facilitate model application. Such a variable is for example the physiological age at which the logistic increase of age resistance (*Mri*) is initiated. *Mri* can be determined by field inoculations of plants with different ages in different agroecozones. Such an experiment is feasible in any institutional environment within which virus problems are of concern and basic virological routine techniques are available (e.g. preparation of inoculum, ELISA techniques).

Aphid-transmitted viruses: The beta function for the simulation of the relative efficiency with which an aphid transmits virus obtained by parametrization simulates an optimal virus transmission at a temperature of 25°C (Fig. 3.4.). The few data reported (13) on the relation between temperature and PLRV transmission record temperature sensitivity of the transmission efficiency for *Myzus persicae* (higher efficiency at 27°C compared to 22°C). The model as it was applied makes no difference between these relationships for different aphid species. This represents a rough approximation to real-

ity which can be avoided as soon as real data are available. The sensitivity of the model to changes of this parameter (REF_{sp}) is, however, not very high (Table 2.7.) indicating that small inaccuracies in the estimation of this parameter do not lead to misleading model outputs.

Validation of the model structure. The model validation as a whole has proven that those biological mechanisms which are relevant for harvest infection under diverse agroecological conditions have been selected. The contribution of a temperature sensitive efficiency of autoinfection to the build-up of harvest infection was confirmed.

It may be argued that, for a long-term prediction, the model does not consider degeneration during tuber storage where the farmer saves his seed. For conditions in the Peruvian highlands it has been demonstrated, however, that degeneration does not increase significantly by applying the farmers' traditional storage practice (1).

The validation of the assumptions which EPIVIT makes on the stochasticity of some components of the pathosystem and on the spatial spread of contact-transmitted viruses was not part of the study presented. Whether the relevant components responsible for the variability of outputs in the real system have been selected still needs to be validated. The simulation of zero primary infections of plants with five and 10 repetitive model runs restricted the number of plots for which model output could be compared statistically with observed values. These zero outputs, however, are not necessarily wrong. The probabilistic model code does not exclude rare, sporadic events such as an infection under conditions of low probability of infection. All respective plots had a low seed infection (2%) or were situated at very high elevations (4000 m.a.s.l.) (Tables 3.2. and 3.3.), i.e. they faced low probabilities of infection in comparison with others with higher seed infection or situated in zones which are more favourable for virus transmission. EPIVIT's zero outputs may represent trends of reality better than the single observation obtained with one particular experiment. If future extensive model runs will yield at least sporadically a primary infection of plants equal to the observed, additional evidence for a realistic model code would be provided. Further observations on stochasticity and spatial pathogen spread related to EPIVIT's code and possible validation techniques have already been discussed elsewhere (2).

The code for some model components needs to be further verified such as the use of the multiple infection transformation for the simulation of the probability of a healthy plant becoming infected by a contact-transmitted virus. EPIVIT's code can easily be changed once evidence exists for another model for the simulation of this probability.

The value of 2.0 for the parameter of the negative binomial distribution (k_{pi}) which was selected to represent best the real system reflects an almost non-noticeable difference to a random distribution of primarily-infected plants around infectious foci (9). The difference from the Poisson distribution which is commonly used by biologists to simulate random distributions is negligible if k_{pi} becomes greater than 2.0 especially at infection levels ranging from 0 to 50%.

EPIVIT needs to be further validated with truly independent data sets. This first, exploratory validation, however, provides considerable information on the characteristics of the components of the pathosystem studied (such as for example the efficiency of autoinfection) and on the interaction between these components. Furthermore, a validation methodology was developed which will facilitate an efficient model validation in the future. The future application of the model to temperature and aphid data of other

seasons will complete EPIVIT's validation. However, these data need to be generated first.

Model application: The multiplication of improved seed in different agroecological zones of Peru. The wide range covered of model outputs is explained by the way the data have been generated: for a model run over 12 successive seasons the same temperature and aphid data were used. Therefore, the range of outputs presented represents the potential difference in long-term degeneration between the two selected seasons at each site. This is suitable for estimating the relevance of the characteristics (temperature, aphids) associated with a particular season in the respective site for seed degeneration by viruses. More homogeneous outputs may be produced by assigning during one particular model run different input data sets to the particular seasons.

The estimation of the long-term trend of seed degeneration with the validated model leads to the same conclusions that have been made from experimental data obtained in two seasons and different agroecozones (3): the highlands are by far to be preferred for the multiplication of improved seed compared to the coastal production zone because of the significantly lower degeneration rate. The long-term trend of harvest infection displayed in Fig. 3.5. is most probably reproducing what happens since centuries in the Peruvian highlands: the harvest infection never reaches 100% in this zones because of the low primary infection of plants and the low efficiencies of autoinfection. It has been demonstrated (4) that virus incidence in tuber lots which farmers used since years without any source of pathogen-free seed tubers is less than 100%.

Regarding above conclusions it appears to be conditional for the success of a programme for the multiplication of improved seed to exploit the advantages which particular environments offer for such a multiplication, especially in countries where it is difficult to run a complex certification scheme. Not respecting these facts would rapidly lead to high degeneration rates and spoil the investment and effort in producing a nucleus of virus-free seed stocks.

Does the model meet its objectives? It was stated that EPIVIT's code satisfies the objectives of the model development (2) from the conceptual point of view. The validation presented demonstrates the practical relevance of the theoretical assumptions made during the model development. The model is highly explanatory as it shows that harvest infection with the utilised cultivar is composed of the outcomes of several pathogen x host genotype x environment and pathogen x vector x environment interactions related to virus multiplication, translocation and transmission, the host growth and the presence of a vector population in the case of aphid-transmitted viruses. EPIVIT's application to data of distinct agroecozones has proven the model's potential of adaptability to different agroecological conditions.

Conclusion. In conclusion, it is recognised that as a whole the objectives of EPIVIT's development have been meet but that the future will judge whether its goals have been achieved: the development of a tool for forecasting epidemics of the most important viruses of the potato crop in the Andes which can be used and which is suitable for potato programme managers. However, the studies undertaken related to the model development have undoubtedly improved the comprehension of the potato virus pathosystem in the Andes and the model has demonstrated its validity as a research tool.

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CONCLUSIONS AND PROSPECTS

The epidemiological experiments of this study have shown large differences between degeneration rates of seed potatoes caused by the aphid-transmitted potato viruses PVY and PLRV in contrasting agroecozones where potatoes are grown in Peru. Much grater degeneration was determined in zones at sea level compared with zones in the highlands. The differences obtained for the contact-transmitted viruses PVX and APMV were smaller but followed the same trend.

The principal, crop related components of the potato virus pathosystem which condition these differences have been quantified in the respective agroecozone during two seasons: the efficiency of autoinfection, the primary infection of plants, and the tuber infection of primarily-infected plants. The efficiency of autoinfection has been described for the first time with the studied viruses being a mechanism which responds greatly to contrasting agroecological conditions. It has been demonstrated that the variability of this mechanism is of practical relevance for the understanding of the pathosystem and the management of virus disease in the Peruvian highlands.

The biologically significant and explanatory simulation model, EPIVIT, was developed from the common knowledge on potato virus epidemiology and the results of these studies. It estimates the percentage of potato tubers infected with a contact- or an aphid-transmitted virus in the harvest of a potato field (harvest infection) with known seed infection. The result of EPIVIT's first, exploratory validation indicates that, as a whole, the conceptual assumptions which have been made for the model development are correct. The model may be used as a research tool or facilitate decision-making in crop management by predicting the percentage of virus-infected tubers in the harvest of a potato plot (harvest infection). Regarding the model development concluded only recently, EPIVIT has been helpful so far in the first respect. The relevance of the findings of this study and future prospects related to the mentioned possibilities for model application are subsequently presented and discussed.

Pathogen x host genotype x environment interactions have been characterised and specified by developing and validating EPIVIT. The model may be suitable also in the future to further study these interactions. The understanding of qualitative and quantitative relationships within pathosystems is helpful for two reasons: It may contribute to the explanation of observations which have not been understood until today and it may also be beneficial for further crop improvement by helping to adjust screening and selection techniques in plant breeding. This may be exemplified by the following:

- Virus incidence in farmers' fields in the Peruvian highlands has been found to be high (4) but not 100%. It is not understood why this incidence did not reach 100% during the many years which the respective cultivars had been multiplied without a source of virus-free seed, until the respective virus survey was made. A reduced efficiency of autoinfection at high altitudes provides the explanation.

- The farmers in the highlands informally move seed between fields at different altitudes as well as between different zones as a means of maintaining seed quality (6). Such traditional seed tuber management may be related to an empirical comprehension of the farmers of how to influence positively the physiology of the seed tubers. The results of this study related to the efficiency of autoinfection suggest a further possible

reason for the farmers' practice: the percentage of healthy tubers in a tuber lot increases slightly by multiplying the lot at high altitudes. This means that the seed tuber lot as a whole would become healthier and its yield potential increase.

- Yield reductions and symptom expression obtained with secondarily-infected plants differ in diverse agroecozones (2, 4, 7). This can be explained by temperature sensitive pathogenicity and virus multiplication rates. EPIVIT recognises and characterises such temperature-sensitivity.

-Breeders who are trying to develop germplasm with high resistance against plant pathogens often observe considerable variability of the resistance level of the germplasm among years. Postulating a strong genotype x environment interaction may help to understand this phenomenon as long as it cannot be explained by the evolution of new pathogen races (fungi, bacteria) or strains (viruses). If experimental evidence confirms such a hypothesis and allows for its quantitative understanding, screening and selection procedures related to plant breeding might be adjusted. Screening scores, for example, might be linked with the relevant environmental variables to estimate more accurately the resistance level provided by the germplasm.

Some aspects related to EPIVIT's structure need still to be looked at (1). Additional model validation with independent data sets is needed. To test EPIVIT's suitability for application to further environments and different potato cultivars is another area of interest. Regarding the time needed for obtaining the necessary input and validation data for the model, efforts may be undertaken to obtain additional data from other zones simultaneously with further model validation with already existing data.

For this purpose, some minimal requirements related to data structure and evaluation methodology must be respected but complex epidemiological field designs can be avoided: daily minimum and maximum temperatures must be recorded. Trap catches of particular aphid species need to be monitored preferably every two or three days. Secondarily-infected seed tubers need to be planted in contrasting agroecozones and their entire tuber harvest tested for virus infection for the determination of the efficiency of autoinfection (approximately 20 tubers in each site). Healthy tubers need to be planted also at the same time in these experimental sites to allow for a later parameter estimation related to the simulation of the physiological age of the crop.

Furthermore, data on harvest infection and seed infection related to particular plots with the same cultivar in the same sites are required. These data can be obtained, if available, from seed certification programmes which test for the percentage of infected tubers in seed multiplication plots with known seed infections. Another possibility is to make small experiments with at least two plots in each site and a known number of randomly distributed secondarily-infected seed tubers in each plot. The harvest infection in these plots can then be determined by a random tuber sample of approximately 200 tubers/plot.

EPIVIT allowed for a generalisation of the results obtained by the epidemiological experiments of this study and to estimate the long-term trend of harvest infection for an average potato field in a determined agroecological zone in Peru. Such a field receives a crop management which is representative for the respective zone and is planted with seed which is carried forward successive growing seasons in this zone by selecting seed tubers from the harvest of the anterior season.

Will EPIVIT provide help for the estimation of the above-mentioned long-term trend in other counties and other environments where farmers practice the outlined

traditional seed management? Will it be a suitable tool for the demarcation of zones for seed multiplication and will it help to identify distribution strategies for improved seed in such environments?

Besides of EPIVIT's technical requirements which need to be satisfied for a successful model application (input data, parameter estimates) other aspects need to be taken into account in this respect: in the Peruvian highlands a considerable number of potato plants are multiply infected (3). The resistance level of a plant to a virus infection is often reduced if it is already infected with another virus (5). Since EPIVIT does not consider multiple infections it is expected that degeneration in reality is somewhat faster than predicted by the model. In all cases, a programme manager will consider further aspects for policy setting additional to the incidence estimates which EPIVIT provides: data on the potential of yield reduction caused by the virus(es) which are important in the respective agroecozones (4, 7), an economic analysis of seed tuber prices, and information on seed tuber management practised by the farmers' families. Only by applying such a holistic approach, can a realistic estimation be made on the frequency with which a traditional farmer needs to refresh his seed stock with improved seed which he is multiplying further with traditional crop management.

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