

**Temporal and spatial analyses of
Citrus Variegated Chlorosis and Coffee Leaf Scorch,
caused by *Xylella fastidiosa***

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List of Abbreviations

α	alpha
$^{\circ}\text{C}$	degree centigrade
CFU	colony forming units
CLS	Coffee Leaf Scorch
cm	centimeter
CVC	Citrus Variegated Chlorosis
df	degrees of freedom
DI	dispersion index
ha	hectar
l	liter
Lat	latitude
Lon	longitude
μm	micrometer
m	meter
m^2	square meter
MPa	Mega Pascal
n	sample size
p	p-value
R^2	coefficient of determination
S	South
s^2	variance
SD	standard deviation
SE	standard error
SI	severity index
US\$	US-Dollar
W	West

Abstract

Temporal and spatial analyses of Citrus Variegated Chlorosis and Coffee Leaf Scorch, caused by *Xylella fastidiosa*

The temporal and spatial progress of Citrus Variegated Chlorosis (CVC) and Coffee Leaf Scorch (CLS), diseases caused by the bacterium *Xylella fastidiosa*, was evaluated in different locations in Brazil and Argentina over several years by visual assessments of the disease symptoms. In some of the citrus plots, the effect of irrigation and chemical vector control was evaluated.

For CLS in coffee, assessed in three plots in the State of Minas Gerais, Brazil, distinct increases in the incidence of CLS symptoms were observed at the end of spring/ beginning of summer (November/ December). A strong relation between the flushing of the coffee trees and the appearance of symptoms was assumed. Low levels in the incidence of symptoms occurred during the autumn and winter months. The temporal disease progress could not be described satisfactory by generally used epidemiological models. The spatial pattern of CLS diseased coffee plants was aggregated at high disease incidences, indicated by ordinary runs analyses, dispersion index and modified Taylor law.

For CVC in citrus, varying results in the different locations were obtained for the temporal CVC progress. Very high escalations of the disease incidence within a short period of time in plots in the State of Bahia, Brazil and the Province Corrientes, Argentina led to the assumption that a reappearance of symptoms was measured. Drought stress or other diseases were assumed to have caused defoliation of the symptomatic leaves prior to the first evaluations. This assumption is supported by a strong decrease of the incidence of symptomatic plants, which remained on a low level in the second part of the evaluations in the plots in the State of Bahia. The lack of clarity on the actual situation of the diseased plants prohibited conclusions on the spatial disease spread. In two plots, located in the Center and South of the State of São Paulo, Brazil, a very slow disease progression from 0 to 3 % and from 0 to 5 % was measured within three years. For two further plots in the North of the São Paulo State, an almost linear disease progress from 17 to 79 % and 12 to 67 % was observed within the same time period. The spatial pattern of CVC diseased plants in these plots was a loose

aggregation with a tendency to randomness in two of the plots, irrespective of the disease incidence level.

A reduction of the disease progress by chemical vector control was not observed. Irrigation led to a better condition of diseased plants compared to the non-irrigated ones.

Keywords: *Xylella fastidiosa*, Citrus Variegated Chlorosis, Coffee Leaf Scorch, temporal progress, spatial spread

Zusammenfassung

Zeitliche und räumliche Analysen von Citrus Variegated Chlorosis und Coffee Leaf Scorch, verursacht durch *Xylella fastidiosa*

Die zeitliche und räumliche Entwicklung von Citrus Variegated Chlorosis (CVC) und Coffee Leaf Scorch (CLS), Krankheiten verursacht durch das Bakterium *Xylella fastidiosa*, wurde in verschiedenen Orten in Brasilien und Argentinien durch visuelle Erhebungen der Krankheitssymptome evaluiert. In einigen der Zitrusflächen wurde der Effekt von chemischer Vektorkontrolle und Bewässerung festgestellt.

Für CLS an Kaffee, erhoben in drei Flächen im Staat Minas Gerais, Brasilien, wurde ein deutlicher Anstieg im Symptomaufreten am Ende des Frühlings/ Beginn des Sommers (November/ Dezember) festgestellt. Ein enger Zusammenhang zwischen dem Austrieb der Kaffeepflanzen und dem Auftreten der Symptome wurde angenommen. Ein geringes Niveau der Symptome trat in den Herbst- und Wintermonaten auf. Der tatsächliche zeitliche Verlauf der Krankheit konnte durch allgemein gebräuchliche epidemiologische Modelle nicht zufriedenstellend beschrieben werden. Die räumliche Verteilung der von CLS befallenen Kaffeepflanzen war bei hohem Krankheitsauftreten aggregiert, was durch den Runs Test, Dispersionsindex und das modifizierte Taylor Gesetz gezeigt wurde.

Für CVC an Zitrus wurden für die unterschiedlichen Orte variierende Ergebnisse des zeitlichen Verlaufs erzielt. Sehr hohe Anstiege des Krankheitsauftretens innerhalb einer

kurzen Zeit in den Flächen im Staat Bahia, Brasilien, und der Provinz Corrientes, Argentinien, führten zu der Annahme, dass ein Wiederauftreten der Symptome gemessen wurde. Es wurde angenommen, dass Trockenstress oder andere Krankheiten einen Abwurf symptomatischer Blätter vor der ersten Erhebung verursacht hatten. Diese Annahme bestärkt, dass in der zweiten Hälfte der Erhebungen in den Flächen im Staat Bahia das Symptomaufreten drastisch sank und auf einem geringen Niveau blieb. Die Unklarheit über die tatsächliche Situation der kranken Pflanzen untersagte Schlussfolgerungen über die räumliche Krankheitsausbreitung. In zwei Flächen in Zentrum und Süden des Staates São Paulo, Brasilien, wurde eine sehr schwache Krankheitszunahme von 0 auf 3 % and von 0 auf 5 % innerhalb von drei Jahren gemessen. In zwei Flächen im Norden des Staates São Paulo wurde ein nahezu linearer Anstieg von 17 auf 79 % und von 12 auf 67 % innerhalb der gleichen Zeitspanne beobachtet. Die räumliche Verteilung der mit CVC befallenen Pflanzen in diesen Flächen war eine lockere Aggregation mit einer Tendenz zur Zufälligkeit in zwei Flächen, unabhängig von dem Niveau des Krankheitsauftretens.

Eine Reduzierung des Krankheitsfortschritts durch chemische Vektorkontrolle konnte nicht beobachtet werden. Bewässerung führte zu einem besseren Zustand der kranken Pflanzen verglichen mit den nicht bewässerten.

Schlagworte: *Xylella fastidiosa*, Citrus Variegated Chlorosis, Coffee Leaf Scorch, zeitliche Dynamik, räumliche Ausbreitung

1. Introduction

Xylella fastidiosa Wells et al. is a xylem-limited bacterium, which colonises the xylem of affected plants. A citrus disease, caused by this bacterium, was first observed in 1987 in Brazilian citrus orchards (ROSETTI ET AL., 1990). Due to the high economic impact of the disease on the Brazilian citrus industry - losses were estimated to exceed 100 million US\$ per year (LARANJEIRA, 1998) - research activities started quickly. Some epidemiological studies have been carried out (GOTTWALD ET AL., 1993; LARANJEIRA, 1997a; 2002; ROBERTO ET AL., 2002; ANDRADE ET AL., 2003), but clear rules for the spread and recommendations thereupon could not be drawn. Furthermore, studies on the measurements and preconditions to avoid or decelerate the disease spread have been carried out, which led to recommendations for the affected growers (RODAS, 1994; UESUGI & UENO, 1999). However, these measurements did not seem to be effective tools to reduce the disease spread, which was reflected in the surveys of citrus plantations in the State of São Paulo. A permanent increase of the incidence of symptomatic trees from 22 % in 1996 to 44 % in 2004 was evaluated. Furthermore, the disease severity and consequently the damage increased steadily. In 1996, 16 % of the trees showed mild and 6 % severe symptoms; in 2004, this proportion has changed to 8 % with mild and 36 % with severe symptoms (FUNDECITRUS, 2004).

Some years after the discovery of *Xylella fastidiosa* in citrus, the pathogen was also detected in Brazilian coffee plantations (PARADELA FILHO ET AL., 1995; BERETTA ET AL., 1996; DE LIMA ET AL., 1996). Since the symptoms of this disease, called Coffee Leaf Scorch (CLS), are difficult to distinguish from nutritional disorders, water stress or other diseases (DE LIMA ET AL., 1996; BARBOSA ET AL., 2002), reduced yields have not been directly contributed to CLS in most cases, which explains the little attention that has been paid (DE LIMA ET AL., 1998). Recent surveys showed a high incidence of CLS in the Brazilian coffee growing regions, ranging from 50% diseased trees in the south of Minas Gerais to 95% in the Federal District (DE LIMA ET AL., 1996; DE SOUZA ET AL., 2000; BARBOSA ET AL., 2002; CASAGRANDE ET AL., 2002; LEITE JR., 2002;). Only few research studies have dealt with epidemiological aspects of the disease (BARBOSA ET AL., 2004), furthermore, recommendations for measurements are rare.

In the frame of an EU funded project with partners from Argentina, Brazil, Spain and Germany, field data on both diseases in citrus and coffee were assessed with the aim to obtain knowledge on the disease progress in time and space and find effective management tools for the diseases.

2. Literature Review

2.1. The Crops

This chapter deals with the biology and cultivation of citrus and coffee as well as with the economical importance of these crops for Brazil and Argentina, where the evaluations for this thesis have been carried out.

2.1.1. Citrus

The generic term 'citrus' summarises a wide range of plants of the Rutaceae family, subfamily Aurantoidae. The economically most important species is *Citrus sinensis* [L.] Osbeck, the sweet orange, followed by mandarins/ satsumas (*C. reticulata* [Blanco] and *C. unshiu* [Marc.]), lemons and limes (*C. limon* [Burm.f.] and *C. aurantifolia* [L.]) and grapefruits (*C. x paradisi* [M.]) (FAO STATISTICS, 2005). Further less important species are kumquat (*Fortunella* spp.), and shaddock or pummelo (*C. maxima* [L.]). As rootstocks mainly rangpur lime (*C. limonia* [Osb.]), trifoliate citrus (*Poncirus trifoliata* [L.]), sour orange (*C. aurantium* [L.]) and rough lemon (*C. jambhiri* [Lush.]) are used (DAVIES & ALBRIGO, 1994; REICHEL, 2002).

Citrus is grown all over the world in a belt of approximately 40° north-south latitude around the equator. Its origin is believed to be southeastern Asia. Temperature has a high impact on the yield as well as water supply; moreover both factors play a role in the flower induction. In general, citrus plants are propagated through grafting, since seed-grown plants have a very long juvenile period, furthermore rootstocks with advantageous properties can be selected for the respective environment (DAVIES & ALBRIGO, 1994). In Brazil, the majority of all citrus plants is grafted on rangpur lime (*C. limonia* [Osb.]) (REICHEL, 2002), since this rootstock is tolerant to Citrus Tristeza Virus, which killed most of the citrus trees grafted on sour orange in the 1930s in Brazil.

Sweet oranges may be separated into four major groups: the round, the navel, the blood and the acidless oranges. The most important group is the round orange, which is also the most popular orange in Brazil. The most important varieties are 'Hamlin', 'Pera', 'Valência' and 'Natal' (DAVIES & ALBRIGO, 1994).

In 2005, the world production of citrus fruit amounted to 105.43 billion tons. Brazil was the largest producer with 20.1 billion tons, of which 17.8 billion tons were sweet oranges (89%). China was the second largest producer of citrus fruit with 16 billion tons, followed by the USA with 10.3 billion tons. Argentina had a citrus production of 2.7 billion tons, of which 50 % were lemons and 30 % sweet oranges (FAO STATISTICS, 2005;).

In 2004, the harvested area in Brazil amounted to 930,379 ha and has slightly decreased in the last years (USDA, 2004; FAO STATISTICS, 2005).

After coffee, citrus was the economically second most important fruit crop in Brazil with a value of 3.9 billion R\$ (approximately 1.3 billion US\$) in 2002. 84 % of the citrus fruit were produced in the southeast of Brazil including the States of São Paulo, Minas Gerais, Espírito Santo and Rio de Janeiro (IBGE, 2002). In Brazil, 30-40 % of the harvest is brought to the fresh market; the remaining 60-70 % are processed for concentrated juice, which is almost exclusively exported, mainly to the European Union. Solely the sector of juice processing employs about 400,000 people in Brazil (ABECITRUS, 2005).

In Argentina, the harvested area amounted to 145,000 ha in 2005 with an increasing tendency in the last years (FAO STATISTICS, 2005). The main production regions in Argentina are located in the northwest with the Provinces Tucuman, Jujuy and Salta and in the northeast with the Provinces Misiones, Corrientes and Entre Rios. In 2004 approximately 23 % of the Argentinean citrus production was exported, mainly (55 %) to the EU (USDA, 2004).

2.1.2. Coffee

The coffee plant belongs to the family Rubiaceae. Approximately 70 species of the genus *Coffea* exist, mainly found in the center of Africa, which is believed to be the origin of the coffee plant. The two species *Coffea arabica* [L.] ('arabica coffee') and *Coffea canephora* [Pierre ex Froehner] var. *Robusta* (syn. *Coffea robusta*) ('robusta coffee') are the most important ones in the world trade. Approximately 80 % of the world's coffee production is 'arabica coffee'. 'Robusta coffee' has a lower quality due to lack of flavour and acidity, resulting in a one third lower price than 'arabica coffee'. However, the yield and caffeine content of 'robusta coffee' is higher than of 'arabica coffee'. Two other species, *Coffea liberica* [Bull ex Hiern.] and *Coffea exelsa* [Chev.] (syn. *Coffea dewevrei*) are also produced and traded, but only on a very small scale, since the obtained coffee is of inferior quality (WILLSON, 1999; WINTGENS, 2004).

Coffee is grown between the latitudes 22° north and 26° south, but the main species, *C. arabica* and *C. canephora* var. *Robusta*, have different requirements to the environment. 'Robusta coffee' has a higher optimum temperature and requires about one third more precipitation than 'arabica coffee'. 'Arabica coffee' is much more

susceptible to pests and diseases than 'robusta coffee' (WILLSON, 1999; DESCROIX & SNOECK, 2004).

In general, 'arabica coffee' is propagated through seeds, which is the easiest and cheapest method of propagation. Due to its self-sterility, 'robusta coffee' has to be propagated vegetatively. Only in regions with nematode problems, both coffee types have to be grafted on tolerant rootstocks (WILLSON, 1999; WINTGENS & ZAMARIPPA, 2004). To ensure a maximum harvest and to shape the coffee trees they are stumped every 4th to 6th year by cutting the tree at a height of approximately 50 cm (WILLSON, 1999).

From the two main varieties of *Coffea arabica* introduced to Latin America, 'Bourbon' and 'Typica', the nowadays most popular varieties in Brazil have been bred: 'Caturra' (a mutant of 'Bourbon'), 'Mundo Novo' (a cross between 'Bourbon' and 'Typica') and 'Catuaí' (a hybrid of 'Mundo Novo' and 'Caturra'), characterized by either yellow ('Catuaí Amarelo') or red berries ('Catuaí Vermelho') (BARISTAGURU, 2005).

In 2005, the world production of green coffee amounted to 7.7 billion tons, Brazil dominated with 2.18 billion tons, followed by Vietnam (1 billion tons) and Indonesia (0.76 billion tons). The harvested area in Brazil covered 2.3 million ha in 2005, with an increasing tendency in the last years (FAO STATISTICS, 2005). Coffee is by far the most important fruit crop in Brazil with a value of 4.6 billion R\$ (approximately 1.5 billion US\$) in 2002, of which 81% was produced in the Southeast including the States São Paulo, Minas Gerais, Espírito Santo and Rio de Janeiro (IBGE, 2002).

2.2. The diseases

This chapter describes the diseases caused by *Xylella fastidiosa* in citrus and coffee, followed by an overview of other *Xylella* diseases.

2.2.1. Citrus Variegated Chlorosis (CVC)

In 1987, ROSETTI ET AL. (1990) detected citrus fruits in orchards located in the States of São Paulo and Minas Gerais, Brazil, with a certain type of unknown lesions on the leaves. The disease was named Citrus Variegated Chlorosis, or 'clorose variegada dos citros' (CVC) in Portuguese; synonymously the name 'amarelinho' is used. This disease was also known before in Argentina as 'pecosita' (BRLANSKY ET AL., 1991). In 1989, bacterial cells inside the xylem of affected plants were found and associated with the disease (ROSETTI ET AL., 1990). The detected cells were similar to the bacterium *Xylella fastidiosa*, recently before described by WELLS ET AL. (1987) as the causal agent of numerous plant diseases. Koch's Postulates for CVC were fulfilled in 1993 and 1994 independently by two research groups (CHANG ET AL., 1993; HARTUNG ET AL., 1994).

CVC is now distributed in all citrus growing regions in Brazil (MIZUBUTI ET AL., 1994; SANTOS FILHO ET AL., 1999; ANDRADE ET AL., 2003; LARANJEIRA ET AL., 2003; POLTRONIERI ET AL., 2003; ZANUTTO ET AL., 2004). Besides Argentina (BRLANSKY ET AL., 1991), the occurrence of the disease was also reported from Paraguay (SEGNANA ET AL., 1998) and Costa Rica (AGUILAR ET AL., 2005).

Affected citrus trees show an interveinal chlorosis on younger leaves, which resembles



Fig. 1: Citrus plant with typical CVC symptoms.

zinc deficiency symptoms. In more developed leaves, the chlorotic spots on the upper leaf surface correspond to brown pustules on the lower side of the leaves. Heavily affected leaves are dropping prematurely and foliar analyses indicate zinc and potassium deficiency (ROSETTI, 2001). Leaves with severe symptoms are found more frequently at the parts close to the main stem of the branch and the distal parts exhibit milder symptoms (ALVES ET AL., 2004). Beside the leaf symptoms, shortened internodes and flowering at abnormal times occur and, in a

more advanced stage of the disease, very small, less juicy fruits with a hard peel are formed. Young affected trees show reduced growth, appear yellowish and show leaf symptoms in the whole plant, while in older trees the symptoms are mainly restricted to some branches of the tree, but can spread through the whole tree canopy after some time. Under normal circumstances the disease is not lethal, but the trees become unproductive: AYRES ET AL. (2000) estimated the yield losses of severely CVC affected trees to be 67 to 84 %. Moreover, the affected fruits have a more acid taste and a very hard peel, which may damage the processing machines. Thus, they are neither marketable for the fresh market nor for juice processing (FONTANEZZI HUANG & CHIARADIA, 1998; BERETTA & LEITE, 2000; ROSETTI, 2001).

Surveys in the citrus growing regions of the State of São Paulo and the region Triângulo Mineiro in Minas Gerais, Brazil, showed an increase of the CVC incidence from 22 % in 1996 to 44 % in 2004. Furthermore, the severity of the disease rose. In 1996, 6 % of the trees showed severe and 16 % mild symptoms, while in 2004 36 % had severe and 8 % mild symptoms (FUNDECITRUS, 2004). For the four most important varieties, AYRES ET AL. (2000) assessed an incidence between 42 % for 'Pera' and 26 % for 'Hamlin'. The results for 'Natal' (39 %) and 'Valência' (36 %) were between these values. In the State of São Paulo, the geographical distribution of the disease intensity was uneven. The southern and western regions had a relatively low disease level with 14 % and 15 %, respectively, whereas in the central, northern and northwestern regions the levels were much higher with 55 %, 61 % and 50 %, respectively.

2.2.2. Coffee Leaf Scorch (CLS)

In 1995, the presence of *Xylella fastidiosa* in 'arabica coffee' plants in the State of São Paulo, Brazil, was confirmed (PARADELA FILHO ET AL., 1995; BERETTA ET AL., 1996) and Koch's Postulates were fulfilled 3 years later (DE LIMA ET AL., 1998). Soon after the detection of the disease, called Coffee Leaf Scorch (CLS), in Portuguese "Requeima do Cafeeiro" or "Atrofia de Ramos de Café", the disease was reported from other coffee growing areas in the State of São Paulo and Minas Gerais (DE LIMA ET AL., 1996) and later from the States of Paraná, Bahia, Rio de Janeiro, Espírito Santo, Goiás and Federal District (CARVALHO ET AL., 2001a; 2001b; BARBOSA ET AL., 2002; CASAGRANDE ET AL., 2002; LEITE JR., 2002). Beside the occurrence of CLS in the Brazilian coffee growing regions, the disease was also reported from Costa Rica (RODRIGUEZ ET AL., 2001). Considering the high degree of dissemination soon after the detection of the

disease, PARADELA FILHO ET AL. (1997) assumed that the disease was already existent, but not recognized a long time before it was detected and the symptoms were attributed to other factors due to the similarity to nutritional disorders, water stress and symptoms of other diseases (DE LIMA ET AL., 1996; BARBOSA ET AL., 2002).

The symptoms are described as clusters of small and sometimes deformed leaves at the tip of the branches, showing an interveinal chlorosis similar to zinc deficiency symptoms. Older leaves of a twig drop prematurely and only the most distal younger leaves remain. The twig appears as a tuft of small, chlorotic leaves with shortened internodes, in some cases also these remaining leaves drop, resulting in a dead twig. Affected branches show a higher number of shoots, sometimes leading to the appearance of a 'witchbroom'. In some regions, leaves with scorched borders could be observed. Affected branches bear smaller coffee berries, leading to a reduced yield (PARADELA FILHO ET AL., 1995; DE LIMA ET AL., 1996).

Surveys showed a high incidence of CLS in the Brazilian coffee growing regions. In the South of Minas Gerais, 50% and in the North of Paraná, 80 % of the trees harboured the bacterium, with a higher incidence in older trees of an age



Fig. 2: Normal coffee twig (below) and twigs with typical CLS symptoms (above).

between 16 and 20 years than in younger ones. The highest incidences were found in the State of Bahia with 90 % infected trees and in the Federal District with 95% (DE LIMA ET AL., 1996; DE SOUZA ET AL., 2000; TAKATSU ET AL., 2001; BARBOSA ET AL., 2002; CASAGRANDE ET AL., 2002; LEITE JR., 2002).

The presence of CLS was confirmed for several 'arabica coffee' varieties, e.g. 'Mundo Novo', 'Catuaí Amarelo', 'Catuaí Vermelho', 'Caturra' and 'Bourbon' (CARVALHO ET AL., 2000), as well as for other coffee species like *C. canephora* var. Robusta, *C. racemosa*, *C. exelsa* and hybrids of these species (YORINORI ET AL., 2000).

2.2.3. Other Xylella diseases

Apart from a report of a pear disease in Taiwan (LEU & SU, 1993) and the detection of Pierce's Disease of grapevines in Yugoslavia (CHEN ET AL., 1996), *Xylella* diseases are restricted to North and South America (PURCELL, 1997) and are furthermore mainly occurring in tropical and subtropical climates (HOPKINS & PURCELL, 2002).

The most prominent symptom of *Xylella* diseases is leaf scorching that occurs in plum, almond, oleander, elm, maple, mulberry, oak, pear, pecan, sycamore and many more plants. Usually scorching begins at the leaf margin and progresses inward, adjacent tissue turns to yellow or red. Further symptoms can include a shortening of the internodes, e.g as in alfalfa, coffee, citrus and peach, and a delayed growth (RAJU & WELLS, 1986). Symptoms of Phony Peach Disease, also caused by *X. fastidiosa*, are different from most other *Xylella* disease symptoms. Beside shortened internodes and a reduced fruit size, the leaves are flattened, dark-green and exhibit a delayed senescence. Affected trees bloom earlier than healthy ones (HOPKINS, 1989).

Tab. 1: Economically important *Xylella* diseases and their occurrence

Disease	Occurrence	References
Almond Leaf Scorch	Argentina, USA	NOMÉ ET AL., 1992; DAVIS ET AL., 1980
Citrus Variegated Chlorosis	Brazil, Argentina, Paraguay, Costa Rica	ROSETTI ET AL., 1990; BRLANSKY ET AL., 1991; SEGNANA ET AL., 1998; AGUILAR ET AL., 2005
Coffee Leaf Scorch	Brazil, Costa Rica	PARADELA ET AL., 1995; BERETTA ET AL., 1996; RODRIGUEZ ET AL., 2001
Elm Leaf Scorch	USA	OLSON ET AL., 2006
Maple Leaf Scald	USA	SHERALD ET AL., 1987
Mulberry Leaf Scorch	USA	KOSTKA ET AL., 1986
Oak Leaf Scorch	USA	BARNARD ET AL., 1998; MCGOVERN 1994; HUANG ET AL., 2004; HARTMAN ET AL., 1991
Oleander Leaf Scorch	USA	GREBUS ET AL., 1996; HUANG ET AL., 2004; WICHMANN ET AL., 2000
Pear Leaf Scorch	Taiwan	LEU & SU, 1993
Phony Peach Disease	USA	WELLS ET AL., 1980
Pecan Leaf Scorch	USA	SANDERLIN, 1998
Pierce's Disease of grapevine	USA, Mexico, Yugoslavia	RAJU ET AL., 1980; CHEN ET AL., 1996
Plum Leaf Scald	Argentina, USA	KITAJIMA ET AL., 1975
Stunting of Sugar Maple and Sweetgum	USA	HARTMAN ET AL., 1996
Sycamore Leaf Scorch	USA	SHERALD ET AL., 1983

Tab. 2: Systemic hosts of *Xylella fastidiosa*

Host plant	Latin name	References
shrubs		
Acacia	<i>Acacia plumosa</i>	HARAKAVA ET AL., 1994
Porcelain berry	<i>Catharanthus roseus</i>	HUANG & SHERALD, 2004 UENO ET AL., 1998; TEXEIRA ET AL., 1996
Elderberry	<i>Sambucus canadensis</i>	COSTA ET AL., 2004
American beautyberry	<i>Callicarpa americana</i>	COSTA ET AL., 2004
Peppervine	<i>Amelopsis arborea</i>	COSTA ET AL., 2004
Himalaya blackberry	<i>Rubus procerus</i>	RAJU ET AL., 1983
Periwinkle	<i>Vinca minor</i>	RAJU ET AL., 1983
Poison Hemlock	<i>Conium maculatum</i>	RAJU ET AL., 1980
Beech Bonsai	<i>Fagus</i> sp.	HUANG ET AL., 2003
weeds		
Alfalfa	<i>Medicago sativa</i>	GOHEEN ET AL., 1973
Amaranth	<i>Amaranthus</i> sp.	HARAKAVA ET AL., 1994, LUDOVICIO ET AL., 2004
	<i>Bidens pilosa</i>	LUDOVICIO ET AL., 2004
	<i>Bracharia decumbans</i>	LUDOVICIO ET AL., 2004
	<i>Chloris</i> sp.	HARAKAVA ET AL., 1994
	<i>Commelina benghalensis</i>	TRAVENSOLA & LEITE, 1996; LOPES ET AL., 1999
	<i>Cynodon dactylon</i>	TRAVENSOLA & LEITE, 1996
	<i>Eleusine indica</i>	TRAVENSOLA & LEITE, 1996
	<i>Emilia sanchifolia</i>	LUDOVICIO ET AL., 2004
	<i>Lepidium</i> sp.	HARAKAVA ET AL., 1994
Lupine	<i>Lupinus aridorum</i>	COSTA ET AL., 2004
Virginia Creeper	<i>Parthenocissus quinquefolia</i>	MCELRONE ET AL., 2001
Wild Strawberry	<i>Fragaria californica</i>	RAJU ET AL., 1983
Miner's Lettuce	<i>Montia linearis</i>	RAJU ET AL., 1983
	<i>Richardia brasiliensis</i>	TRAVENSOLA & LEITE, 1996, LUDOVICIO ET AL., 2004
	<i>Rynchelitrum repens</i>	TRAVENSOLA & LEITE, 1996
	<i>Sida</i> sp.	TRAVENSOLA & LEITE, 1996; HARAKAVA ET AL., 1994
Solanaceous herb	<i>Solanum</i> sp.	TRAVENSOLA & LEITE, 1996, LUDOVICIO ET AL., 2004
Dandelion	<i>Taraxacum officinale</i>	LUDOVICIO ET AL., 2004, LEITE ET AL., 1997
	<i>Veronica</i> sp.	TRAVENSOLA & LEITE, 1996
grasses		
Dallis grass	<i>Paspalum dilatatum</i>	RAJU ET AL., 1980
Johnson Grass	<i>Sorghum halepense</i>	WELLS ET AL., 1980
Sickle pod	<i>Cassia tora</i>	WELLS ET AL., 1980
Watergrass	<i>Echinochloa crus-galli</i>	LOPES ET AL., 1999
Umbrella Sedge	<i>Cyperus eragrostis</i>	RAJU ET AL., 1980

Besides the economically important *Xylella* diseases (Tab. 1), many hosts are known to harbour *Xylella* populations. Infections are classified in systemic, in most cases with symptom production, and non-systemic or symptomless infections. The list of alternative hosts grows constantly with the availability of more precise molecular techniques and with the awareness of the importance of such unrecognised sources of *X. fastidiosa*. The systemic hosts (Tab. 2) pose a greater threat as infection sources than non-systemic hosts (PURCELL & HOPKINS, 1996).

2.3. The bacterium

This chapter provides background information of the history of the bacterium, requirements for growth, infection mechanisms and pathogenicity factors as well as the effects of the pathogen on the host plant.

Xylella fastidiosa [Wells et al.] is a gram-negative, rod-shaped bacterium with a rippled cell wall and a size of 0.3 – 0.5 µm in diameter and 1 - 3.5 µm in length (GOHEEN ET AL., 1973; CHAGAS ET AL., 1992). It was first discovered in 1973 as the causal agent of Pierce's Disease of grapevine and Alfalfa Dwarf (GOHEEN ET AL., 1973), and shortly thereafter of Phony Peach Disease (HOPKINS ET AL., 1973b). Due to certain similarities, the bacterium was first assigned to the family Rickettsiaceae (GOHEEN ET AL., 1973), however, twelve years later these similarities were found to be coincidental (KAMPER ET AL., 1985; WELLS ET AL., 1987), and from 1987 on *Xylella fastidiosa* was referred to as a 'xylem-limited bacterium' (XLB) (WELLS ET AL., 1987). *X. fastidiosa* was the first plant pathogen of which the genome was fully sequenced (SIMPSON ET AL., 2000).

The first successful cultivation of *X. fastidiosa* outside the plant was carried out by DAVIS ET AL. (1978). As its name implies, the bacterium is very fastidious, furthermore the growth is very slow, which complicates the cultivation outside the plant. For *X. fastidiosa* in grapevine, an optimum growth temperature of 28°C was determined and no growth was observed below 12°C (FEIL & PURCELL, 2001). Several media compositions were proposed by different authors, for instance Periwinkle Wilt medium and BCYE agar (UCHIBABA ET AL., 1992). CHANG and DONALDSON (2000) found the inorganic salts K₂HPO₄ and MgSO₄ x 7H₂O to be essential for the culture of the Pierce's Disease strain on artificial medium. Furthermore, growth was supported by amino acids with 6 uncharged polar R groups (asparagine, cysteine, glutamine, glycine, serine and threonine), and by citrate, alpha-ketoglutarate, succinate, fumarate, malate and oxalacetate.

Inside some plant species, the bacteria mainly colonise the thick-walled tracheids of the xylem (HOPKINS ET AL., 1973b; KITAJIMA ET AL., 1975), occasionally they are found in the intercellular spaces of the xylem (GOHEEN ET AL., 1973). In citrus and grapevine, the bacteria are evenly distributed in the lumen of the tracheary elements (MOLLENHAUER & HOPKINS, 1974; CHAGAS ET AL., 1992). Inside the plant, the xylem sap flow provides the bacteria with a wide range of nutrients, although the concentration of solids is relatively low (1-20 mg/ ml) compared to the phloem sap (50-300 mg/ ml). The concentration and

the composition of the xylem sap vary with plant species, location within the plant, time of the day, plant age, seasonal cycle, plant nutritional stage and health status of the plant (PATE, 1976).

X. fastidiosa is capable of degrading the pit membrane of the xylem vessels, what is of crucial importance for the movement of the bacteria within the plant (SIMPSON ET AL., 2000). The bacterium moves passively with the flow of the xylem to the upper parts of the plants, but many scientists also reported the movement of the bacterium in the opposite direction, although *X. fastidiosa* has no flagellum and is consequently not able to move actively (HILL & PURCELL, 1995b; HE ET AL., 2000; ALMEIDA ET AL., 2001). In sweet orange flushes, HE ET AL. (2000) observed a movement in the direction of the xylem sap flow of 1 cm/ day under greenhouse conditions and 3 cm/ day under field conditions. The same rate for the movement opposite to the xylem flow was assumed, which was confirmed by ALMEIDA ET AL. (2001) for citrus seedlings in greenhouse experiments: one week after inoculation, *X. fastidiosa* was detected 4 cm above and below the inoculation point. For the systemic movement of the pathogen, a high bacterial population was not required. In grapevine, more susceptible cultivars allowed a faster movement of the bacteria (HILL & PURCELL, 1995b). OLIVEIRA ET AL. (2002) confirmed this finding also for citrus.

In citrus, other endophytic bacteria seem to have an influence on the xylem colonisation with *X. fastidiosa* and the symptom expression of the host. In liquid culture, growth of *X. fastidiosa* appeared to be stimulated by *Methylobacterium extorquens*, a common endophytic bacterium colonising citrus vessels. On the other hand, *M. mesophilicum* and *Curtobacterium flaccumfaciens* inhibited *X. fastidiosa* growth in vitro (LACAVA ET AL., 2004). The latter bacterium could be isolated more frequently from asymptomatic CVC infected citrus plants than from those showing symptoms (ARAUJO ET AL., 2002).

The bacterium's ability to biofilm formation is one important pathogenicity factor (SIMPSON ET AL., 2000; DA SILVA ET AL., 2001; MARQUES ET AL., 2002; MIYASAWA ET AL., 2003; OSIRO ET AL., 2004; DE SOUZA ET AL., 2004). Different strains and microenvironmental conditions produce varying biofilm morphologies (MARQUES ET AL., 2002). These biofilms are composed of the bacteria, embedded in a translucent matrix, which mainly consists of self-produced extracellular polysaccharides; fimbria-like structures enhance the attachment between the bacteria, to the plant's xylem surface or to the inner foregut of the vectoring insects (CHAGAS ET AL., 1992; SIMPSON ET AL., 2000; MARQUES ET AL., 2002). Serial subculturing of the bacteria sometimes leads to the loss

of the biofilm formation ability and the virulence (HOPKINS, 1989). This biofilm formation may also be an important factor for the induction of disease symptoms, since clumps of aggregated bacteria are suspected to occlude the xylem vessels and cause water stress to the plant (MARQUES ET AL., 2002). On the other hand, FRY and MILHOLLAND (1990) suggested that the occlusion of xylem vessels in grapevine is largely due to pectins produced by the plant as an effort to restrict the bacteria in their movement.

The amount of occluded xylem vessels greatly depends on the host plant and the symptom severity. In symptomatic citrus plants, 5 to 10 % of the vessels showed colonisation by *X. fastidiosa* (MIZUBUTI ET AL., 1994). In symptomatic coffee plants, QUEIROZ-VOLTAN ET AL. (2003) found 5.5 % occluded xylem vessels. ALVES ET AL. (2004) differentiated between severe and mild symptoms of affected leaves and found the percentage of plugged xylem vessels in citrus to be 12 and 8 %, respectively. The same authors detected a xylem occlusion of 52 % in coffee leaves with severe symptoms and 26 % in leaves with mild symptoms. In almond, this relation ranged from 10 to 15 % (MIRCETICH ET AL., 1976) and in plum from 11 to 38 % (ALVES ET AL., 2004). In grapevine, up to 18% occluded vessels were found (HOPKINS & THOMPSON, 1984). In symptomatic citrus leaves, ALMEIDA ET AL. (2001) observed 10^2 to 10^3 times less bacteria than in grapevine, but similar amounts as in symptomless hosts of the Pierce's Disease bacterium, like blackberry, french broom and elderberry. Furthermore, ALVES ET AL. (2002; 2004) could not detect a connection between the bacterial population and the percentage of plugged xylem vessels as well as the symptom severity in citrus, whereas the symptom severity in coffee and plum was well related to the amount of plugged xylem vessels. Although the amount of occluded xylem vessels in citrus is relatively low, OLIVEIRA ET AL. (2000) found a reduction of 56 % in daily sap flow in infected, non-symptomatic sweet orange plants.

Another factor in the induction of symptoms is the toxin production of the bacterium, which was hypothesised because of the partly low number of occluded vessels in symptomatic plants and a lack of visible wilting symptoms (MIRCETICH ET AL., 1976; ANDERSEN & FRENCH, 1987; GOODWIN ET AL., 1988; RIBEIRO ET AL., 2003). SIMPSON ET AL. (2000) found several genes responsible for toxin production, but it is still unknown under which circumstances and to which amount toxins are produced and, moreover, to which extent they contribute to the symptom appearance.

One hypothesis explaining the presence of symptoms despite the low number of occluded vessels is the growth regulator imbalance theory. For Phony Peach Disease,

FRENCH AND STASSI (1978) assumed an influence of a *Xylella* infection on the endogenous level of plant growth regulators, especially gibberellic acid. Applications of this plant hormone partly reversed typical disease symptoms.

RIBEIRO ET AL. (2004) found a negative influence of a *X. fastidiosa* infection on the photosynthetic activity of citrus plants: CO₂ assimilation rates were reduced in infected citrus plants; the difference between healthy and infected plants grew with increasing growth and leaf temperatures. The authors suggested that this effect may be partly due to the water restriction caused by xylem blockage leading to stomatal closure, but additionally toxins may be involved. MC ELRONE ET AL. (2004) could show that water stress and bacterial infection both reduced photosynthate production of *Parthenocissus quinquefolia*. Moreover, the authors assumed that water stress increases the severity of the symptoms and the rate of spread of the pathogen, since the carbon gain capacity was reduced by water stress and hence the ability of the plant to outgrow the pathogen and produce effective defenses. When exposed to water stress, diseased citrus plants showed a lower stomatal conductance and higher abscisic acid contents than healthy ones (GOMES ET AL., 2003).

The described strains of the genus *Xylella* are summarised in one species *Xylella fastidiosa*. According to the bacterium's wide distribution in the new world and the high number of host plants, many different strains were found. Scientists are trying to reveal relationships between the strains from different host plants and to group them according to the degree of similarities in the genome by molecular techniques (WICHMANN ET AL., 2002). Another method to detect relationships are reciprocal transmission tests, which evaluate whether strains isolated from one host plant induce symptoms in other known host plants of *X. fastidiosa*.

The genomes of the citrus and coffee strains are closely related (DE LIMA ET AL., 1998; ROSATO ET AL., 1998; COSTA ET AL., 2000b; MEHTA ET AL., 2000) and moreover, citrus strains can induce symptoms in coffee (LI ET AL. 2001). PARADELA FILHO ET AL. (1995) assumed that *Xylella fastidiosa* may have disseminated from coffee to citrus, since the region where CVC first appeared was previously cultivated with coffee, where the remaining coffee plants showed a very high incidence of CLS. However, in cross inoculation tests with one *Xylella* strain from coffee, PRADO (2003) failed to get infections of citrus plants. Beside molecular tests, symptom expression of tobacco plants are used to distinguish the strains infecting coffee and citrus (LOPES ET AL., 2003a; 2003b).

Strains from citrus and coffee could also infect grapevine, although the symptoms were milder than the ones evoked by original grape strains (LI ET AL., 2002). One identical strain induced symptoms in almond and grapevine (MIRCETICH ET AL., 1976). Approaches to group the strains according to their genetical relationships are controversial. QIN ET AL. (2001) considered coffee and citrus strains to belong to one natural group, another one was formed by grape, almond and ragweed strains, and a third by elm, oak and plum strains. On the other hand, WICHMANN ET AL. (2002) classified citrus and coffee to the 'Pierce's Disease group'. Reciprocal transmission between elm and sycamore failed, although the trees commonly occur close together in North America (SHERALD, 1993).

The existence of different sweet orange varieties did not play a role in the population structure of *X. fastidiosa* (COLETTA FILHO & MACHADO, 2002). However, the strains were related to the region where they have been isolated: strains from the southern parts of Brazil could be distinguished from the ones isolated from the States of São Paulo and Sergipe (MEHTA ET AL., 2000). A CVC strain maintained the virulence after passing through *Cataranthus roseus* plants (TEIXEIRA ET AL., 2001).

As a general rule, symptomatic plants harbour more bacteria than asymptomatic, but infected ones (WELLS ET AL., 1980; ALDRICH ET AL., 1992; LACAVA ET AL., 2004). Inside the plant, the distribution of the bacteria seems to depend on the host and/ or the strain. In roots of affected peach, the populations of *X. fastidiosa* were much higher than in the aerial plant parts, although symptoms were expressed in the canopy. From most of the other hosts it is known that the bacterial titer is highest in the upper plant parts (WELLS ET AL., 1980; HOPKINS ET AL., 1991; HE ET AL., 2000). In citrus, the distribution of the bacteria within the plant is thought to be irregularly (CHANG ET AL., 1993). *X. fastidiosa* is translocated to the roots of infected citrus depending on the rootstock, which may differ in susceptibility to the bacterium. In some rootstocks, the bacterium could not be detected by molecular methods. However, a translocation of the bacteria to the roots is not essential for the symptom development of the upper part (HE ET AL., 2000). In non-grafted coffee plants, the bacteria can be easily detected in the twigs, leaves and roots of infected trees (DE LIMA ET AL., 1996)

Apart from the host and the strain, the bacterial titer seems to depend on the season and the meteorological conditions. In North American peach, a higher amount of bacteria could be observed during the spring quarter, the lowest levels were obtained during the autumn and winter quarters (WELLS ET AL., 1980). In Brazilian citrus, no clear

seasonal pattern in the bacterial titer could be found (PEREIRA, 2000; LARANJEIRA, 2002). Within coffee plants, PARADELA FILHO ET AL. (1997) observed the lowest bacterial concentrations during the late summer months (January to March), the time with the highest precipitation in this region of Brazil.

Usually grapevine develops symptoms of Pierce's Disease at the end of the growing season when the plants are senescing. The hormonal balance, in particular abscisic acid changes, enhances symptom development (HOPKINS, 1985). MC ELRONE & FORSETH (2004) assumed that an infection with *X. fastidiosa* leads to an accelerated senescence of the plant.

2.4. The transmission

For analyses of the epidemiology of a disease, the knowledge of the transmission pathways is of crucial importance. This chapter deals with the different pathways of plant-to-plant transmission of *Xylella fastidiosa* diseases.

Like all systemic bacterial plant pathogens, *Xylella fastidiosa* can be transmitted through graftings of an infected rootstock with a healthy scion and vice versa (DE LIMA ET AL., 1996). Additionally, a transmission through natural root grafts, formed by the plant, is possible. In greenhouse experiments with young citrus plants, cultivated in the same pot for two years, a transmission rate of 31 % was observed (HE ET AL., 2000).

The bacterium could be found in germplasms from infected coffee and citrus plants (YORINORI ET AL., 2003) and for citrus the transmission of the pathogen from seeds to seedlings has been proven (LI ET AL., 2003).

The main way of plant-to-plant spread of *Xylella fastidiosa* inside the field is the transmission by xylem-sucking insect vectors, like sharpshooter leafhoppers (Homoptera, Cicadellidae, subfamily Cicadellinae, tribes Cicadellini and Proconiini) and spittlebugs (Cercopidae).

For citrus, the sharpshooters *Dilobopterus costalimai* [Young], *Agroconia terminalis* [Young] and *Oncometopia facialis* [Signoret] (Homoptera, Cicadellidae, subfamily Cicadellinae) were the first identified vectors of *Xylella fastidiosa* (LOPES ET AL., 1996; ROBERTO ET AL., 1996). Further identified vectors of the same subfamily are the species *Pleisommata corniculata* [Young], *Bucephalogonia xanthopis* [Berg], *Sonesimia grossa* [Signoret], *Homalodisca ignorata* [Melichar], *Ferrariana trivittata* [Signoret], *Macugonalia leucomelas* [Walker], *Parathona gratiosa* [Blanchard], and *Agroconia virescens* [Metcalf] (ROBERTO ET AL., 1996; YAMAMOTO ET AL., 2002). BRLANSKY ET AL. (2002) found *Oncometopia nigricans* [Walker], a sharpshooter frequently occurring in Californian citrus groves, to be capable of transmitting the CVC strain, however, up to now the disease has not been reported from the USA.

From sharpshooters (Homoptera: Cicadellidae, subfamily Cicadellinae) caught in Brazilian coffee plantations, the species *Agroconia citrina* [Marucci & Cavichioli], *Bucephalogonia xanthopis* [Berg], *Dilobopterus costalimai* [Young], *Macugonalia cavifrons* [Stal], *Molomea consolidata* [Schröder] and *Scopogonalia subolivaceae* [Stal] were proven to carry the bacterium (PAIÃO ET AL., 2002a; 2002b, 2003a; 2003c).

However, MARUCCI ET AL. (2001) could prove a *X. fastidiosa* transmission only for *D. costalimai*; the sharpshooters *B. xanthopis*, *H. ignorata* and *O. facialis* were not successful in transmitting the pathogen. The insects *Dorisiana virides* [Olivier], *D. drewseni* [Stal], *Carineta matura* [Distant] and *Carineta* sp. (Homoptera: Cicadidae) also harboured populations of the bacterium (PAIÃO ET AL., 2003b).

In Brazil, two spittlebug species captured in plum orchards, *Deois schach* [F.] and *D. flavopicta* [Stal] were found to carry *Xylella fastidiosa* (HICKEL ET AL., 2001), however, for citrus and coffee, no CVC or CLS transmission by spittlebugs was proven up to now. Generally, these insects are considered as less important for *X. fastidiosa* transmission (REDAK ET AL., 2004).

The sharpshooters of the subfamily Cicadellinae have five nymphal stages. At $25\pm 2^\circ\text{C}$ the mean duration of the nymphal stages on citrus plants of *Dilobopterus costalimai*, *Oncometopia facialis* and *Homalodisca ignorata* was around 60, 71 and 53 days, respectively (ALMEIDA & LOPES, 1999; PAIVA ET AL., 2001). The longevities of adult *D. costalimai* and *O. facialis* were 36 and 16 days, respectively (PAIVA ET AL., 2001).

Xylella fastidiosa is transmitted through a noncirculative mechanism, since no measurable latent period exists and infectivity is lost after molting (PURCELL & FINLEY, 1979). The bacteria are located in the cibarium, the apodemal groove of the diaphragm and the precibarium of the sharpshooters; these parts are removed when the insect is molting (BRLANSKY ET AL., 1983). Sharpshooters can acquire and transmit the bacteria in less than 2 h and infected adults retain the ability to transmit *Xylella* their whole life (PURCELL & FINLEY, 1979). There is no vertical transmission to the progenies, but nymphs can also acquire the bacterium by sucking on diseased plants and transmit with the same efficiency as adult insects (RAJU & WELLS, 1986).

The postinoculation incubation period, i.e. the time needed before a vector can acquire the pathogen for further transmission after the inoculation of a plant, varies from plant to plant. In greenhouse experiments, this period was four days in grapevine, 22 days in blackberry, 29 days in mugwort and 25 days in watergrass (HILL & PURCELL, 1997). Inside the plant, the bacteria have to reach a threshold population before they can be sufficiently acquired and transmitted by the insects. HILL and PURCELL (1997) found 4 CFU/ g to be the minimum bacterial population required for transmission in grapevine, which was also close to the threshold population for detection by cultivation. With a population of 6 CFU/ g the transmission efficiency was 40 %.

On the other hand, HILL and PURCELL (1995a) showed that the transmission efficiency of the blue green sharpshooter (*Graphocephala acropunctata*), an insect transmitting Pierce's Disease, was not related to the population size of the bacteria inside the insects' head and moreover, high bacterial populations did not ensure transmission.

Different sharpshooter species show different efficiencies in transmitting the bacterium, also depending on the plant. A recently to Californian vine growing regions introduced insect, the glassy winged sharpshooter (*Homalodisca coagulata*), has a transmission efficiency for Pierce's Disease of 15-20 %, whereas the same insect transmits *X. fastidiosa* to almonds with only 2 % efficiency. The blue green sharpshooter transmits Pierce's Disease of grapevines with an efficiency up to 90 % (PURCELL & SAUNDERS, 1999) and Oleander Leaf Scorch with up to 83 % (COSTA ET AL., 2000a).

In citrus, the transmission efficiency of the known vectors ranges from 1.1 % (*O. facialis*) to 30 % (*H. ignorata*). In coffee, transmission rates of 1.2 to 7.2 % were observed for four of the known species. The vectors had almost the same transmission efficiency for both plants, apart from *H. ignorata*, which transmitted *X. fastidiosa* to coffee with only 2.2 % efficiency, whereas it was much higher for citrus (30 %) (MARUCCI ET AL., 2003). The reason for a relatively low transmission efficiency in citrus and coffee is unknown; possible explanations are the low acquisition and/ or inoculation efficiency of the vectors, or alternatively, a low persistence rate of the infections after inoculation. Another factor may be the low concentration of bacteria inside the plants, which limits the acquisition of the bacteria by the insects (LOPES, 1999).

The abundance of the sharpshooters inside the citrus or coffee groves depends on several aspects. The species *D. costalimai* and *O. facialis* are occurring accidentally in citrus groves; in most cases, adult insects immigrate from surrounding vegetation to the crop. Citrus plants are not a suited host for the rearing of the nymphs, as high nymphal mortality rates indicate (ALMEIDA & LOPES, 1999; YAMAMOTO & GRAVENA, 2000; MILANEZ ET AL., 2001). *H. coagulata* showed a distinct seasonal preference for certain host plants, despite the possibility to use a wide range of hosts (MIZELL & FRENCH, 1987). In citrus groves, GARCIA JR. ET AL. (1997) found higher sharpshooter populations in lowlands than in higher elevated groves. Irrigation increased the number of caught sharpshooters inside the field; furthermore the abundance was extended into the dry months compared to non-irrigated orchards, where no sharpshooters were caught in the dry season. Greenhouse experiments revealed that a soil water deficit negatively affected the rate of xylem sap ingestion and the survival of the sharpshooter *O. facialis*

on citrus seedlings (PEREIRA ET AL., 2005; PEREIRA, 2005). These observations are congruent with an experiment of *H. coagulata* feeding on *Lagerstroemia indica*. The insect fed on the plant only during daytime and the feeding rate was reduced exponentially as a function of xylem tension; consequently the feeding rates were higher on irrigated compared to non-irrigated plants (ANDERSEN ET AL., 1992). However, within a normal physiological range of xylem tension (e.g. 0-1.8 MPa), the authors assumed that the ratio of amides to total organic compounds predominantly influences the feeding behaviour of the sharpshooters.

The physiological state of the plant seems to have an influence on the transmission efficiency, as BRLANSKY ET AL. (2002) detected that small, actively growing citrus plants are more susceptible to a *X. fastidiosa* infection than larger plants. Mechanical inoculations of citrus yielded a higher infection rate in summer and autumn than in winter and spring. Inoculations under water stress and low temperatures were less successful. Moreover, the bacterial colonisation of the plant was faster during spring and summer (PEREIRA, 2005).

2.5. Management of the disease

The main goal of disease management is to prevent a disease from spreading. One principle rule is to avoid introduction into a region or orchard, and if this has already occurred, to reduce the spread of the disease inside the field and to adjacent fields and to minimise the damage caused by the infection.

2.5.1. Curative measurements

Although grapevines can be protected and cured from Pierce's Disease with tetracycline treatments (HOPKINS & MORTENSEN, 1971; HOPKINS ET AL., 1973a), remedial measures of bacterial infections are not practicable. A registration of this antibiotic in the United States in 1974 was extended in 1997 only for the use in peach and pear as fruit crops, all other crop trees were just allowed to be treated if no harvestable fruits within twelve months after the application would be borne. Due to the risk that plant pathogens develop resistances against these antibiotics and transfer them to human pathogens, the application is forbidden in many countries of the world and, if allowed, strongly restricted (SCHNEIDER, 2000). For the treatment of Xylella diseases, no antibiotic is registered in Brazil (BERGAMIN FILHO, personal communication). DE LIMA ET AL. (1996) found no effect of a soil treatment with antibiotics on the survival of the bacteria in young infected citrus plants.

Curative measurements like heat therapy, as applied for Pierce's Disease and Alfalfa Dwarf (GOHEEN ET AL., 1973), or cold therapy of Pierce's Disease (PURCELL, 1980) are possible in experiments under controlled conditions, but no solutions in practice, especially for frost sensitive plants like citrus and coffee. Exposure of 3-year-old, CVC infected citrus plants to 5°C for up to 90 days or 52°C for up to 30 minutes had no effect on the survival of the bacterium (DE LIMA ET AL., 1996).

2.5.2. Pruning

In coffee, pruning is a common measurement to shape the trees, but concrete recommendations to prune CLS symptomatic branches have not been given.

The aim of pruning CVC infected citrus plants is to reduce the inoculum sources. Citrus groves should be inspected regularly for CVC symptoms from January to July, since at this time the symptoms can be detected more easily. For citrus trees of an age below 1.5 years, the complete removal of the plant was recommended, if CVC symptoms appeared, since experience has shown that they will not recover from the disease and

will not bring an economically satisfactory yield. The same was recommended for trees of 2 to 4 years with severe symptoms. For older trees, the pruning of the affected branches is considered as sufficient, since symptoms are mainly restricted to certain branches. If symptoms are detected in the first three months of the year, the branch has to be cut 50 cm below the last symptomatic leaf, in April and May the distance has to be 70 cm and in June and July 100 cm (RODAS, 1994; GARCIA JR. ET AL., 1995; FONTANEZZI HUANG & CHIARADIA, 1998).

The success of pruning is doubtful, especially at an advanced stage of the disease. Severe pruning of CVC affected citrus trees 50 cm above the graft union led to a rapid development of new flushes, which started to show symptoms in the same season, part of them already one month after the pruning (HE ET AL., 2000). DE LIMA ET AL. (1996) found four to five prunings necessary to maintain symptomless trees, but the bacteria itself were not eliminated by this measure, they frequently reoccurred on shoots emerging from the pruned sites. UESUGI & UENO (1999) compared the CVC incidences of two orchards, in one grove pruning of symptomatic branches was applied. Two years after this measurement no difference in the amount of symptomatic plants could be detected. The authors concluded that in this case the pruning caused higher economic losses. However, this measurement was carried out when already 22 % of the plants showed CVC symptoms.

It has to be considered that infected citrus plants may not be the only source for infections. The vectors can also acquire the bacterium from other herbaceous plants or shrubs inside and at the borders of the grove (PURCELL & SAUNDERS, 1995).

2.5.3. Irrigation/ nutrition

The CVC disease severity greatly depends on the ambiental conditions of the plant, as there are climate, nutrition and hydric balance (DE LIMA, 1995; DE LIMA ET AL., 1996). Several researchers suggested that drought stress enhances the symptom severity of Xylella diseases (GOODWIN ET AL., 1988; HOPKINS, 1989; TARGON ET AL., 2004). MC ELRONE ET AL. (2001) found a clear relationship between abiotic factors like drought and heat and the severity of the symptoms for *Parthenocissus quinquefolia*. Since no effective curative measure is available, the authors recommended maintaining well-watered conditions to slow the disease progression, reduce the symptom severity and extend the life of infected plants.

Additionally to the irrigation, a balanced fertilisation may help to reduce the losses caused by the disease. Mineral fertilisation led to lower disease incidence levels in *X. fastidiosa* infected tobacco plants (TORRES ET AL., 2002).

2.5.4. Vector control

In the case of CVC, the earliest recommendations were to use healthy trees for plantings by preventing infections of nursery trees and using healthy budwood. Furthermore, nurseries should not be located in areas with heavy CVC infections and vectors should be kept away (GARCIA JR. ET AL., 1995). A prevention of the young plants from an infection with CVC can be arranged by a protection of the culture with insect nets, alternatively by an insecticide application every 7 to 15 days and windbreak barriers like Napier grass to avoid a drift of the vectors into the nursery (RODAS, 1994; GARCIA JR. ET AL., 1995).

Citrus is not the primary host of the known sharpshooter vectors. Especially *Agroconia* sp. and *B. xanthopis* can be observed feeding or ovipositing on other plants, in particular trees and shrubs adjacent to citrus groves, including coffee (LOPES, 1999). Under certain circumstances the insects enter the citrus groves. In the State of São Paulo, an increase in the population occurs with the beginning of the raining season in spring, reaching a peak at the end of summer (PAIVA ET AL., 1996; ROBERTO ET AL., 1996; GARCIA JR. ET AL., 1997). Therefore, the control of the vegetation inside and adjacent to citrus groves may help to reduce the sharpshooter population. Encouraging results were obtained in studies of Pierce's Disease, where the main breeding hosts of the sharpshooters like elderberry, blackberry and wild grape were removed and replaced by non-hosts of the insects, which reduced their activity in the grapevine groves by 70 to 99 % (ANONYMOUS, 2003).

The ratio of amides to total organic compounds in the xylem sap predominantly influences the feeding behaviour of the leafhoppers within a normal range of xylem tension (ANDERSEN ET AL., 1992). Since the xylem fluid chemistry can be influenced by some factors, e.g. fertilisation and rootstock, there is potential for making host plants less attractive for feeding and thus decreasing the probability of infection (REDAK ET AL., 2004).

Systemic insecticides, e.g. imidacloprid and acetamiprid, ensure a satisfactory control of the sharpshooters (YAMAMOTO ET AL., 2003). Apart from the commonly applied spraying, the insecticides can be applied on the trunk, because the active component is

systemically distributed within the plant (NAKANO, 2004), which is an environmentally friendly alternative to the common spraying. Acetaprimid has a faster, but shorter effect on the insects (YAMAMOTO, 2000).

2.5.5. Selection of resistant/ tolerant species

One approach to live with a disease is to select resistant or tolerant species. In the case of *Xylella fastidiosa*, all sweet orange (*C. sinensis*) varieties are susceptible to *X. fastidiosa*, but exhibit different grades in the economical damage caused by the disease. The varieties 'Barão', 'Pera', 'Lima', 'Rubi', 'Berna', 'Valência' and 'Cadenera 17' and 'S1' showed the highest yield reduction, 'Gardner', 'Pineapple', 'Sunstar', 'Folha Murcha' and 'Baianinha' were less affected, and the economic losses for 'Lue Gim Gong' and 'Westin' were lowest (LARANJEIRA & POMPEU JR., 2002). In evaluations of the CVC symptom severity of four sweet orange varieties, ZANUTTO ET AL. (2004) found 'Folha Murcha' to be less affected by the disease compared to 'Valência', 'Natal' and 'Pera', while the latter one showed the highest symptom severity.

Besides sweet oranges, the species *C. aurantium* and *C. reticulata* were susceptible to CVC. The citrus species *C. volkameriana*, *C. jambhiri*, *C. limonia*, *C. latifolia* and *C. paradisi* are resistant to CVC, as well as *Poncirus trifoliata* and *Fortunella margarita* (BERETTA ET AL., 1993; LARANJEIRA ET AL., 1998; BERETTA & LEITE, 2000).

DE LIMA ET AL. (1998) suggested that the 'arabica coffee' variety 'Mundo Novo' is more tolerant to CLS than 'Catuaí Vermelo', but QUEIROZ-VOLTAN ET AL. (2004a) could not find differences in the amount of obstructed xylem vessels as well as in the symptom severity. However, grafted plants of both varieties showed a lower symptom severity than non-grafted plants (QUEIROZ-VOLTAN ET AL., 2004b).

2.6. Epidemiological aspects

The application of adequate management strategies requires a proper knowledge of the dissemination procedure of the disease. The dissemination can be divided into the section of the progress in time and the spread in space.

2.6.1. Incubation period

The incubation period is defined as the time required for symptoms to develop after the infection (GILLIGAN, 1994). Environmental factors and plant aspects influence the duration of this period. In greenhouse experiments with citrus seedlings or young graftings from infected trees, the plants started to show CVC symptoms two months after inoculation (ALMEIDA ET AL., 2001; HE ET AL., 2000). Under field conditions, the incubation period lasts between 6 and 14 months (LOPES ET AL., 1996). Inside a CVC affected field, newly established young citrus plant did not show symptoms up to one year after the introduction. After 18 months 5-10 % and after 27 months 10-60 % of the plants had developed symptoms (DE LIMA ET AL., 1996).

In greenhouse experiments, coffee plants developed CLS symptoms 3 to 6 months after an artificial inoculation (DE LIMA ET AL., 1998; LI ET AL., 2001).

2.6.2. Temporal aspects of CVC and CLS

Soon after the detection of CVC, evaluations started aiming to understand the dissemination of the disease inside a citrus grove and to find effective measurements to control the disease. No similarities were found to Pierce's Disease of grapevines, an epidemiologically well-evaluated *Xylella* disease. The temporal development of this disease shows a monomolecular progress, since the inoculum sources lie outside the grapevine orchards and a spread within a grove is unlikely. Wild plants adjacent to the grapevine orchards play a major role in the epidemiology of Pierce's Disease (RAJU ET AL., 1983; HOPKINS & PURCELL, 2002; ANONYMOUS, 2003).

PALAZZO & CARVALHO (1992) found two periods of higher increase of the incidence and the severity of the symptoms, one between September and October and the other one in March. The authors concluded a relation of the increase in symptom severity with higher temperatures, increasing precipitation and the flushing of the citrus trees. LARANJEIRA (1997a; 2002) and ROBERTO ET AL. (2002) confirmed this assumption by determining spring and summer as the times of the highest disease increase. In the

autumn and winter months, leaf symptoms rarely were found (RODAS, 1994; LARANJEIRA, 1997a).

Evaluations of the temporal disease progress yielded extremely varying results. Reports of an increase of the disease incidence from 4.2 % to 99.8 % within 13 months (LARANJEIRA, 2002) exist as well as incidences remaining below 10 % after 18 evaluation months (LARANJEIRA, 1997a). From yearly assessments, GOTTWALD ET AL. (1993) calculated that, after the introduction of a single infected tree into a grove, an incidence of 100 % occurs after 10 to 13 years. Other evaluations detected increases from 0.1 % to 15 % within two years (ROSETTI ET AL., 1990), from 14.4 % to 32.2 % within nine months (BERETTA ET AL., 1994) and from 11 % to 84 % within two years (ROBERTO ET AL., 2002). In the latter case, the authors explained the high rate with the absence of vector control in the grove. LARANJEIRA (2002) detected an increase of the disease incidence from 21.4 % to 96.6 % and from 6.2 % to 92.6 %, respectively, within 2 ½ years. As mentioned before, he measured a steep rise from 4.2 % to 99.8 % within 13 months. The author explained this with the planting of already infected nursery trees. Different authors tried to find an epidemiological model, which describes the disease progress of CVC. Several authors (GOTTWALD ET AL., 1993; ZANUTTO ET AL., 2004) found the Gompertz model to be appropriate to describe the temporal development of the disease. LARANJEIRA (1997a) determined a model of double sigmoid pattern to describe the CVC disease progress. In later evaluations, none of the generally used models gave a satisfactory fit to CVC epidemics (LARANJEIRA, 2002).

To the authors knowledge, epidemiological studies on the temporal progress of CLS in coffee have not been done up to now.

2.6.3. Spatial distribution

The spatial pattern of CVC diseased plants within a field was described as loosely aggregated, whereas at an initial stage of the disease random patterns were observed (LARANJEIRA, 2002; ROBERTO ET AL., 2002). An influence of the wind direction as well as of machines on the disease spread was not detected (LARANJEIRA, 1997a). Several authors stressed the limited dispersion range of the vectoring insects (GOTTWALD ET AL., 1993; LARANJEIRA 1997a; 2002; ROBERTO ET AL., 2002). LARANJEIRA (1997a) detected a tendency of CVC disease foci to occur at groves edges, especially when other diseased plots were adjacent.

ROBERTO ET AL. (2002) found initially a random distribution of infected plants, which formed aggregated foci after some time less than 10-14 m apart.

For CLS in coffee, BARBOSA ET AL. (2004) found an aggregated pattern of diseased coffee plants, whereas symptomatic plants randomly appeared at the beginning of the epidemic. At high incidence levels, disease foci coalesced, leading to a low number of foci with a high number of plants.

3. Materials & Methods

3.1. Field descriptions

An overview of the field descriptions as well as information on the assessments is given in Tab. 7, Annex.

3.1.1. Coffee plots in the State of Minas Gerais, Brazil

For the assessments of the CLS incidence, plantations in the county of São Gotardo (Lat 46°S, Lon 19°2'W) and in the county of Ervália (Lat 42°6'S, Lon 20°9'W) in the State of Minas Gerais, Brazil, were selected.

In São Gotardo, two adjacent plots of a *Coffea arabica* plantation, variety 'Catuaí Vermelho', were assessed, which were parts of a great coffee plantation (Fig. 39, Annex). In both plots, the distance between the rows was 3 m, the distance between the plants within one row approximately 0.7 m. Fungicides to control coffee rust were applied regularly, but no insecticide treatments were carried out.

The first plot (SG1) consisted of 7712 coffee plants, planted in 38 rows. The number of plants per row varied from 98 to 304. At the beginning of the evaluations, the plants had an age of 3 years after stumping. One side of the plot adjoined a pasture, the other three sides coffee plants.

The second plot (SG2) consisted of 6000 coffee plants in 20 rows with 300 plants per row. At the beginning of the assessments, the plants had an age of 3 years after stumping. All sides of the plot were adjacent to coffee plants.

The plot at Ervália consisted of 894 *Coffea arabica* plants, variety 'Catuaí Vermelho', planted in 15 rows with 14 to 93 plants per row. The plot was lying on a steep hillside exposed to the north. The trees were planted in s-shaped rows along the hillside, the distance between the plants inside the rows was approximately 1 m, and varied from 1.50 m to 2.50 m across the rows. A big gap of approximately 4 m between row 7 and 8 enlarged to 5 m between row 7 and 9.

Three sides of the experimental plot were adjacent to other coffee plantings. One side adjoined natural vegetation, which consisted of endemic trees, ferns and grasses. Inside the experimental plot, other plants, e.g. papaya, palms and citrus, were occurring sparsely distributed. Apart from fungicides against coffee rust, no pesticide treatments were applied. At the beginning of the assessments the plants had an age of 2 years after stumping.

3.1.2. Citrus plots in the State of São Paulo, Brazil

All selected orchards in the State of São Paulo were planted with sweet oranges (*Citrus sinensis* Osb.) of the variety 'Pera', grafted on rangpur lime (*C. limonia* Osb.). In all plantations, the plants had an age of 4 years at the beginning of the evaluations.

The first plot was located at São Carlos in the south of the State of São Paulo (Lat 47°9'S, Lon 22°W). It consisted of 1610 plants in 10 rows with 154 to 167 plants per row. The distance between the rows was 7 m, between plants within the rows 3 m. The plot was adjacent to other sweet orange groves to three sides and at one side to a pasture. No insecticides were applied.

The second plot was located at Engenheiro Coelho in the center of the São Paulo State (Lat 46°9'S, Lon 22°5'W). It consisted of 1149 plants in 38 rows with 3 to 49 plants per row. The plants were spaced 7 m between the rows and 4 m inside the rows. To three sides, the field was surrounded by other sweet orange groves. Two of them were directly adjacent and one was separated by a street. One side of the orchard was bordered by farm housings. No insecticid treatments were carried out.

In the region of Bebedouro in the north of the State of São Paulo (Lat 48°5'S, Lon 21°W), two orchards were selected for the CVC assessment. The first ('B untreated') consisted of 425 plants in 12 rows, each with 34 to 36 trees. The distance between the plants inside the rows amounted to 4 m, between the rows to 7 m. No chemical insecticides to control the vectors of CVC have been applied. A street adjoined the grove to one side, the other three sides were adjacent to other sweet orange plantations.

The second orchard ('B treated') included 2130 plants in 49 rows with 1 to 74 plants per row. The distance between the plants within the rows amounted to 3.5 m and to 7 m between the rows. Chemical insecticides to control the vectors of CVC were applied regularly. Two sides of the orchard bordered streets, one side a pasture and the other side another sweet orange plantation.

3.1.3. Citrus plots in the State of Bahia, Brazil

An orchard planted with the sweet orange 'Pera' grafted on rangpur lime was selected at Itapicuru, located in the northeast of the State of Bahia (Lat 38°2' S, Lon 11°1'W). At the beginning of the evaluations, the trees had an age of 12 years. The distance of the plants between the rows was 3.5 m, within the rows 1.5 m. No insecticides were applied.

Half of the orchard was irrigated with mini-sprinklers. In the irrigated area, one rectangular plot of 900 plants, consisting of 18 rows with 50 plants per row, was assessed. In the non-irrigated part, two plots of 364 (14 x 26 plants) (non-irrigated 1) and 362 plants (9-16 x 30) (non-irrigated 2) were selected. The position of the plots inside the orchard is shown in Fig. 38, Annex.

3.1.4. Citrus plots in the Province Corrientes, Argentina

Four orchards in the Province Corrientes, located in the northeast of Argentina, were selected for the evaluations of the CVC incidence, all planted with sweet oranges of the variety 'Valência Late' grafted on rangpur lime. The fields A and B were close to the location Lomas Sur (Lat 28°33'S, Lon 59°02'W), the irrigated and non-irrigated plot close to Bella Vista (Lat 28°27'S, Lon 58°59'W). No insecticides to control the vectors were applied in the plots.

The orchard A consisted of 320 (10 x 32), 12 years old plants, spaced 3 m inside the rows and 5 m between the rows. To the three sides, the field was bordered by other sweet orange 'Valência Late' plantings, separated at two sides by a *Pinus* curtain. A planting of eucalyptus trees was adjacent to the other side.

The orchard B consisted of 289 (17 x 17) plants, which had an age of 13 years at the beginning of the assessments. The distance of the plants was 3 m within and 5.5 m between the rows. To three sides, other 'Valência Late' plantings bordered the plot. A street behind a *Pinus* curtain adjoined the other side.

The two other selected orchards were experimental plots from a research station, originally planted to evaluate influences of different tree spacings. The spacings between the plants inside the rows were 2, 3, 4 and 5 m and between the rows 4, 5, 6 and 7 m. Both plots included 398 plants in 13 rows with 12 to 16 plants per row and had an age of 12 years at the beginning of the evaluations. In one plot (irrigated), drip irrigation was applied three times a week during the dry season. In the other plot (non-irrigated), no additional irrigation was applied. Both plots were zoned by a street and to the other directions surrounded by other sweet orange and mandarine plantings of different varieties.

3.2. Data Assessment

An overview of the assessment information together with the field descriptions is given in Tab. 7, Annex.

3.2.1. CLS assessment in the State of Minas Gerais, Brazil

The coffee plantations at São Gotardo as well as at Ervália have been assessed every second to third month. In the plot SG1, evaluations were carried out over a period of 31 months from April 2003 until November 2005. In the plot SG2, the assessments ran over 20 months from March 2004 until November 2005.

In Ervália, the first evaluation took place on 20th May 2003 for the rows 8-15, the rows 1 to 7 were assessed two weeks later on 4th June 2003. Due to the great time interval to the second evaluation on 24th September 2003, these two weeks difference within the first assessment were not considered in the analyses. The evaluations were carried out over a period of 20 months, from May 2003 until January 2005. After January 2005, no further evaluations were carried out due to the stumping of the plants.

The coffee plants were visually assessed for symptoms of Coffee Leaf Scorch. The plants were classified as symptomatic when at least three branches in different parts of the plant showed interveinal chlorosis additionally to shortened internodes.

The mean monthly temperature and the monthly precipitation were recorded from a weather station close to the experimental plantation in São Gotardo. For Ervália, no meteorological data were available.

3.2.2. CVC assessment in the State of São Paulo, Brazil

Assessments in the four citrus orchards in the State of São Paulo were carried out monthly or bimonthly. In the orchards at São Carlos and Engenheiro Coelho, the evaluations covered a period of 35 months from October 2002 until September 2005 and in the two orchards at Bebedouro a period of 33 months from December 2002 until September 2005.

Binary data were obtained by visually classifying the plants as CVC symptomatic or non-symptomatic. CVC symptoms considered were leaf chlorosis with brown pustules on the adaxial leaf side, shortened internodes and, at a severe stage of the disease, small, hard fruits. Additionally to the presence of symptoms, the symptom severity was visually assessed over 24 months from September 2003 until September 2005. A scale ranging from 0 to 3 was applied to discriminate the symptom severity:

- 0 no symptoms
- 1 initial symptoms
- 2 some affected branches
- 3 severe leaf and fruit symptoms

The occurrence of other diseases was also noted in the maps. Meteorological data were provided from weather stations close to the respective experimental plots. These included the daily precipitation and the daily minimum and maximum temperatures.

3.2.3. CVC assessment in the State of Bahia, Brazil

In the orchard in the State of Bahia, evaluations were carried out monthly from June 2003 until October 2005, resulting in an assessment period of 28 months. The presence of symptoms as well as the symptom severity was visually assessed over the whole period. The scale for the severity of the symptoms conformed to the one used for the assessments in the State of São Paulo.

Meteorological information like the mean temperature and precipitation on a monthly basis was recorded from a weather station close to the orchard.

3.2.4. CVC assessment in the Province Corrientes, Argentina

The evaluations were carried out monthly from April 2004 until September 2005, covering a period of 17 months. Additionally to the presence of symptoms, the severity was visually assessed over the whole period. A scale ranging from 0 to 4 was applied to discriminate the severity of the symptoms:

- 0 no symptoms
- 1 few leaves with mild symptoms
- 2 more than two branches with symptoms
- 3 four to five branches with symptoms
- 4 more than six branches and fruits with symptoms

Meteorological data were obtained from a weather station close to the irrigated and non-irrigated experimental plots.

3.3. Processing of the meteorological data

For most of the locations, the mean monthly temperature was provided. For the four plantations in the State of São Paulo, only the minimum and maximum temperature per day was available. To establish a comparable basis to the other locations, the mean

temperature was calculated as the mean of the minimum and maximum temperature. From the meteorological station in the Province Corrientes, Argentina, the minimum and maximum temperature was available as well as the mean daily temperature. The calculation of the mean temperature using the above mentioned method resulted in an error of 0.38°C (SD 0.73, n=368), which justifies the procedure for the data of São Paulo.

Information on the precipitation was provided partly on a daily and partly on a monthly basis. The sum of the precipitations per month was divided by the days per month, leading to the mean daily precipitation on a monthly basis.

3.4. Temporal analyses

The binary data, indicating the status (symptomatic/ non symptomatic) of each plant, served to calculate the incidence of symptomatic plants in percent. Additionally, cumulative maps were generated in which a plant, once showing symptoms, was considered as diseased for all further assessments, accommodating the persistence of the pathogen. This resulted in the incidence of diseased plants in percent.

In epidemiology, a wide range of models is used to describe temporal disease progress curves. The selection of an appropriate model should help to compare epidemics, predict the further progress of a disease and evaluate control strategies (CAMPBELL & MADDEN, 1990). The following models were applied in the attempt to fit the cumulative data by the least square regression method:

monomolecular $y(t) = a(1 - \exp[b - ct])$

logistic $y(t) = a/(1 + \exp[b - ct])$

double sigmoid logistic $y(t) = a_1/(1 + \exp[b_1 - c_1t]) + a_2/(1 + \exp[b_2 - c_2t])$ with

$y(t)$ disease incidence at time t

t time (days)

a parameter, asymptotic capacity of the disease

b parameter, initial disease level

c parameter, progress rate of the disease

The value of the coefficient of determination R^2 served to judge the adequacy of the regression equation to describe the data. Additionally to the coefficient of determination, the pattern of the residuals was considered. Several successive points lying on one side of the regression curve reflected a systematic pattern of the residuals, showing the

inappropriateness of the model to describe the disease progress properly (CAMPBELL & MADDEN, 1990).

3.5. Times of the highest increase of the incidence of symptoms

To detect the times of the year, in which changes of the incidence of symptomatic plants occurred, differences in the incidence of symptomatic plants from one assessment to the next were calculated. Positive values indicated an increase, negative values a decrease and values equal to zero no change in the incidence of symptomatic plants.

3.6. Persistence of the symptoms

For the plots in the State of Bahia and the irrigated and non-irrigated plot in the Province Corrientes, the duration a plant showed symptoms was determined. For the other citrus plots, this analysis was not applied, since the plants almost consistently showed symptoms.

The alteration of the disease status of each plant within a plot was evaluated. The amount of plants, which changed from asymptomatic to symptomatic, was set to 100 % for the respective assessment date. For the following six assessments, the percentage of plants was determined, which became asymptomatic again.

3.7. Severity of the disease symptoms

To facilitate a comparison of the disease severities, a severity index (*SI*) was calculated through

$$SI = \frac{(a + (b * 2) + (c * 3))}{y} \quad \text{for the orchards in the States of São Paulo and Bahia, Brazil,}$$

and

$$SI = \frac{(a + (b * 2) + (c * 3) + (d * 4))}{y} \quad \text{for the orchards in the Province Corrientes, Argentina,}$$

with

- a* percentage of plants with severity grade 1
- b* percentage of plants with severity grade 2
- c* percentage of plants with severity grade 3
- d* percentage of plants with severity grade 4
- y* total incidence of symptomatic plants in percent

The severity index S_I can adopt values between 1 and 3 or 4, respectively, for the Argentinean orchards. The higher the index, the more symptomatic plants showed symptoms of a higher severity grade

3.8. Spatial analyses

For the spatial analyses, cumulative data were used, since they should rather reflect the disease status of the plants than the incidence of symptomatic plants. In some of the plots, the incidence of diseased plants reached asymptotic levels close to 100 % soon after the beginning of the evaluations. Further assessments did not yield additional information on the epidemic as well as on the spatial distribution, but were carried on to obtain information on the symptom appearance and disappearance. To avoid a bias of the results, which would have resulted if the majority of data points were obtained from these high disease levels, assessments were neglected in the analyses after the incidence level 85 % exceeded.

3.8.1. Ordinary runs analysis

With the ordinary runs analysis the aggregation of diseased plants in one direction of a plot can be tested. A run (R) is defined as a succession of plants with the same disease status (diseased or healthy) within or across a planting row. The expected numbers of runs $E(R)$ and the variance s^2 of R were given by

$$E(R) = 1 + \frac{2m(N-m)}{N}$$

$$s^2 = \frac{2m(N-m)[2m(N-m)-N]}{N^2(N-1)} \quad \text{with}$$

N number of plants per row

m number of diseased plants per row

The value $Z(R)$ is given by

$$Z(R) = \frac{(R + 0.5 - E(R))}{s} \quad \text{in which the value 0.5 is a continuity correction.}$$

Values of $Z(R) \leq -1.64$ indicate an aggregated pattern of diseased plants within the tested rows ($p < 0.05$). The use of this test is restricted to rows with $N \geq 20$ (CAMPBELL & MADDEN, 1990). Furthermore, rows with $m < 2$ and $N = m$ were excluded from the analysis. For rows with $N \leq 20$, the critical R values were taken from a table according to Swed, Frida and Eisenhart (SACHS, 1999).

The analyses were carried out on the basis of the cumulative maps. The observed number of runs per row (R) was calculated by a self-established Microsoft EXCEL Visual Basic macro.

3.8.2. Quadrat based analyses

The cumulative maps of the fields of each assessment date were partitioned into quadrats of different sizes by the use of a Microsoft EXCEL Visual basic macro (GOTTWALD, unpublished data).

For the coffee plots SG1, SG2 and Ervália, only one-dimensional quadrats of the sizes 1 x 3, 1 x 5, 1 x 8 and 1 x 10 were applied, due to the great distances between the plants across the rows (for SG1 and SG2 3.5 m, for Ervália varying) compared to the distances between the rows (for SG1 and SG2 0.7 m, for Ervália varying).

For the citrus plots, the sizes 3 x 3, 4 x 4 and 5 x 5 were applied. Since the distance of the plants between the rows was greater than inside the rows, the sizes 3 x 5 and 5 x 3 served to establish, whether a greater degree of aggregation existed in one direction of the field.

For each assessment date, the proportion of diseased plants (p), the expected number of diseased plants per quadrat ($N \cdot p$) and the theoretical binomial variance (v_{bin}), assuming a random distribution, were calculated for each applied quadrat size. With the actual number of diseased plants per quadrat (x_i) the observed variance (v_{obs}) was calculated.

$$p = \frac{\sum x_i}{NQ \cdot N}$$

$$v_{obs} = \frac{\sum (x_i - Np)^2}{N^2(N-1)}$$

$$v_{bin} = \frac{p(1-p)}{N} \quad \text{with}$$

p proportion of diseased plants in the plot

x_i observed number of diseased plants in the i -th quadrat

NQ number of quadrats

N number of plants per quadrat

Np expected number of diseased plants per quadrat

3.8.2.1. Dispersion index DI

Numerous indices to describe the degree of aggregation of diseased plants inside a field exist, but no unique agreement on the best method to use (MADDEN & HUGHES, 1995).

The quotient of the observed and the estimated binomial variance, the dispersion index DI , can serve as a measure of aggregation, calculated by

$$DI = \frac{v_{obs}}{v_{bin}}$$

with the null hypothesis $H_0 : v_{obs} = v_{bin}$ or $DI = 1$

and the alternative hypothesis $H_a : v_{obs} > v_{bin}$ or $DI > 1$

The significant departure of DI from 1 was tested by the Chi-Square test (α , $df=NQ-1$).

The rejection of the null hypothesis indicates an aggregated pattern of the diseased plants for the tested quadrat size at the certain assessment.

3.8.2.2. Application of the modified Taylor law

The dispersion index DI can detect aggregated patterns of diseased plants for single assessments. From the modified Taylor law, one can obtain information on the trend of the spatial pattern inside a plot for the whole assessment period. For binary data, MADDEN and HUGHES (1992) suggested a modified form of the power law written as

$$\log(v_{obs}) = \log(A) + b \log(v_{bin})$$

in which A and b are parameters. The significance of the relation between v_{obs} and v_{bin} was tested by the F-test. The parameters A and b were estimated by linear least-square regressions. The appropriateness of the modified Taylor law to describe the data points was determined by the coefficient of determination R^2 and the pattern of the residuals, as described in 3.3.

A random distribution of diseased plants is indicated by $b = 1$ and $A = 1$, or $\log(A) = 0$, respectively, which indicates that v_{obs} equals v_{bin} . The parameter b , which is also the slope of the linear equation, suggests an increasing grade of aggregation as a function of the disease incidence if $b > 1$. If $A > 1$ and $b = 1$, the degree of aggregation depends on the value of A and is constant throughout the assessment period. The significant departure of $\log(A)$ from zero ($H_0: \log(A) = 0$, $H_a: \log(A) > 0$) and b from 1 ($H_0: b = 1$, $H_a: b > 1$) was tested by the T-test (α , $df=(\text{number of assessments})-1$).

3.9. Analysis of foci dynamics and structure

This type of data analysis has been proposed by NELSON (1996) as a simple method to detect differences in the spatial distribution of diseased plants. A disease focus is defined as a site of one or more diseased plants within a field. Adjacent plants, sharing an edge (rook's case) or a corner (bishop's case) in the spatial map, are considered to belong of the same focus.

The number of foci and the mean number of plants per focus were calculated by a self-established Microsoft EXCEL Visual Basic macro. The number of foci depends on the disease incidence and the size of the plot. To eliminate the influence of the plot size and establish a comparable basis between the plots, the number of foci was calculated per 100 plants. The relative focus size was calculated as the number of plants per focus in relation to the total number of plants per plot. If all plants of the plot were diseased, this would result in a relative focus size of 100.

4. Results

4.1. Temporal aspects of the diseases

4.1.1. Temporal development of CLS in the State of Minas Gerais, Brazil

The temporal development of the CLS incidence of the symptomatic and diseased plants in the plots SG1 and SG2 at São Gotardo is presented in Fig. 3. In the plot SG1, the assessment started at day 113 (April 2003) with an incidence of symptomatic plants below 10 %, in the plot SG2 one year later at day 451 (March 2005) with 15 %. In both plots, the incidence of symptomatic plants was highest around November and December, the springtime in South America, which is in this region characterized by the rise of the temperatures and the start of the raining season. Throughout the rest of the year, especially in autumn and winter (March-September) the incidence of symptomatic plants was on a much lower level. The local maximum incidences for the plot SG1 were 78 % in 2003 (day 342) and 94 % in 2004 and 2005 (day 681 and day 1049).

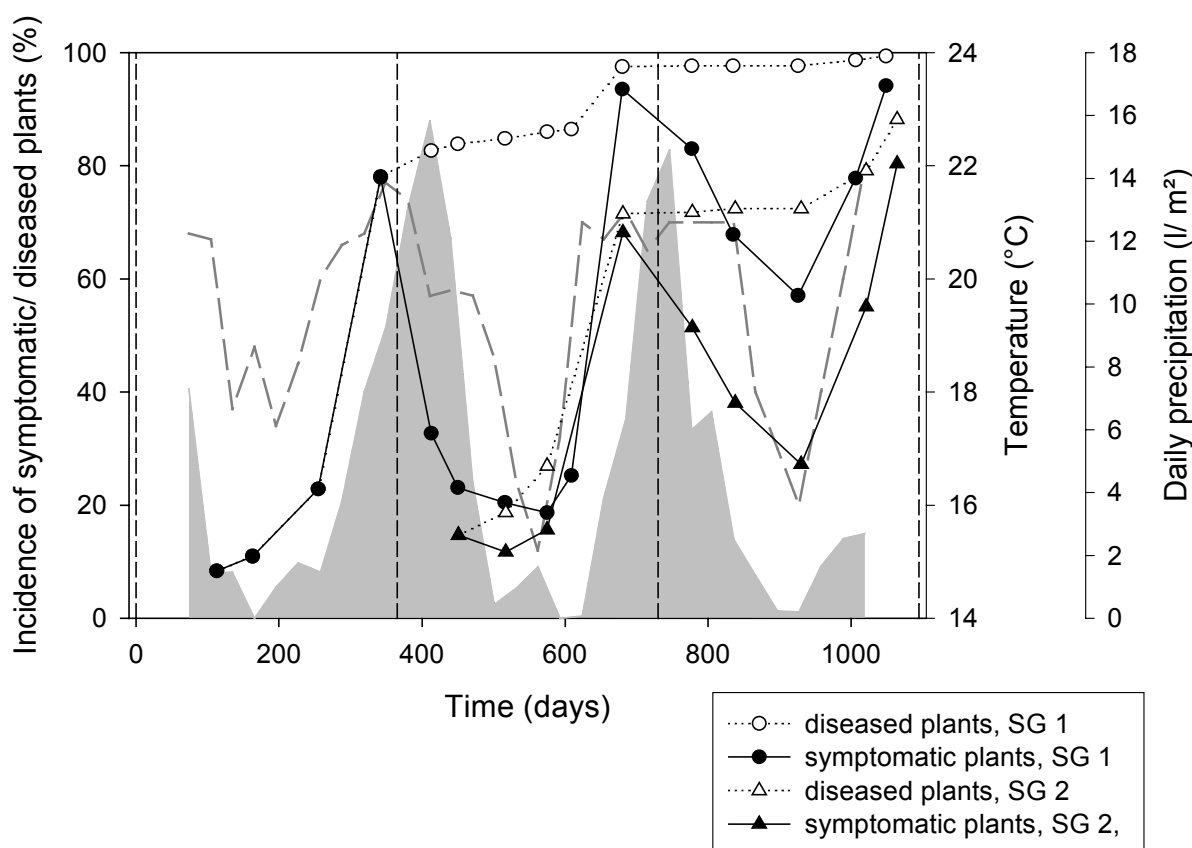


Fig. 3: Incidence of CLS symptomatic and diseased coffee plants (black lines), mean temperature (grey broken line) and daily precipitation (grey area) on a monthly basis in the plots **SG1** and **SG2** at **São Gotardo**. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2003

The incidence of symptomatic plants in the plot SG2 reached 68 % in 2004 (day 681) and 80 % in 2005 (day 1049). The curves of both plots were almost parallel from day 681 on. In the plot SG1, the decline in 2005 (day 731-1095) was not as distinct as in the previous year.

In the plot SG1, the first dispartment of the curves of the symptomatic and the diseased plants occurred after the first peak at day 342 (December 2003). At the end of 2004 (day 681), the incidence of diseased plants reached almost 100 %. In the plot SG2, the curves split up already at the second assessment date at day 517 (June 2004), indicating the disappearance of symptoms in part of the plants and the new appearance of symptoms in other plants, reflected by the slight increase of the diseased plants curve. After the first peak at day 681, the percentage of diseased plants remained constant until day 926 (July 2005). At the last assessment at day 1049 (November 2005), the incidence of diseased plants reached 88 %.

For both plots, a double sigmoid logistic function gave a satisfactory fit to the data points of the diseased plants ($R^2=0.99$), although a non-random pattern of the residuals resulted. This is caused by the fact that the modelling of the long constant phase between the two escalating phases of the disease incidence was inadequate (Figs. 40 and 41, Annex).

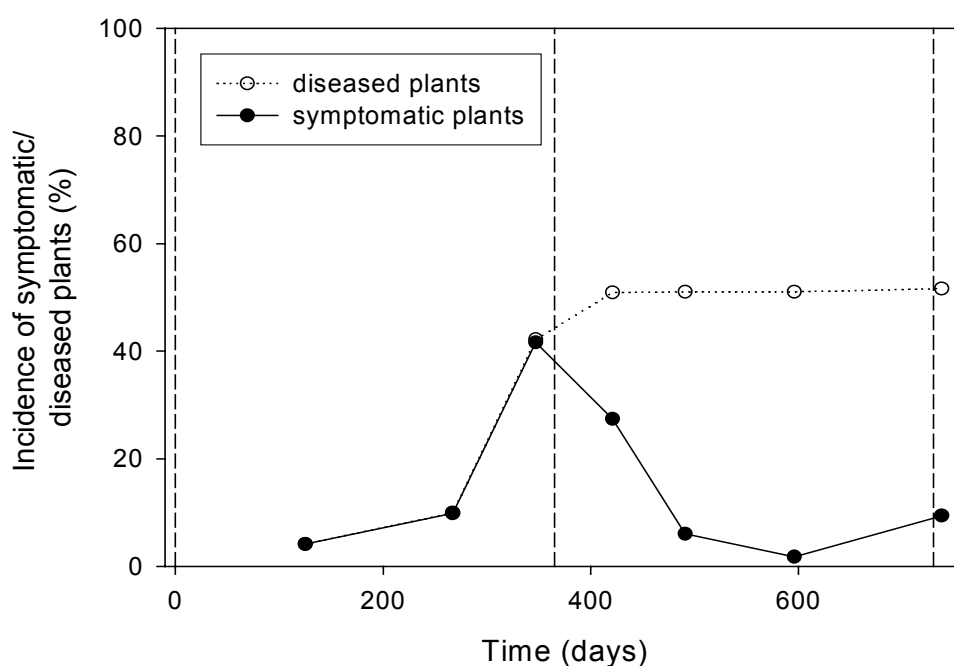


Fig. 4: Incidence of CLS symptomatic and diseased coffee plants in the plantation at **Ervália**. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2003

In the plantation at Ervália, the assessments started in May 2003 with an incidence of 5 % symptomatic plants (Fig. 4). As in the coffee plantation at São Gotardo, the percentage increased at the end of the year 2003 and dropped during the following assessments. Only a slight rise of the incidence of symptomatic plants was measured at the end of 2004. However, as 140 days passed between the assessment in August 2004 and the last one in January 2005, the period for the increase of the symptoms could have been missed.

The curves of the symptomatic and diseased plants departed after the peak at the end of 2003, from day 41 (February 2004) on, the incidence of diseased plants remained constantly at 50 %.

4.1.2. Temporal development of CVC in the State of São Paulo, Brazil

In the orchard at São Carlos (Fig. 5) the evaluation started in October 2002 with 0.06 % plants showing symptoms. Until mid of 2003, a relatively steep increase of the amount of symptomatic plants to 2 % was observed. A slight drop occurred at day 614 (September 2003) and another stronger decrease at day 677 (November 2003). Later, the incidence of symptomatic plants increased in a staircase-like manner. A slight decrease in the amount of symptomatic plants was measured after day 1256 (May 2005).

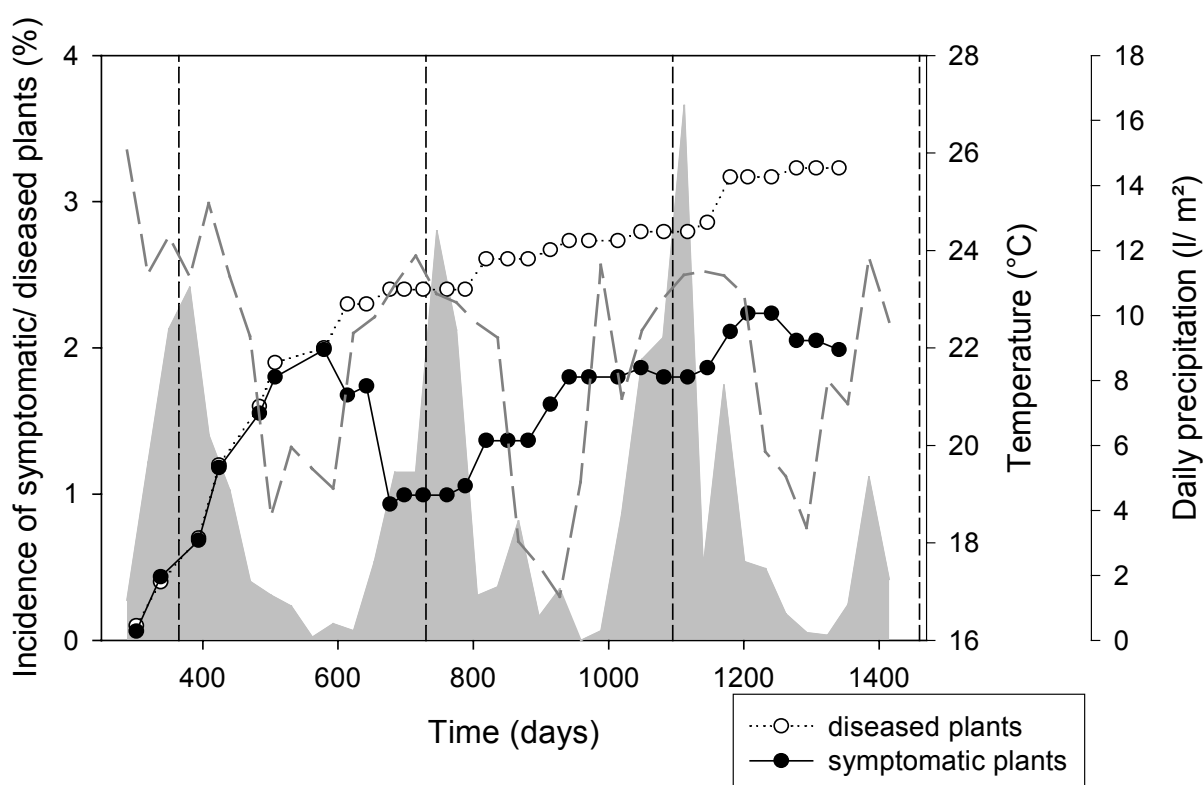


Fig. 5: Incidence of CVC symptomatic and diseased citrus plants (black lines), mean temperature (grey broken line) and mean daily precipitation (grey area) on a monthly basis in the plot at **São Carlos**. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2002

Throughout the three assessment years, the incidence of symptomatic plants tended to increase in the first half of the years and to decrease or remain constant in the second half of the years. In relation to the meteorological data, less new symptoms developed during the dry and relatively cold period at the end of winter (July/ August) and in spring (September - November) and more new symptoms appeared in summer and autumn (December-May).

The distinction between the curves of the symptomatic and diseased plants mainly resulted from the drop of the curve of symptomatic plants at the days 614 and 677 (September and November 2003). Until the decline at day 1278 (July 2005), this difference has diminished from 1.43 to 0.81 % of the total incidence.

The cumulative incidence of the diseased plants reached 3 % at the end of the evaluation. Within the first evaluation year, the incidence increased by 2.3 %, in the two following years only a rise of 0.7 % was observed. A monomolecular function gave a good fit to the data of the diseased plants ($R^2=0.98$), but the pattern of the residuals was not satisfactory (Fig. 42, Annex). Other models also failed to describe the data appropriately, mainly due to the two 'steps' at the beginning of 2004 and 2005.

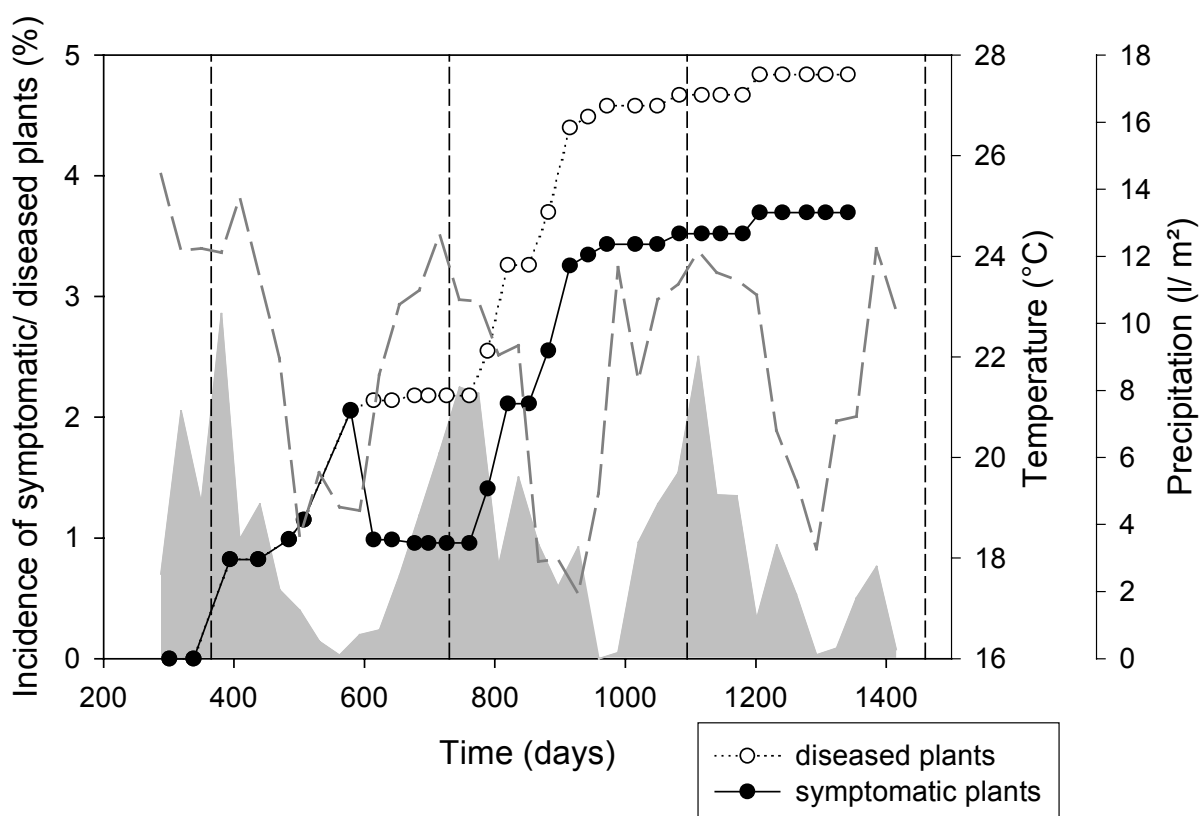


Fig. 6: Incidence of CVC symptomatic and diseased citrus plants (black lines), mean temperature (grey broken line) and mean daily precipitation (grey area) on a monthly basis in the plot at **Engenheiro Coelho**. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2002

At Engenheiro Coelho, the progress curve of the symptomatic plants (Fig. 6) was very similar to the one of the orchard at São Carlos (Fig. 5). Starting with 0 % incidence of symptomatic plants, a relatively steep escalation occurred at the beginning of the evaluations, followed by a decrease at day 614 (September 2003). After this point,

constant phases of varying duration were observed, interrupted by increases which were restricted to one or two months, mainly occurring in the first half of the years. However, the increase at the beginning of 2005 was very weak.

The difference between the curves of the symptomatic and the diseased plants, resulting from the decrease of the symptomatic plants in September 2003, remained almost constant for the rest of the evaluations.

The total increase of the incidence of diseased plants over the assessment period of three years amounted to 5%. A double sigmoid logistic function well fitted the data points of the diseased plants ($R^2=0.99$). However, in the asymptotic phase of the curve the residuals showed a systematic pattern.

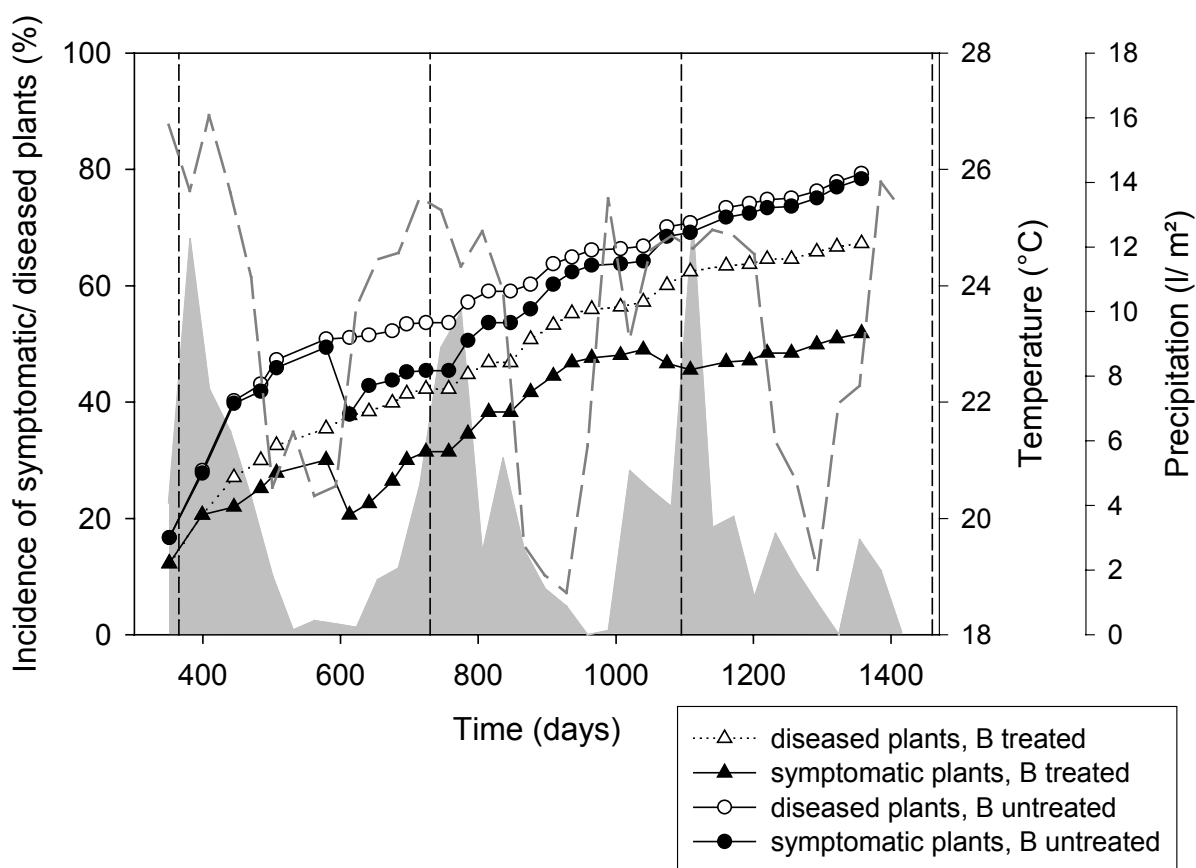


Fig. 7: Incidence of CVC symptomatic and diseased citrus plants (black lines), mean temperature (grey broken line) and mean daily precipitation (grey area) on a monthly basis in the plots 'B treated' and 'B untreated' at Bebedouro. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2002

The assessments of the CVC incidence in the two orchards at Bebedouro with ('B treated') and without ('B untreated') chemical insect control started at day 351 (December 2002) (Fig. 7). At the beginning, the orchard 'B treated' had a slightly lower incidence of symptomatic plants than the orchard 'B untreated'. In both plots, a drop at

day 614 (September 2003) followed a relatively steep increase of the curves in the first half of 2003, as in the other two orchards in the State of São Paulo. However, the trend of the curves to grow in the first half of the years and to remain constant or decrease in the second half was not observed in both plots in the second and third evaluation year. From day 614 (September 2003) until day 1094 (November 2004), the curve of symptomatic plants had a staircase pattern similar to the progression in the orchard at São Carlos (Fig. 5). Apart from these 'waves', the curves of the diseased plants linearly increased from day 579 (August 2003) on. Disregarding the first four evaluations, linear regression lines gave a good fit to the data of the plot 'B untreated' ($R^2=0.99$) and 'B treated' ($R^2=0.96$). A systematic pattern of the residuals was observed in both plots, mainly resulting from the 'waves' (Fig. 44, Annex). The common non-linear functions used in epidemiology failed to properly describe the complete data of the diseased plants for both plots.

The difference between the curves of the symptomatic and the diseased plants in the orchard 'B untreated', which resulted from the drop in September 2003, diminished continuously, reaching almost the same level at the end of the evaluations. In the orchard 'B treated', 132 plants had been rogued and replaced by young ones between day 399 and day 484 (February to April 2003). As 118 of these removed plants had shown CVC symptoms prior to their removal, they remained in the cumulative incidence as diseased additionally to the newly planted trees, which caused a dispartment of the curves. The difference, which resulted from the decline at day 614 (September 2003), decreased during the following evaluations like in the plot 'B untreated'. At the end of 2004, the distance rose again due to the pruning of symptomatic plants.

After the roguing between the days 399 and 484, 126 new trees, assumed not to be infected with CVC, were planted in the plot 'B treated'. The first one of these trees showed CVC symptoms approximately 20 months later (November 2004). Until the end of the evaluations in September 2005, 25 of these plants showed CVC symptoms (19.8 %).

4.1.3. Temporal development of CVC in the State of Bahia, Brazil

The symptomatic and the diseased plants in the irrigated and non-irrigated plots in Bahia (Fig. 8) showed a completely different progress than the ones in the citrus orchards of São Paulo. In the irrigated plot, the incidence of symptomatic plants increased from the beginning of the evaluation at day 166 (June 2003) until day 491 (May 2004) with a slight decrease and a constant phase in between. After day 491, the curve decreased almost steadily until day 780 (January 2005) from 80 % to 10 %. Until the end of the evaluation, the incidence remained low, varying between 6 and 18 %.

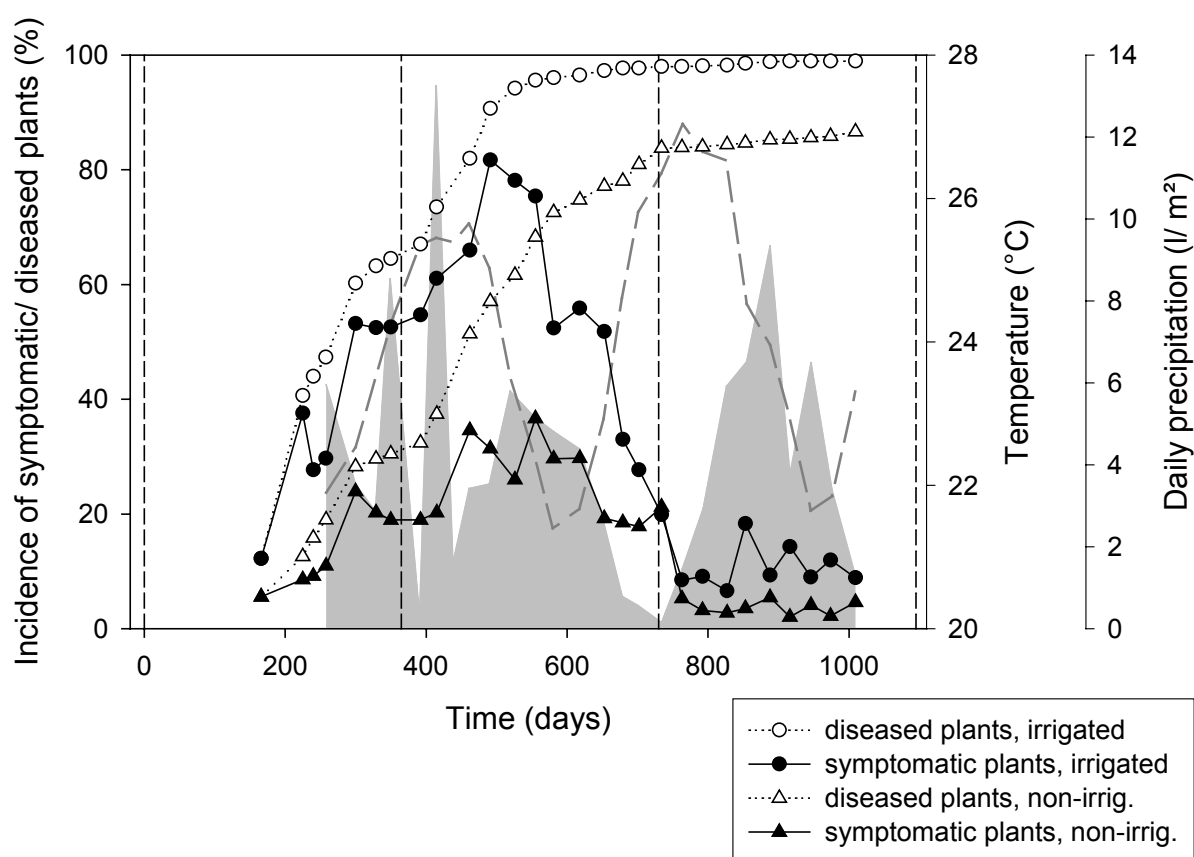


Fig. 8: Incidence of CVC symptomatic and diseased citrus plants (black lines), mean temperature (grey broken line) and daily precipitation (grey area) on a monthly basis in the plots **irrigated** and **non-irrigated** (1 & 2) in **Bahia**. Vertical lines represent the 1st January of the years. Day 0= 1.1.2003

The plots non-irrigated 1 and non-irrigated 2 were considered together in the temporal analyses. In this part of the orchard, a similar tendency as in the irrigated plot, but on a much lower level was observed. A rise in the incidence of symptomatic plants at the beginning of the evaluation was interrupted by a slight decrease after day 300 (October 2003) and a constant phase until day 415 (February 2004). The curve reached a local maximum at day 462 (April 2004) and, after a slight decrease in between, again at day

555 (July 2004), followed by an almost continuous decrease. From day 763 (January 2005) until the end of the evaluations in October 2005, the incidence of symptomatic plants varied around 5 %. Neither a seasonal pattern nor a relation to the meteorological conditions could be detected to explain the progress of the curves.

The cumulative incidence of the diseased plants increased monotonously in all plots, even if the symptomatic plants curve decreased. This indicates a contrary behaviour of the trees in the symptom appearance and disappearance. Despite a disease incidence of 99 % (irrigated plot), respectively 87 % (non-irrigated plots), only a very small part of the plants showed symptoms in the last evaluation year.

A double sigmoid logistic function well described the data of both plots, with a random pattern of the residuals. For the irrigated plot, an R^2 of 0.998 was obtained, for the non-irrigated plots, an R^2 of 0.997 (Fig. 45, Annex).

4.1.4 Temporal development of CVC in the Province Corrientes, Argentina

In the plots A and B in the Province Corrientes (Fig. 9), a very steep escalation of the incidence of symptomatic plants in the first four evaluation months (days 106 to 197, April to August 2004) was observed. In both orchards, the increase slowed down between day 197 and 259 (July/ September 2004) and reached asymptotic levels from day 289 (October 2004) on. This level was 89 % incidence in the orchard A and 99 % in plot B. In plot A, the incidence of symptomatic plants slightly decreased during the following evaluations, leading to small differences between the incidence of symptomatic and diseased plants. In plot B, both curves are nearly identical. Neither a seasonal pattern nor a relation to meteorological conditions was observed.

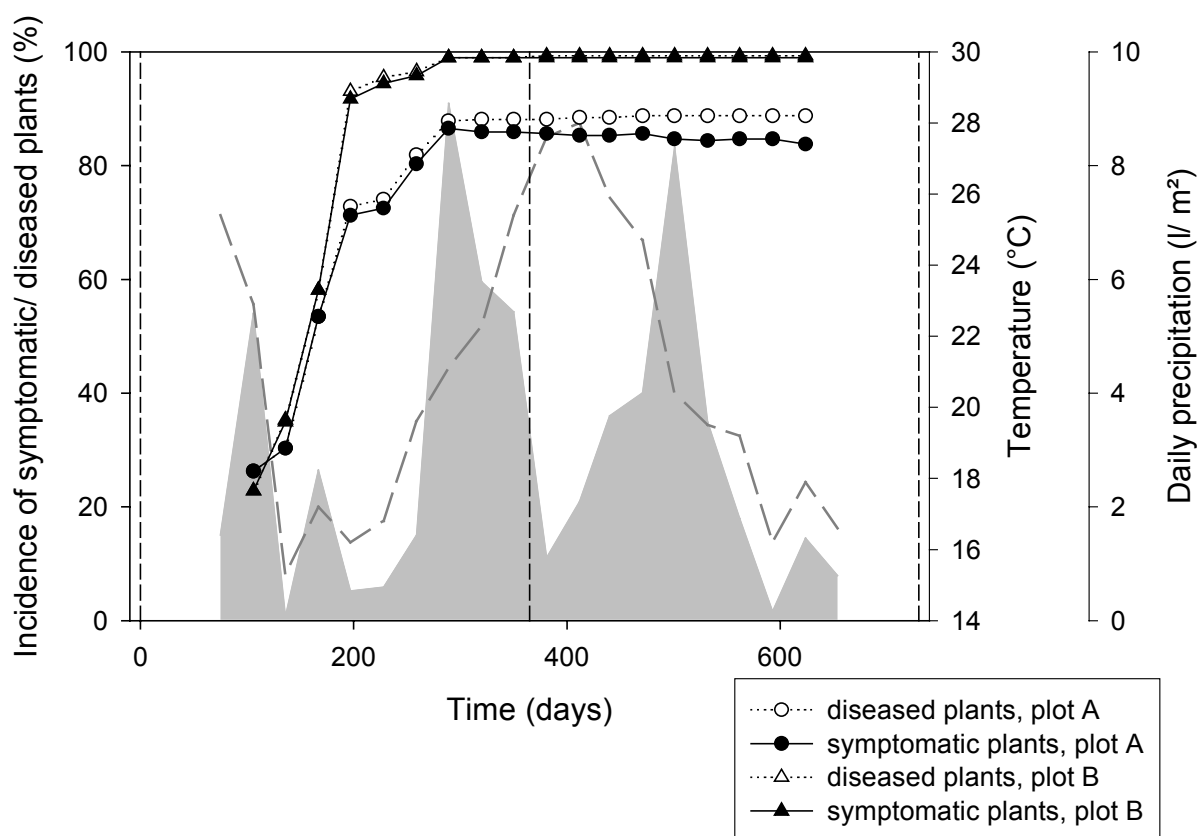


Fig: 9: Incidence of CVC symptomatic and diseased citrus plants (black lines), mean temperature (grey broken line) and daily precipitation (grey area) on a monthly basis in the plots **A** and **B** in the Province **Corrientes**. Vertical lines represent the 1st January of the years. Day 0= 1.1.2004

Logistic functions were successfully fitted to the data of the diseased plants of both plots. For plot A ($R^2 = 0.99$) as well as for plot B ($R^2 = 0.98$) the residuals were randomly distributed (Fig. 46, Annex).

In the irrigated and non-irrigated plot (Fig. 10), the incidence of symptomatic plants showed a higher variation than in the orchards A and B (Fig. 9). The non-irrigated orchard started from a much lower level than the irrigated one. From day 412 (February 2005) on until the end of the evaluations, both plots showed almost the same incidence of symptomatic plants. Three peaks were observed: one around day 167 (June 2004) in the irrigated plot and around day 228 (August 2004) in the non-irrigated plot, a second one at day 381 (January 2005) and a third one around day 501/ 532 (May/ June 2005) in both plots. The increasing parts of the curves coincided more or less with the times of high precipitation and the drops occurred in the dry periods. A relation between the temperatures and the occurrence of symptoms could not be clearly detected.

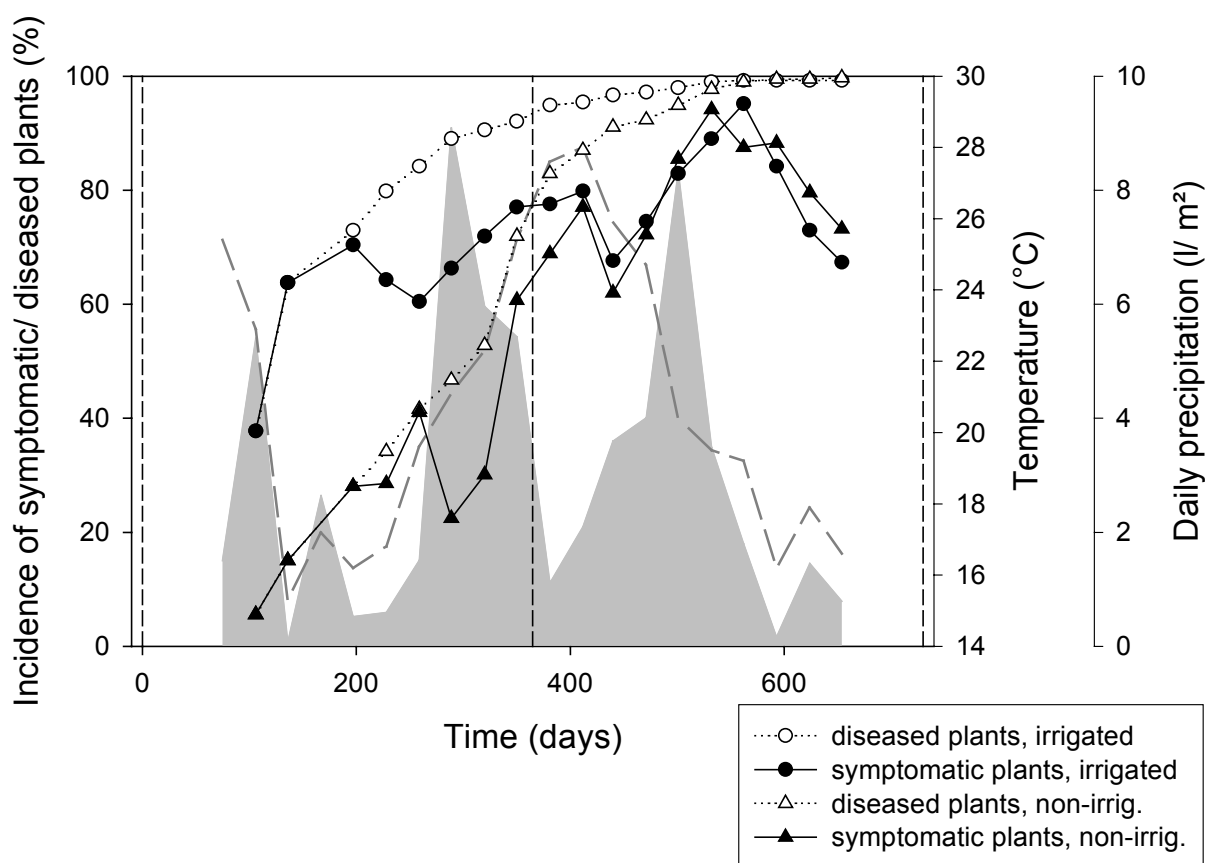


Fig. 10: Incidence of CVC symptomatic and diseased citrus plants (black lines), mean temperature (grey broken line) and daily precipitation (grey area) on a monthly basis in the **irrigated** and **non-irrigated** plot in the Province **Corrientes**. Vertical lines represent the 1st January of the years. Day 0= 1.1.2004

The difference between the curves of the symptomatic and diseased plants varied according to the declines of the curve of symptomatic plants. However, even if the amount of symptomatic plants dropped, the amount of diseased plants increased, more

distinct in the non-irrigated orchard. As in Bahia, this indicates a contrary behaviour of the appearance and disappearance of the symptoms.

The progress of the diseased plants in the irrigated plot could be well described by a logistic function ($R^2 = 0.97$), with a random pattern of the residuals. A double sigmoid logistic function gave a good fit to the data of the non-irrigated plot ($R^2=0.99$), without a systematic pattern of the residuals (Fig. 47, Annex).

Between day 300 and 400, the increase of the symptoms incidence seemed to be related to the increase of the temperature. However, the following rise from March to July 2005 (days 433-562) occurred at a time with falling temperatures.

4.2. Times of the highest increase of the symptom incidence

This type of data presentation aimed to better visualize the times at which the incidence of the symptoms increased or decreased.

4.2.1. Symptom incidence of CLS in the State of Minas Gerais, Brazil

In the coffee plots SG1 and SG2 at São Gotardo, a very strong seasonal pattern in the incidence of symptomatic plants could be detected. Increases of the incidence of symptomatic plants were observed exclusively in the second half of the years, indicated by the positive values for the difference in the incidence of symptomatic plants (Fig. 11). The highest positive values occurred at the end of the years, shortly after the rise of the temperature. In the first half of the years, only negative values were obtained, indicating a decrease of the symptoms incidence. The distinct drops at the beginning of the years occurred during the raining season.

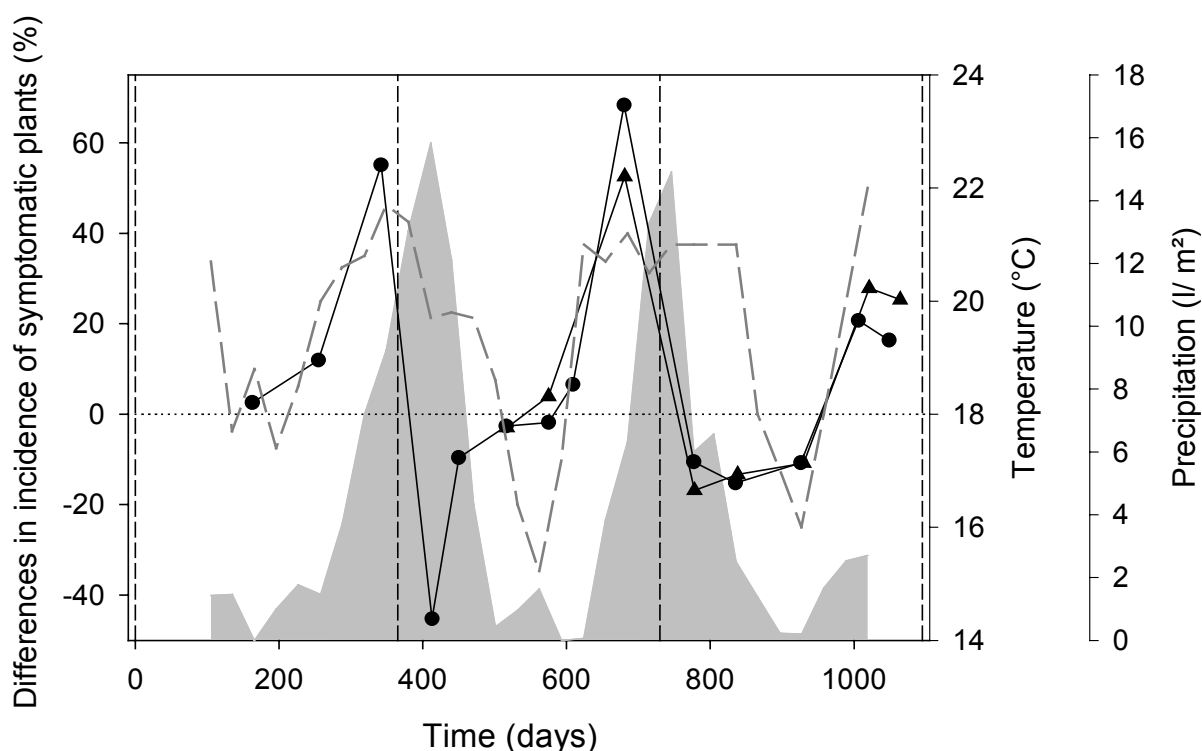


Fig. 11: Differences in the incidence of CLS symptomatic coffee plants (black lines), mean temperature (grey broken line) and mean daily precipitation (grey area) on a monthly basis in the plots **SG1** (●) and **SG2** (▲) at **São Gotardo**. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2003

4.2.2. Symptom incidence of CVC in the State of São Paulo, Brazil

For the plot at São Carlos, positive values were obtained from the beginning of the evaluations until the mid of 2003. In the second half of 2003, two distinct negative values were observed, indicating a strong decrease of the incidence of symptomatic plants (Fig. 12). These drops occurred at the end of the dry and colder season (day 614, September 2003) and at the beginning of the raining season (day 677, November 2003) with rising temperatures, respectively. In the year 2004, positive values were obtained for February and March (day 788 and day 819), which was the end of the raining season, as well as for July and August (day 914 and day 942), which was the end of the dry season and the time of the year at which the strong decline in the occurrence of symptoms was observed in the previous year. In 2005, the increasing tendency of symptomatic plants in February, March and April (days 1146 – 1206) was repeated. A negative value occurred at the beginning of July (day 1278), coinciding with the time of the lowest temperature and precipitation in that year.

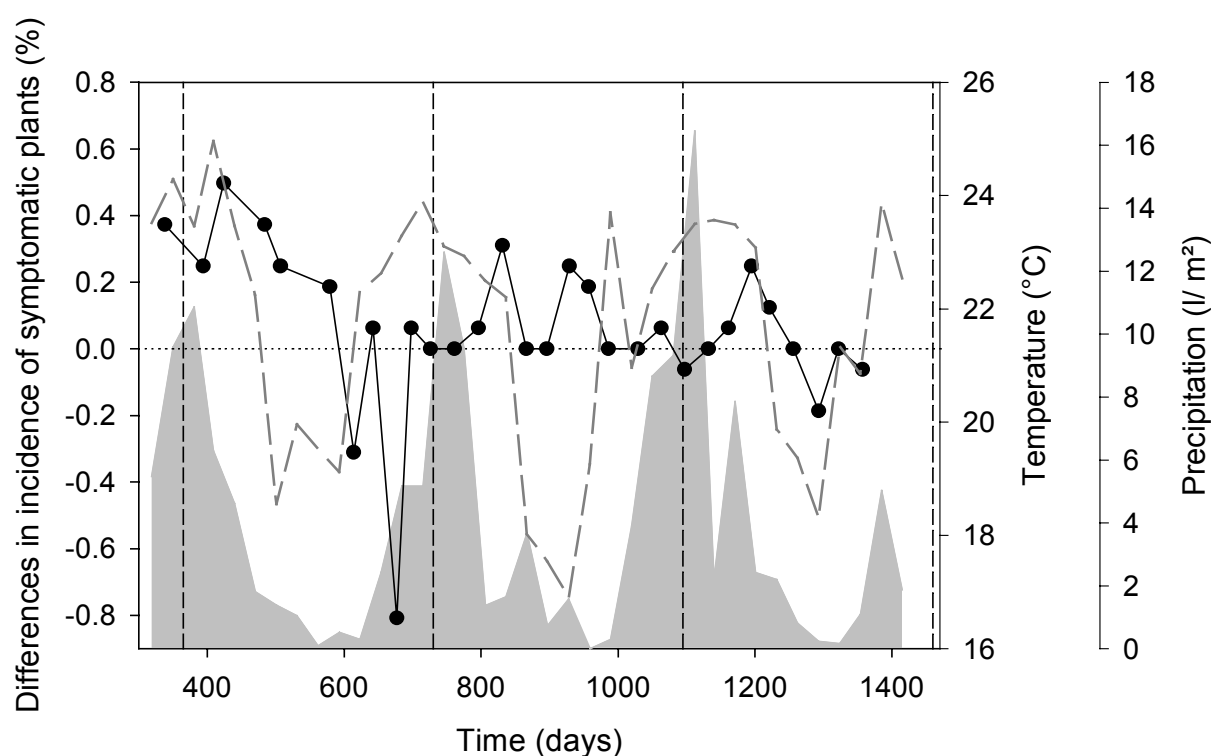


Fig. 12: Differences in the incidence of CVC symptomatic citrus plants (black line), mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the plot at **São Carlos**. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2002

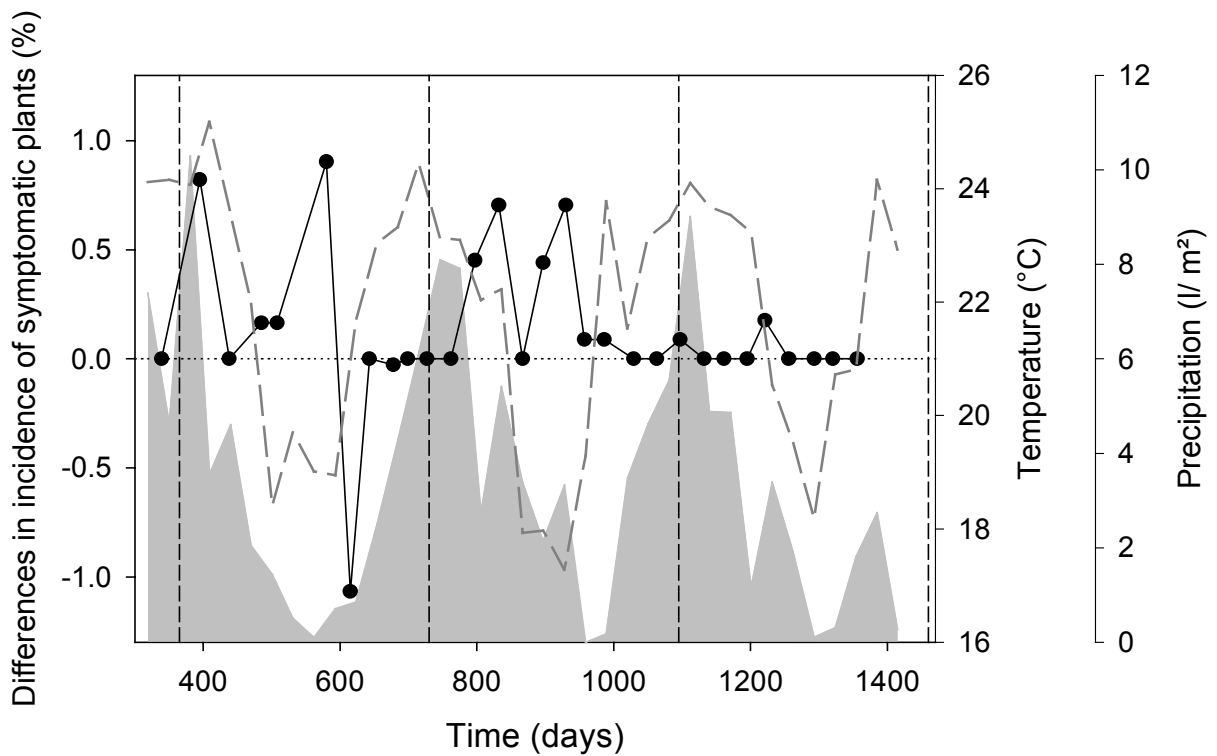


Fig. 13: Differences in the incidence of CVC symptomatic citrus plants (black line), mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the plot at **Engenheiro Coelho**. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2002

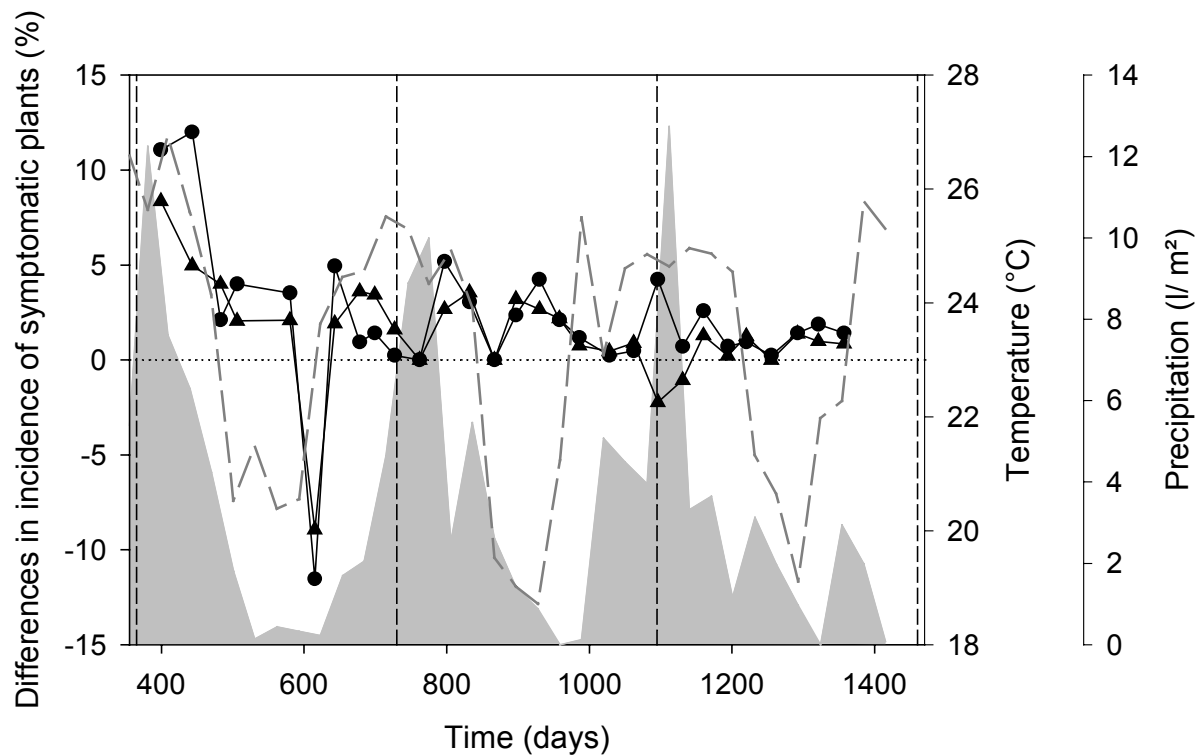


Fig. 14: Differences in the incidence of CVC symptomatic citrus plants (black lines), mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the plots '**B untreated**' (●) and '**B treated**' (▲) at **Bebedouro**. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2002

For the plot at Engenheiro Coelho, only one negative value was obtained for day 614 (September 2003), which was the end of the dry season with lower temperatures (Fig. 13). High positive values were obtained for January and beginning of August 2003 (day 394 and day 579) and for March and July 2004 (day 820 and day 915), respectively. In 2005, the only positive value could be observed in April (day 1206). The peaks in January 2003, March 2004 and April 2005 occurred during or at the end of the wet and warm season. The peaks in August 2003 and July 2004 occurred during the colder season, with very low precipitation during this time in 2003, but not in 2004.

In the plots 'B untreated' and 'B treated' at Bebedouro, negative values were obtained for September 2003 (day 614) (Fig. 14), as in the other two plots. At the end of 2004, negative values occurred in the plot 'B treated' due to the pruning of symptomatic plants. Positive values were observed for the complete first evaluation year, apart from the drop in September. The values were highest in February and March (day 399 and day 443). In 2004, the highest values were again obtained for February and March (day 797 and day 832), as well as for June, July and August (days 897 – 958). In the plot 'B untreated', a third peak occurred in December (day 1096). In the year 2005, higher values were observed again around February (day 1160) and July/ August (day 1292/ 1321).

4.2.3. Symptom incidence of CVC in the State of Bahia, Brazil

In the State of Bahia, a pattern of periods with high and low values was difficult to detect. Negative values occurred in the irrigated plot in August 2003 (day 239), from May until July (days 486 – 549) and from October until December 2004 (days 645 – 670), as well as in January (725), March (784), May (818), July (878) and September 2005 (day 935) (Fig. 15). In the non-irrigated plots, negative values were observed in November 2003 (day 326), April (457), May (486), July (549) and September 2004 (610), and January (725), February (735), June (844) and August 2005 (906). In the same way, the positive values are distributed all over the years without a visible pattern. A relation with the meteorological conditions could not be detected clearly. The relatively hot and dry period from October 2004 until February 2005 (days 680-800) cannot explain the frequent occurrence of negative values during this time, since a different reaction could have been expected from the irrigation. Moreover, many negative values were also observed during times of higher precipitation.

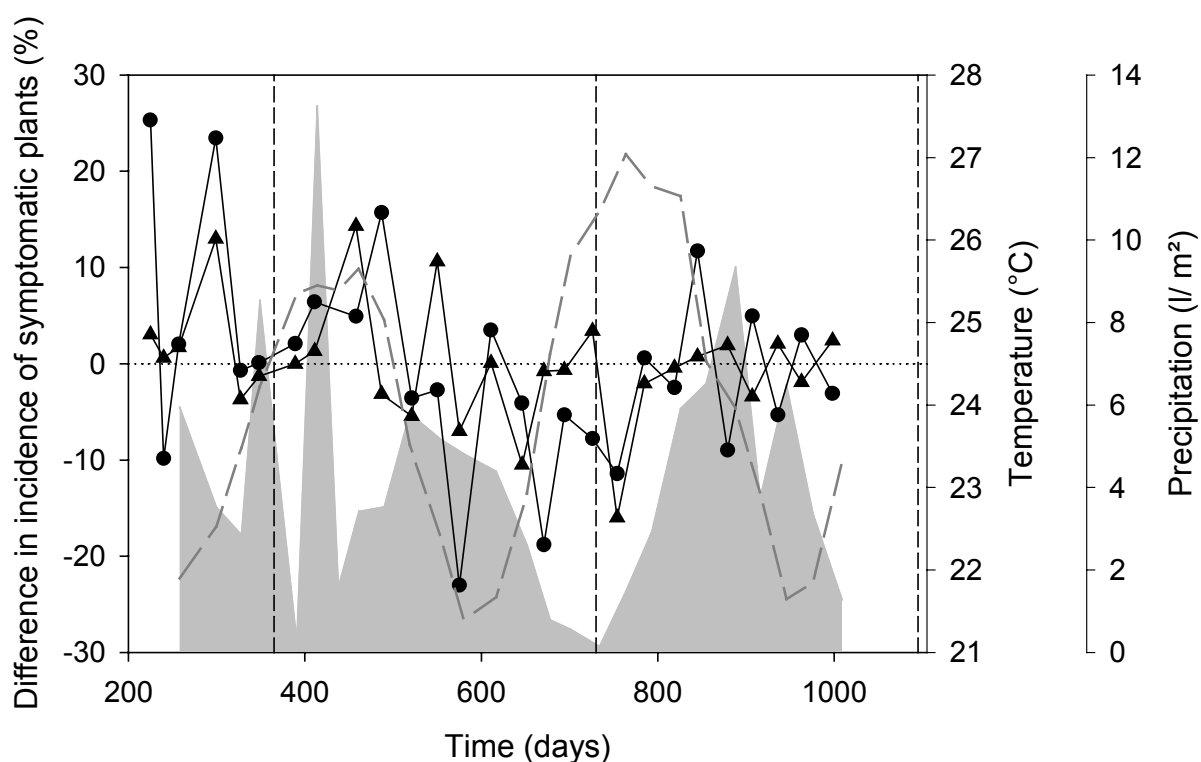


Fig. 15: Differences in the incidence of CVC symptomatic citrus plants (black lines), mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the plots **irrigated** (●) and **non-irrigated** (1 & 2) (▲) in **Bahia**. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2003

4.2.4. Symptom incidence of CVC in the Province Corrientes, Argentina

For the plots A and B, positive values were obtained for the first assessments from May to October 2004 (days 136 – 289). From November 2004 until September 2005 (days 320 – 624), slightly negative (plot A) or values near zero were observed (data not shown). Neither a seasonal pattern nor a relation to the meteorological conditions could be detected.

For the irrigated and non-irrigated plot, negative values were obtained in July/ August (days 197/ 228) (irrigated) and September 2004 (day 259) (non-irrigated), in February 2005 (day 412) and in June and August (day 532 and day 593) (non-irrigated) and July/ August 2005 (days 562/ 593) (irrigated) (Fig. 16). At the first and second decline, the temperatures were relatively low, but the drop in February 2005 coincided with the maximum temperatures of the year. Negative differences mainly occurred at times of low precipitation. The first drop was less distinct in the irrigated plot, possibly as an effect of the irrigation, for the other points no difference was observed.

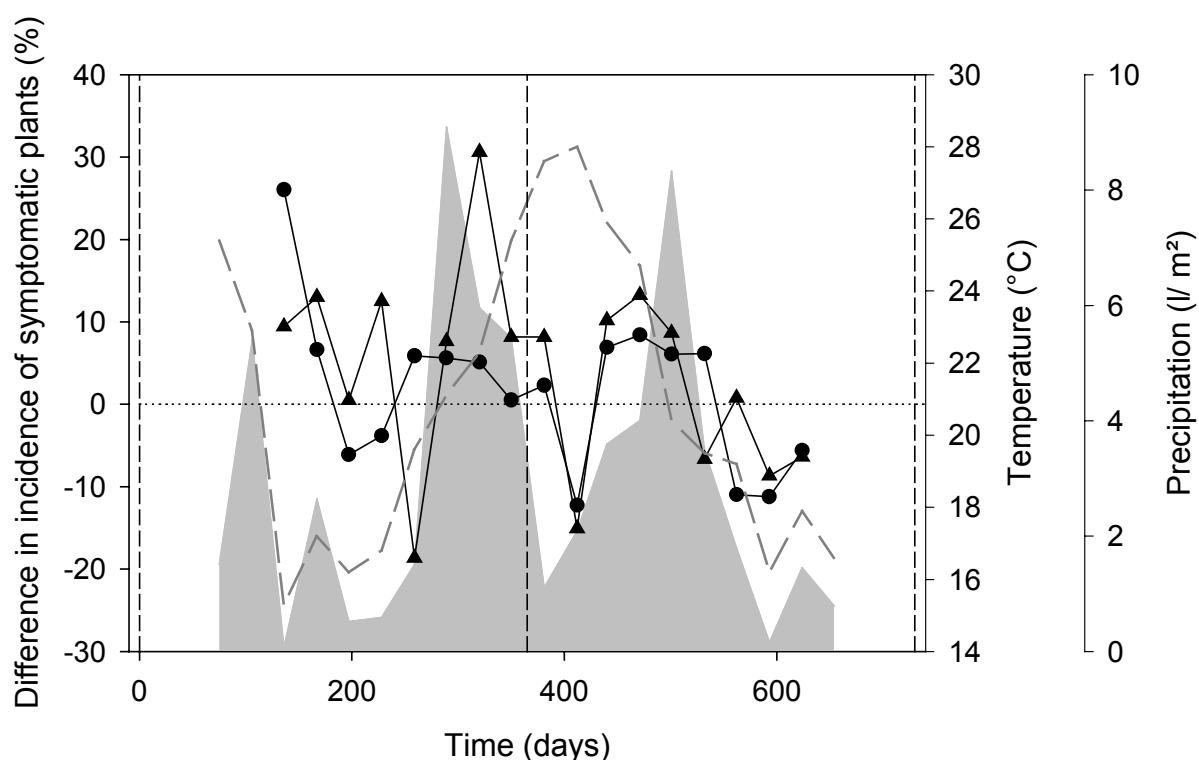


Fig. 16: Differences in the incidence of CVC symptomatic citrus plants (black lines), mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the plots **irrigated** (●) and **non-irrigated** (▲) in the Province **Corrientes**. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2004

High increases in the incidence of symptomatic plants, indicated by positive values, occurred in the irrigated plot in May and June 2004 (day 136 and day 167) as well as from March until June 2005 (days 440 – 532), and from August until October 2004 (days 228 – 289). In the non-irrigated plot, the peaks could be observed at May/ June (136-167), August (228) and from October until December 2004 (days 289 – 350). In the year 2005, the peaks occurred from March until May (days 440 – 501), almost coinciding with the ones of the irrigated plot. Apart from the beginning of the evaluation, the incidence of the symptoms increased at the beginning or during times of higher precipitation.

4.3. Persistence of the symptoms

This analysis aimed to detect how long the plants are showing the symptoms. It was only applied to the plots in the State of Bahia as well as to the irrigated and non-irrigated plot in the Province Corrientes, because in the other plots, decreases in the incidence of symptoms were not observed to a large extent and over a long period of time.

4.3.1. Persistence of the CVC symptoms in the State of Bahia, Brazil

In the irrigated plot in the State of Bahia, the symptoms of the plants remained for a shorter period of time in the second part of the evaluations compared to the initial phase (Fig. 17). However, even in the first part of the evaluations, symptoms disappeared to a large extent. From the symptomatic plants detected from day 166 until day 326 (June–November 2003), approximately 50 % remained symptomatic at the following six assessments.

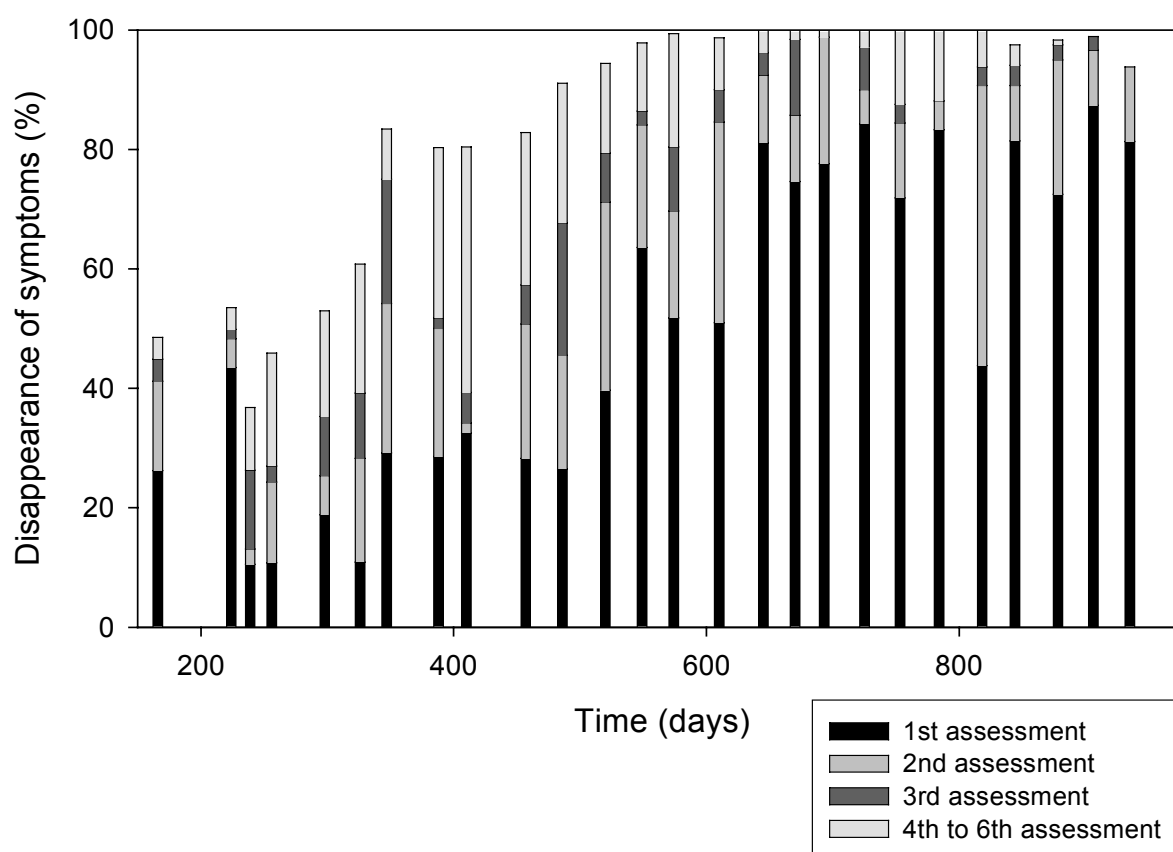


Fig. 17: Percentage of plants, which became asymptomatic within one of the following six assessments in the **irrigated** plot in **Bahia**. Each bar represents the assessment date, at which the plants were classified as symptomatic. The height of the bars reflects the total percentage of plants, which became asymptomatic again; the color indicates the following assessment at which the symptoms disappeared. Day 0 = 1.1.2003

From all plants ranked as symptomatic from day 520 (June 2004) on, 70 % were asymptomatic one or two evaluations later. In 90 % of these plants, the symptoms have disappeared until the sixth assessment. For the complete period, 67 % of the plants were asymptomatic within the following two evaluations and 85 % within the next six assessments on average.

In the non-irrigated plots (Fig. 18), the disappearance of symptoms was much more intense than in the irrigated plot. The percentage of plants, which became asymptomatic within the first two assessments after the symptoms have appeared, was always above 40 % and the average amounted to 78 %. After six evaluations, 95 % of all plants were asymptomatic. As already observed in the irrigated plot, the disappearance of symptoms was much faster in the second than in the first half of the evaluations.

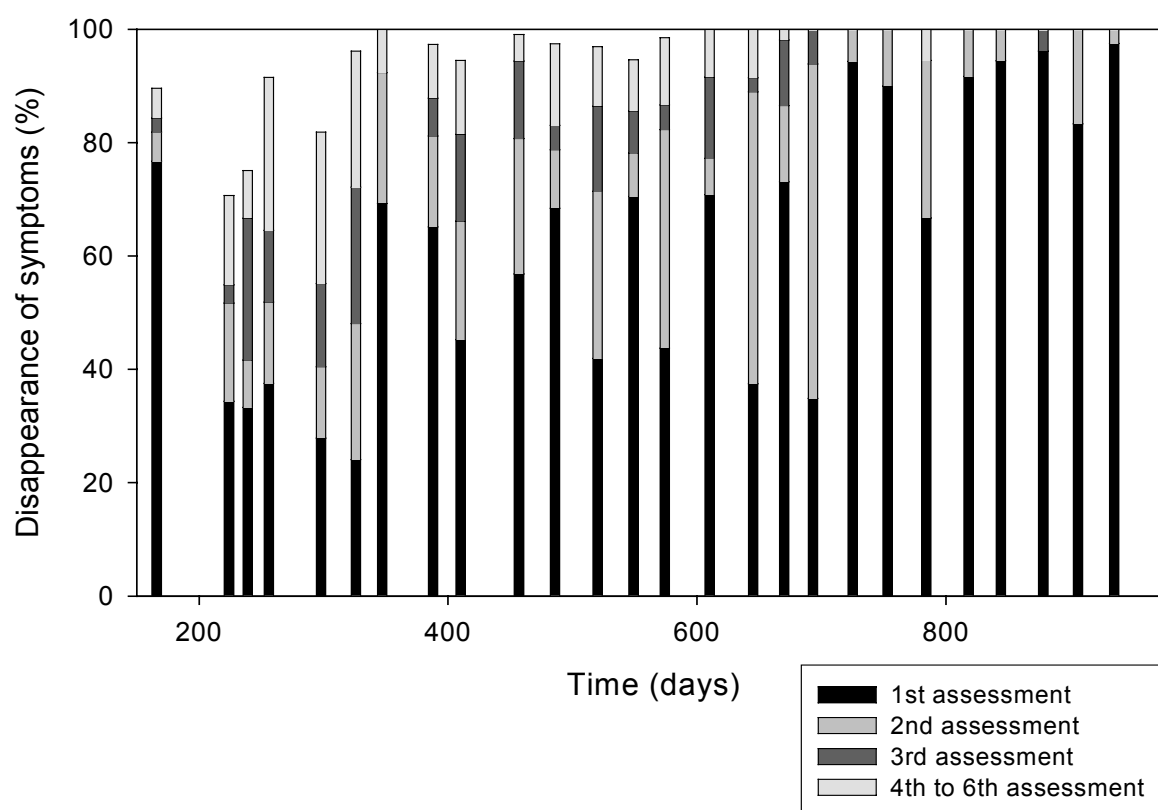


Fig. 18: Percentage of plants, which became asymptomatic within one of the following six assessments in the **non-irrigated** (1 & 2) plots in **Bahia**. Each bar represents the assessment date, at which the plants were classified as symptomatic. The height of the bars reflects the total percentage of plants, which became asymptomatic again; the color indicates the following assessment at which the symptoms disappeared. Day 0 = 1.1.2003

4.3.2. Persistence of the CVC symptoms in the Province Corrientes, Argentina

In the irrigated plot in the Province Corrientes (Fig. 19), the alteration of the disease status of the plants was not as extreme as in the plantation in the State of Bahia. On average, 29 % of the symptomatic plants became asymptomatic within the following two evaluations and 56 % within the next six ones. High values around 80 % were obtained for plants, which became symptomatic at the days 501 until 561 (May – July 2005).

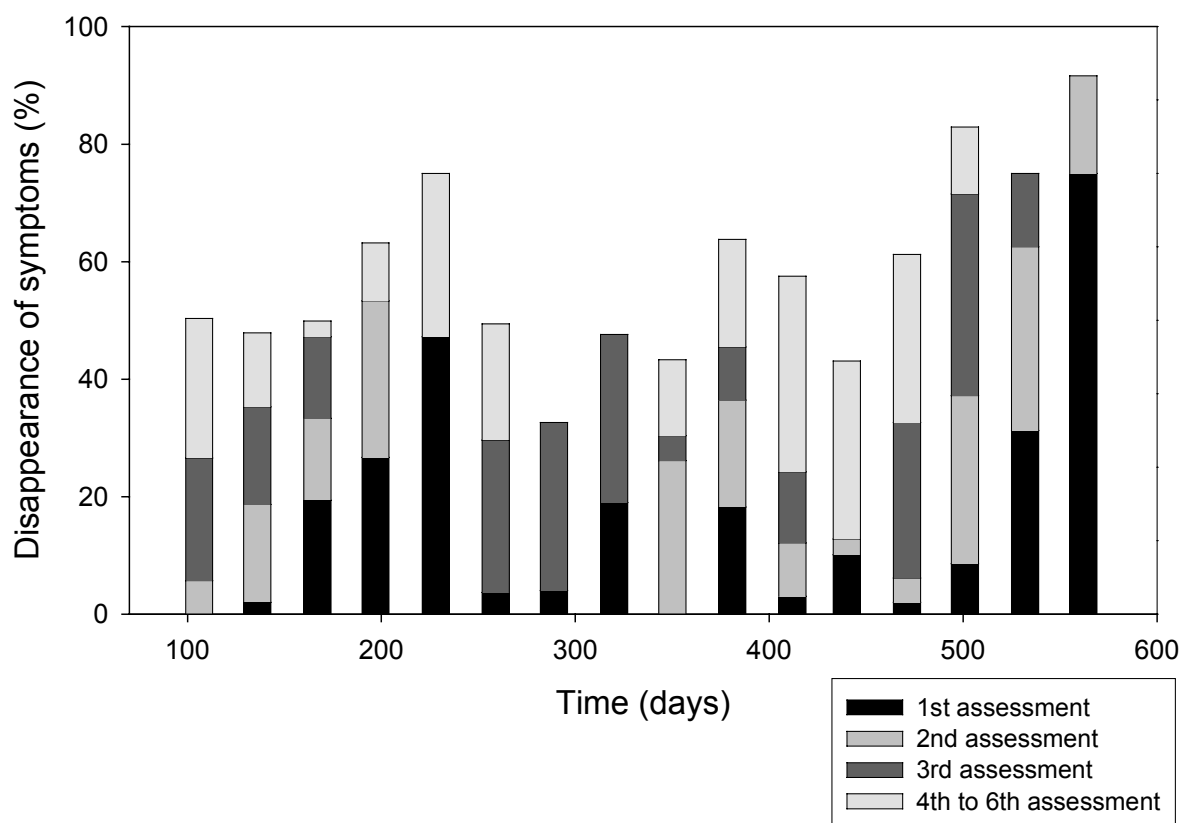


Fig. 19: Percentage of plants, which became asymptomatic within one of the following six assessments in the **irrigated** plot in the Province **Corrientes**. Each bar represents the assessment date, at which the plants were classified as symptomatic. The height of the bars reflects the total percentage of plants, which became asymptomatic again; the color indicates the following assessment at which the symptoms disappeared. Day 0 = 1.1.2004

In the non-irrigated plot (Fig. 20), the amount of plants in which the symptoms disappeared within the following six assessments after the initial detection was similar to that of the irrigated plot. However, a different pattern was observed. The highest values for the disappearance of symptoms occurred on plants, which became symptomatic from day 136 until day 228 (May – August 2004). The mean percentage of the plants, which became asymptomatic, was 33 % for the following two assessments and 55 % up to the sixth assessment.

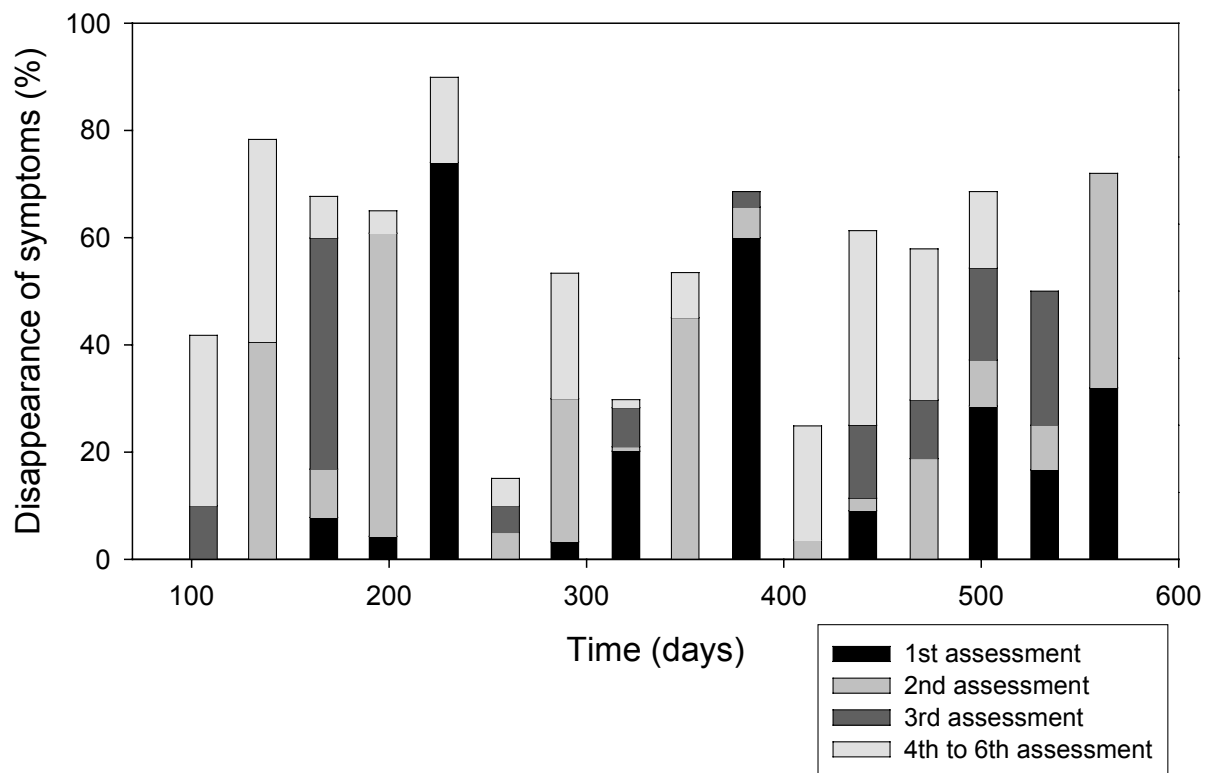


Fig. 20: Percentage of plants, which became asymptomatic within one of the following six assessments in the **non-irrigated** plot in the Province **Corrientes**. Each bar represents the assessment date, at which the plants were classified as symptomatic. The height of the bars reflects the total percentage of plants, which became asymptomatic again; the color indicates the following assessment at which the symptoms disappeared. Day 0 = 1.1.2004

4.4. Severity of the disease symptoms

The evaluation of the symptom severity in different grades aimed to detect seasons with favourable conditions for the symptom development additionally to the presence of symptoms. In the coffee plots, the symptom severity was not assessed.

4.4.1. Severity of the CVC symptoms in the State of São Paulo, Brazil

In the orchard at São Carlos, about 50 % of the symptomatic plants almost consistently showed symptoms of the severity grade 3 and only a small part of the symptomatic plants was considered as grade 2 (Fig. 21). Severity indices above 2 for all assessments were calculated, the highest values occurred between day 698 and day 796 (November 2003 and February 2004). At this time, the total incidence of symptomatic plants had decreased, indicating the disappearance of symptoms predominantly of the severity grade 1.

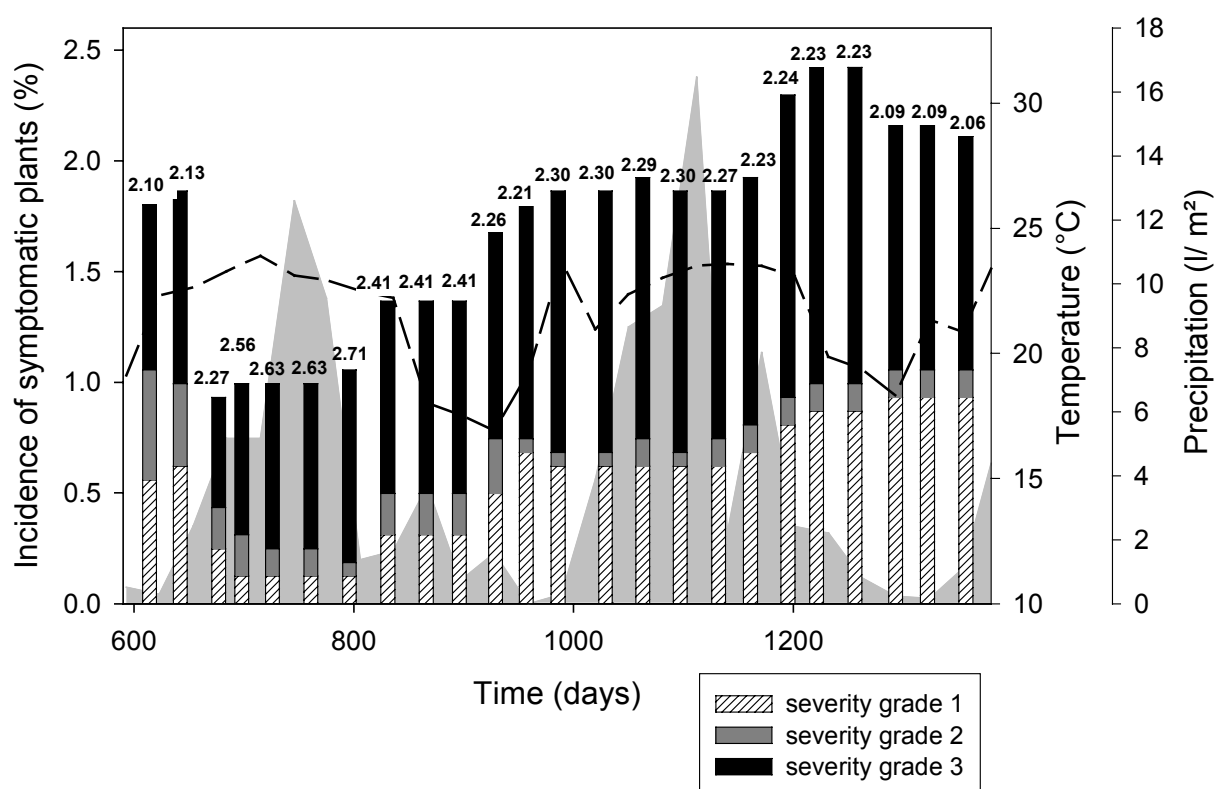


Fig. 21: Frequency distribution of the CVC symptom severity and the severity indices, mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the plot at **São Carlos**. Day 0 = 1.1.2002

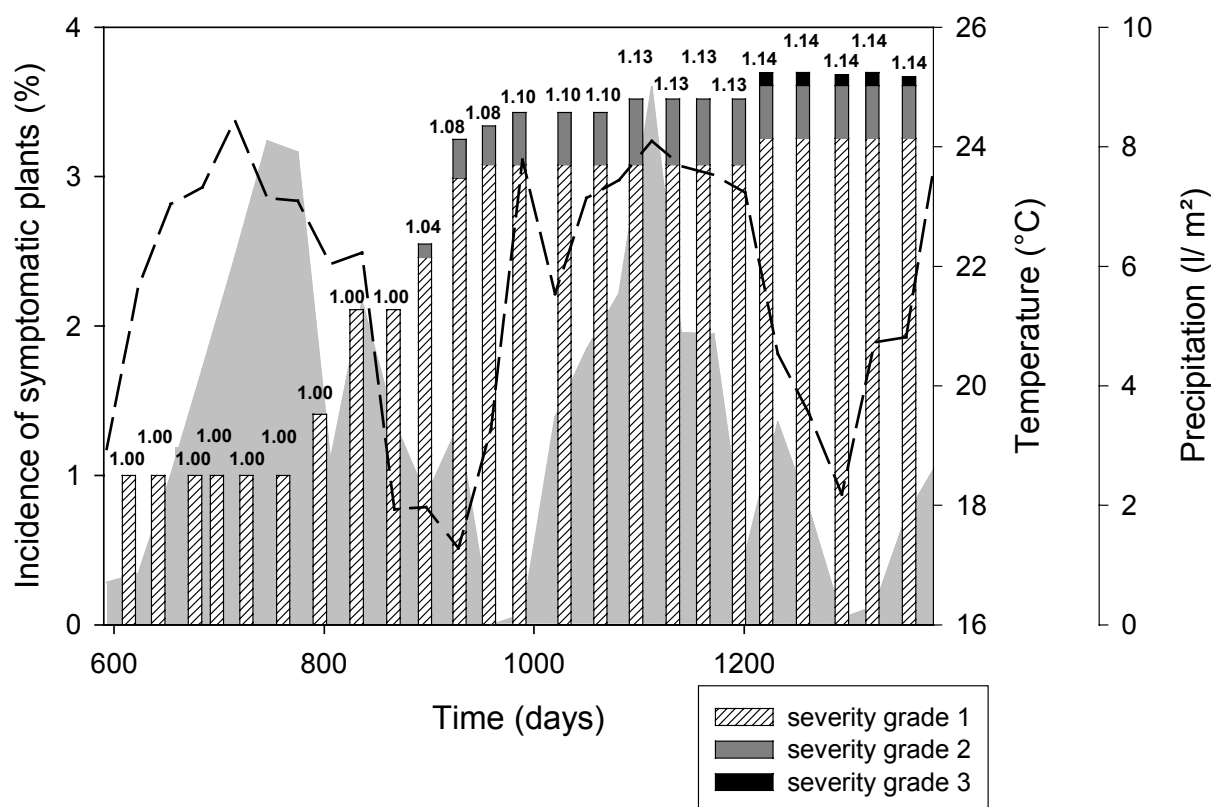


Fig. 22: Frequency distribution of the CVC symptom severity and the severity indices, mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the plot at **Engenheiro Coelho**. Day 0 = 1.1.2002

Although the total incidence of symptomatic plants in the orchard at Engenheiro Coelho (Fig. 22) was comparable to that at São Carlos, the plants showed a much lower symptom severity throughout the assessment period with severity indices ranging from 1.00 to 1.14. At day 869 (May 2004), the first plants with symptoms of the severity grade 2 were observed; plants with the severity grade 3 were first identified at day 1221 (April 2005), but only to a very low extent. At all assessments, plants showing symptoms of the grades 2 and 3 amounted to less than 10 % of the total symptomatic plants.

In the two orchards at Bebedouro, the total incidence of symptomatic plants was on a much higher level than in the orchards at São Carlos and Engenheiro Coelho. Throughout the evaluation, the severity indices of the orchard 'B untreated' increased steadily from 1.40 to 1.90 (Fig. 23), which was mainly due to the increase of the incidence of plants with the severity grade 3 from 4 % at the beginning to 23 % at the end of the severity grade assessment. The incidence of plants with the grade 2 remained almost constant. The percentage of plants with mild symptoms (grade 1) slightly increased throughout the evaluation.

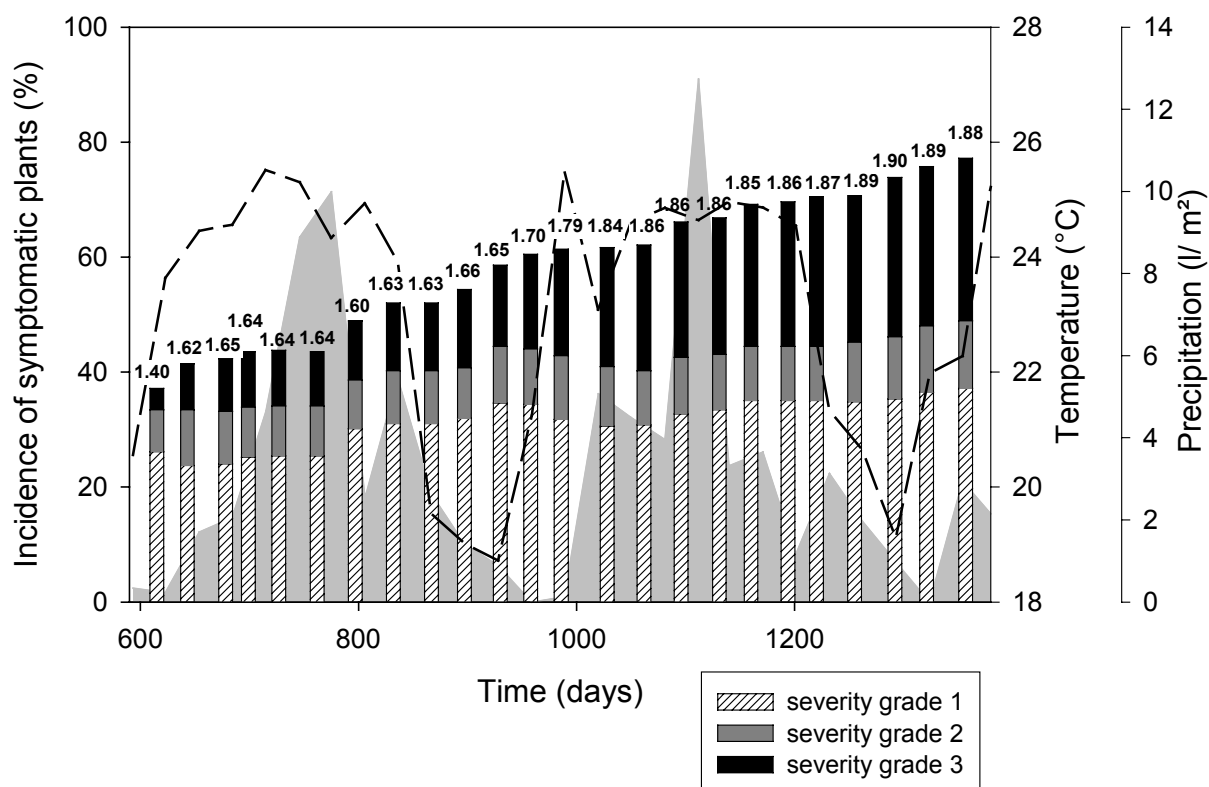


Fig. 23: Frequency distribution of the CVC symptom severity and the severity indices, mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the plot '**B untreated**' at **Bebedouro**. Day 0 = 1.1.2002

In the plot '**B treated**' (Fig. 24), the severity indices were slightly lower than in the plot '**B untreated**' (Fig. 23). The severity index increased from 1.43 at the first assessment to 1.63 at day 1096 (December 2004). The following decrease between days 1096 and 1131 was due to the pruning of plants, mainly of those with symptoms of the severity grade 3. Thereafter, the severity index showed again an increasing tendency. The incidence of plants with grade 1 rose continuously from 15 to 35 % during the assessments, those with grade 2 from 2.5 to 9 %. The incidence of plants with grade 3 also increased from 3 to 12 %, but only until day 1062 (November 2004). From day 1131 on, again an increasing trend of the incidence of plants with grade 3 was observed. For this orchard it has to be considered, that diseased plants had been removed at a time when the severity had not been assessed (between days 399 and 484).

In all evaluated plots in the State of São Paulo, a significant positive correlation of the incidence of symptomatic plants with the number of trees of the three severity grades exists (Pearson, $p < 0.05$). This indicates a rise of the incidence of the severity grades together with the incidence of symptomatic plants.

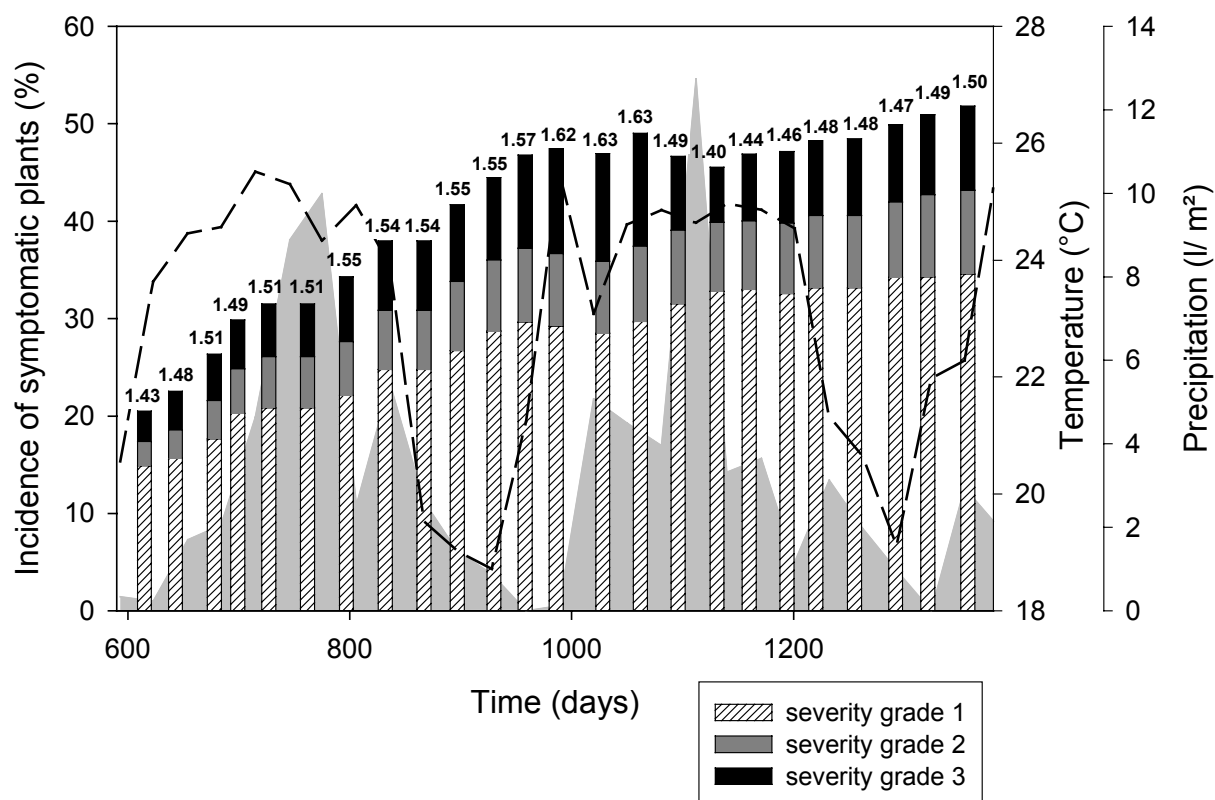


Fig. 24: Frequency distribution of the CVC symptom severity and the severity indices, mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the plot 'B treated' at Bebedouro. Day 0 = 1.1.2002

4.4.2. Severity of the CVC symptoms in the State of Bahia, Brazil

The symptom severity of the plants in the irrigated plot in Bahia is shown in Fig. 25. In this plot, the severity indices varied between 1.00 and 1.95 throughout the evaluations. At the beginning, only plants with symptoms of the grade 1 were observed. From the fifth assessment on, when plants with grade 2 occurred, the severity index increased up to day 457 (March 2004). Only at that date, plants with grade 3 were identified to a large extent, which led to a severity index near 2. In the following three assessments, the total amount of symptomatic plants was higher, but the severity index already started to decrease and fell almost monotonously until the end of the evaluations. As for the temporal analyses, a relation of the meteorological conditions to the severity indices is difficult to detect. The highest severity index occurred at a relatively dry period and high temperature.

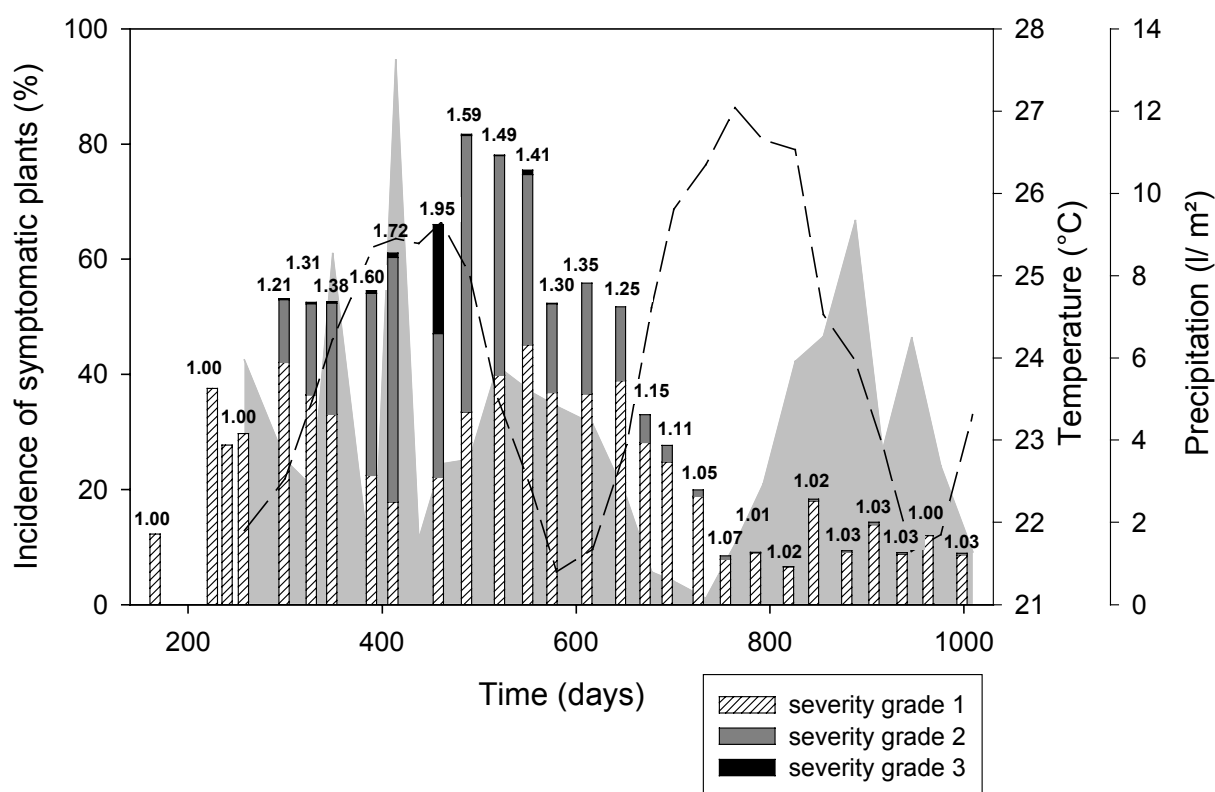


Fig. 25: Frequency distribution of the CVC symptom severity and the severity indices, mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the **irrigated** plot in **Bahia**. Day 0 = 1.1.2003

The symptom severity in the non-irrigated plots (Fig. 26) gave similar results as in the irrigated plot. At the beginning and at the end of the evaluation, only plants showing symptoms of the grade 1 were observed. From day 298 (October 2003) on, plants with grade 2 and, to a very small extent, grade 3 occurred. The symptom severity was lower than in the irrigated plot, as severity indices between 1 and 1.48 indicate. The greatest incidence of plants with grade 3 occurred at day 457 (March 2004), as in the irrigated plot.

The incidence of symptomatic trees was significantly correlated with the symptom severity only in the irrigated plot for the grades 1 and 2. (Pearson, $p < 0.01$).

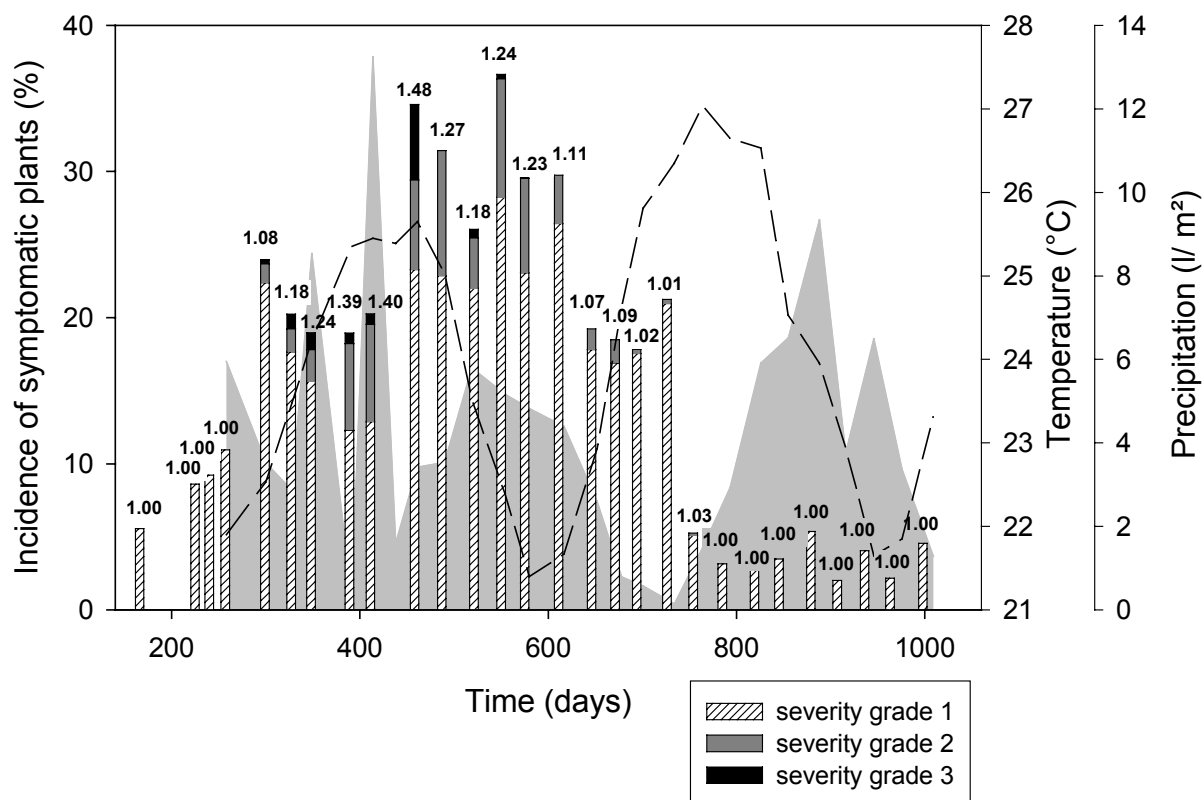


Fig. 26: Frequency distribution of the CVC symptom severity and the severity indices, mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the **non-irrigated** (1 & 2) plots in **Bahia**. Day 0 = 1.1.2003

4.4.3. Severity of the CVC symptoms in the Province Corrientes, Argentina

For the orchards in the Province Corrientes, four severity grades were applied; hence, the severity indices cannot be directly compared with the ones from the Brazilian citrus plots.

In the first two assessments in the orchard A, only plants with symptoms of the severity grades 1 and 2 were observed (Fig. 27). From the seventh assessment at day 289 (October 2004) on, the incidence of symptomatic plants remained almost constant at 95 %, but the severity of the symptoms changed during this time. The highest symptom severity occurred at day 350 (December 2004), when less than 10 % of the symptomatic plants showed mild symptoms (grades 1 and 2). This period was characterised by increasing temperatures and high precipitations. In the following two evaluations, peaks in the severity indices were observed at the days 471 (April 2005) and 562 (July 2005), which were within and at the end of the raining season. As a tendency, lower severity indices occurred at the end of the dry seasons.

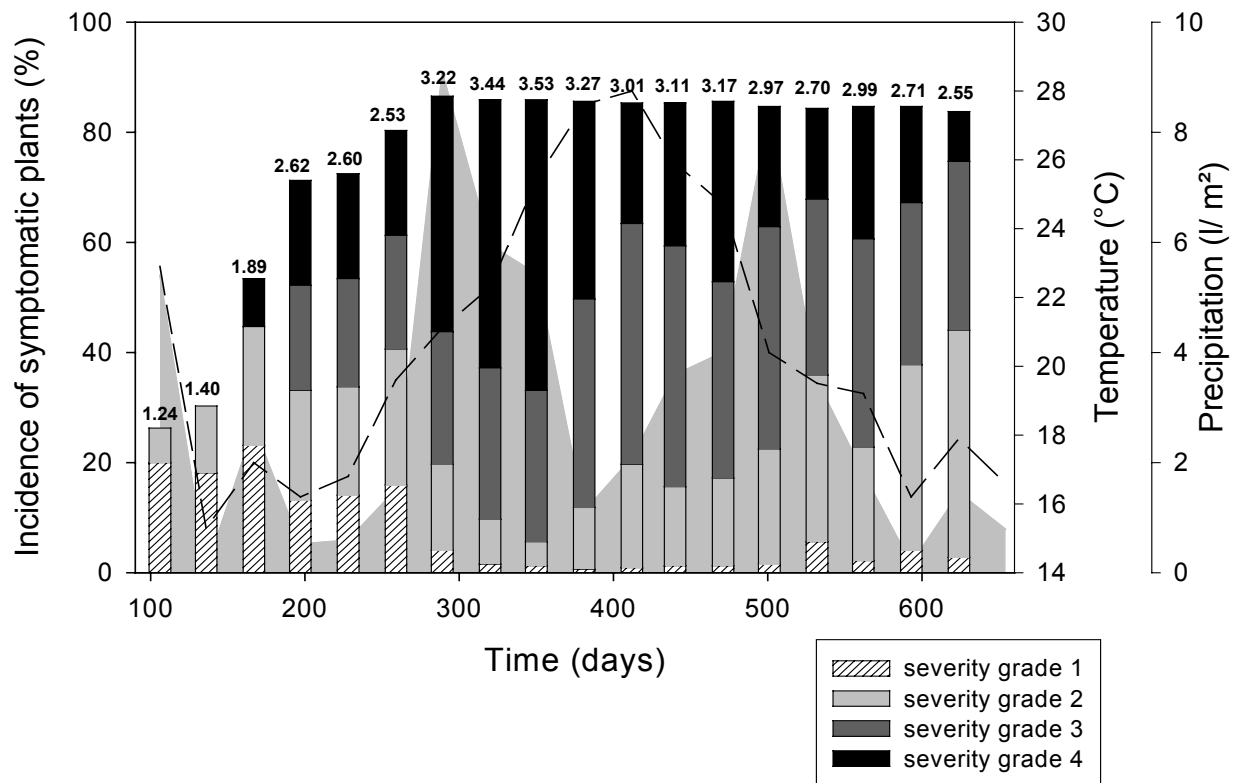


Fig. 27: Frequency distribution of the CVC symptom severity and the severity indices, mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in plot **A** in the Province **Corrientes**. Day 0 = 1.1.2004

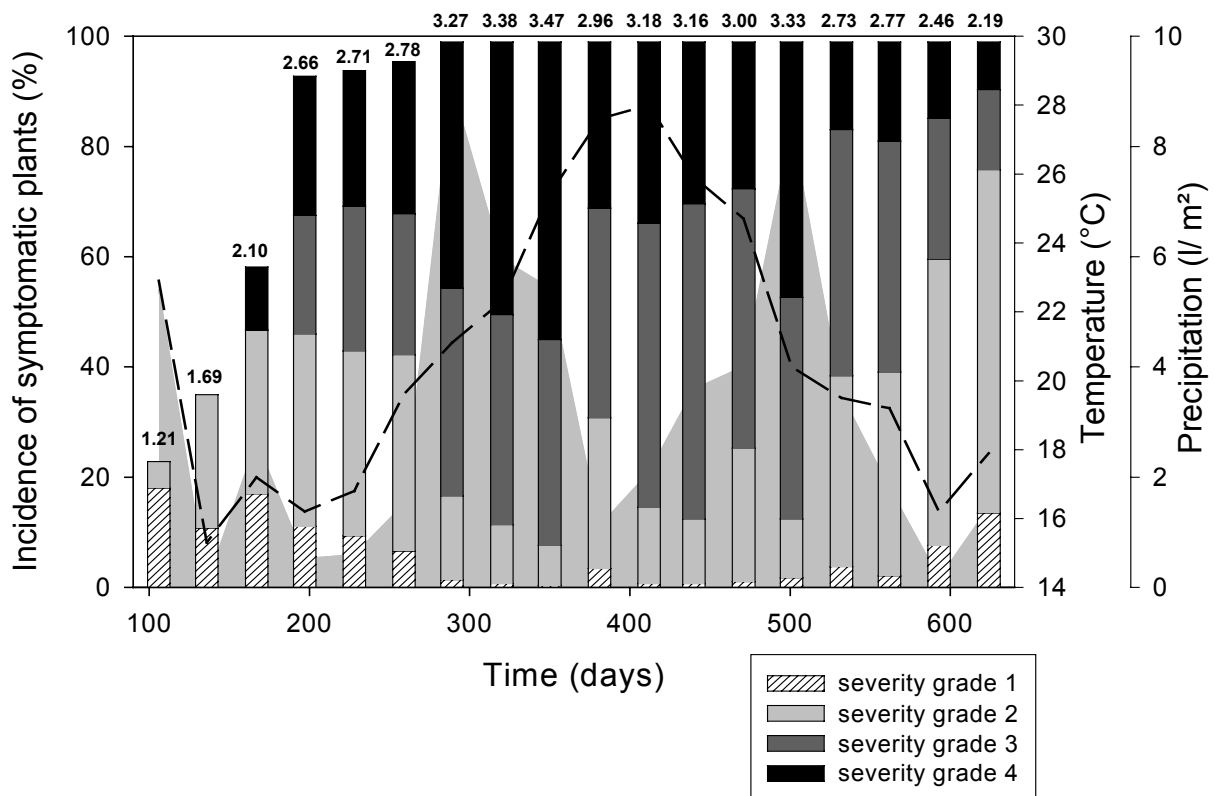


Fig. 28: Frequency distribution of the CVC symptom severity and the severity indices, mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in plot **B** in the Province **Corrientes**. Day 0 = 1.1.2004

In orchard B (Fig. 28), a similar trend was observed as in orchard A. From the beginning of the evaluations in April 2004 (day 106) on, the severity indices increased steadily until day 350 (December 2004), at which the highest severity index of 3.47 was determined. In the following assessments, the symptom severity was at a lower level around 3.0, but slightly increased again on day 501 (May 2005) to 3.33. In the last four assessments, the severity indices declined down to 2.19. As in the plot A, higher severity indices occurred during periods with high precipitation and vice versa.

For both plots, a significant positive correlation was calculated between the incidence of symptomatic plants and the severity grades 3 and 4. In addition, the symptom incidence and the severity grade 1 were significantly negative correlated ($p < 0.01$, Pearson).

In contrary to the orchards A and B, plants with severity grade 1 predominated in the irrigated orchard (Fig. 29). Plants with the grades 3 and 4 rarely occurred. Consequently, the severity indices remained below 2 throughout the evaluations. The highest severity index was determined in the mid of the raining season at day 532 (June 2005), which was also the time of the highest incidence of symptomatic plants.

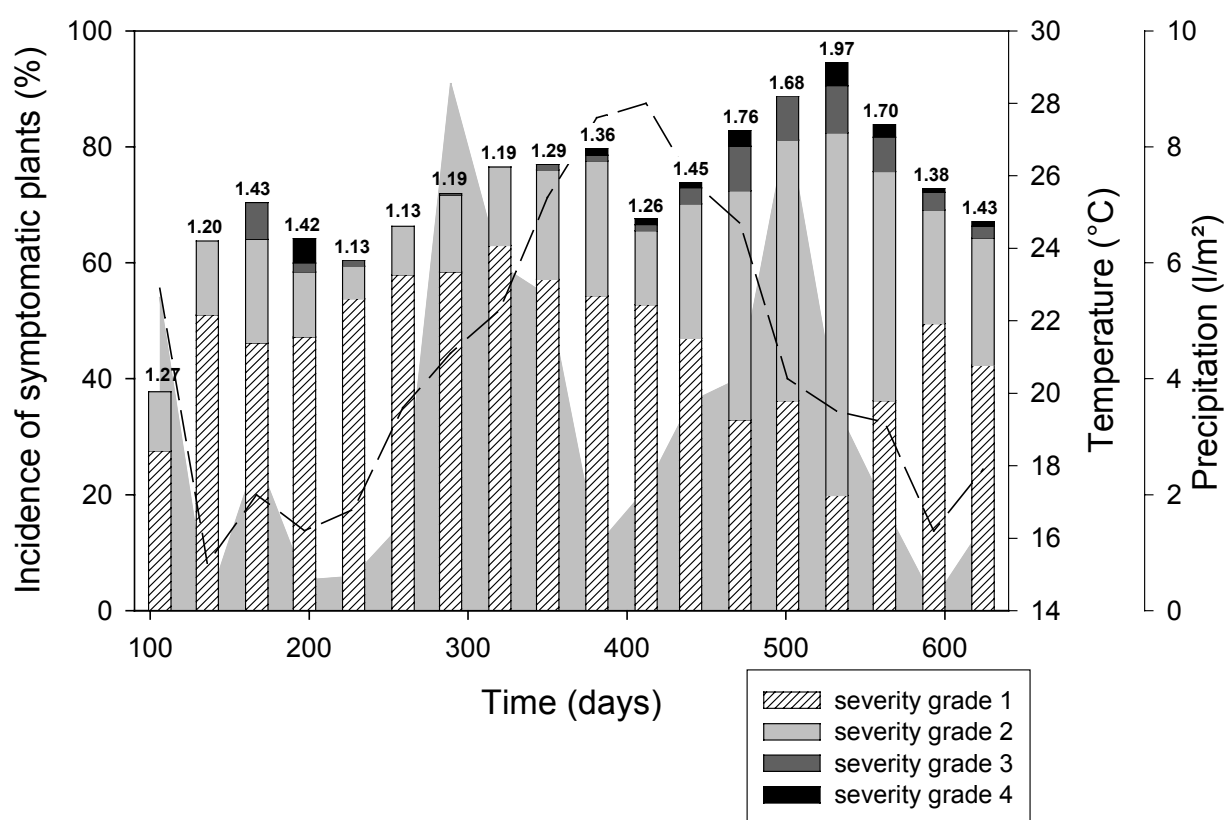


Fig. 29: Frequency distribution of the CVC symptom severity and the severity indices, mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the **irrigated** plot in the Province **Corrientes**. Day 0 = 1.1.2004

In the non-irrigated orchard (Fig. 30), the incidence of symptomatic plants and the severity indices were lower than in the irrigated plot in the first seven assessments (days 106 to 289, April to October 2004). From day 320 (November 2004) on, the incidence of symptomatic plants increased as well as the severity index.

The highest symptom severities were calculated at the days 532 and 562 (June and July 2005), the end of the wet period, with indices of 2.19 and 2.14, respectively.

A significant positive correlation between the incidence of symptomatic plants and the severity grades 2 and 3 was determined for both plots; in the non-irrigated plot a significant positive correlation also existed for the severity grade 4 ($p < 0.05$, Pearson).

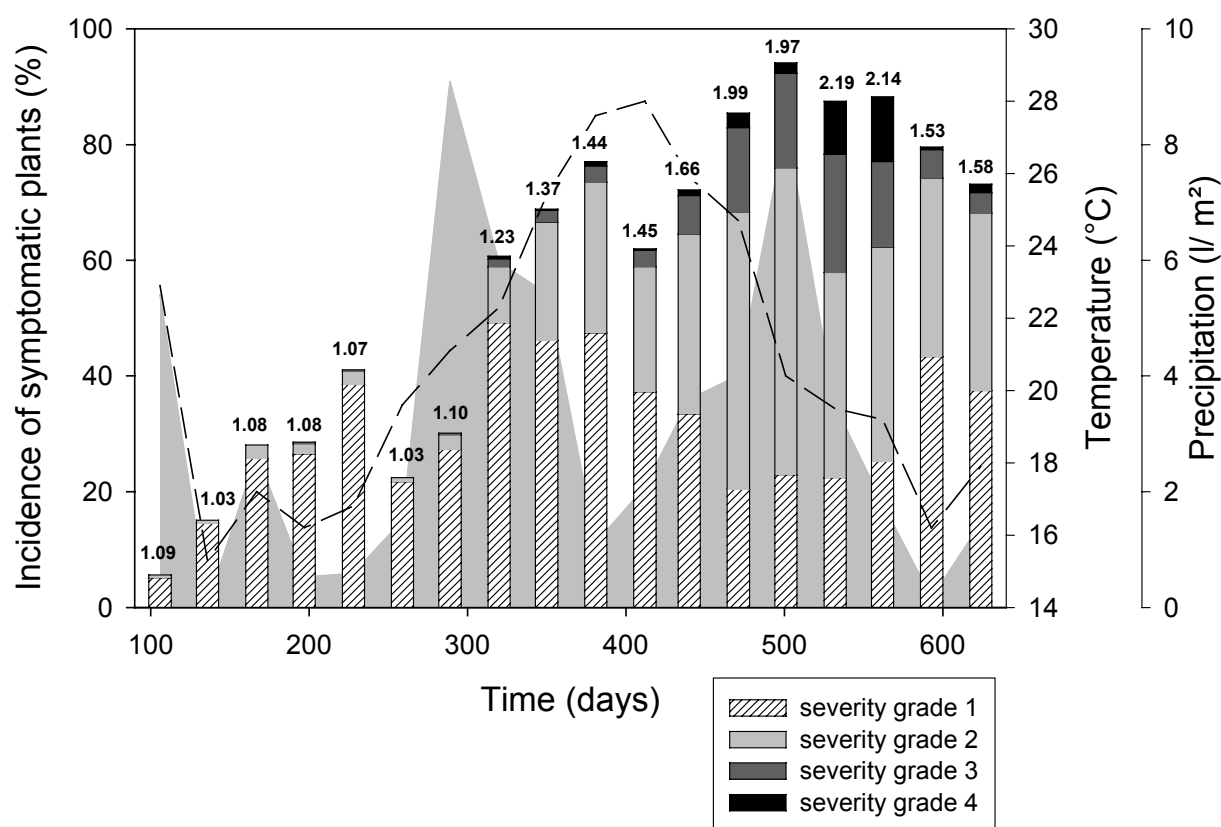


Fig. 30: Frequency distribution of the CVC symptom severity and the severity indices, mean temperature (broken line) and mean daily precipitation (grey area) on a monthly basis in the **non-irrigated** plot in the Province **Corrientes**. Day 0 = 1.1.2004

4.5. Spatial analyses of disease patterns

For the spatial analyses, cumulative data were used, since the incidence of diseased plants should rather reflect the disease status of the plants than the incidence of symptomatic plants.

4.5.1. Spatial pattern of CLS in the State of Minas Gerais, Brazil

For the plot SG1, the ordinary runs test indicated an aggregation of diseased plants within the rows for 56 to 89 % of all tested rows during the observation period, with the highest percentage at a disease incidence of 23 % at the third assessment (September 2003). In the plot SG2, all tested rows at all assessment dates showed a significant aggregation of diseased plants. In the plantation at Ervália, the percentage of rows with an aggregation of diseased plants ranged from 38 to 64 % with the highest values at disease incidences around 50 % (Tab. 8, Annex).

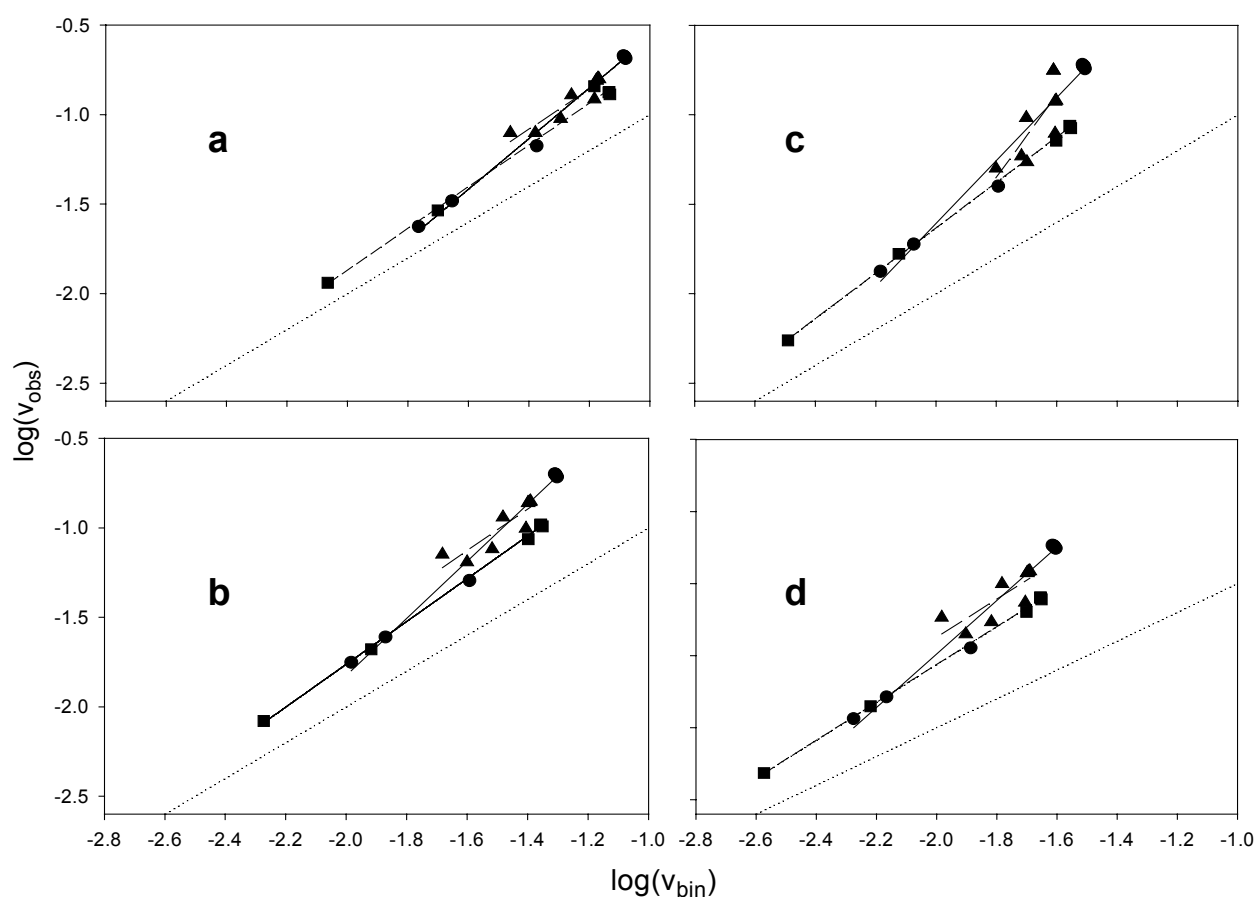


Fig. 31: Relationship between the logarithms of the observed (v_{obs}) and the estimated binomial variance (v_{bin}) in a quadrat based analysis with quadrat sizes of 1x3 (a), 1x5 (b), 1x8 (c) and 1x10 (d) plants in the plots **SG1** (●, solid regression lines), **SG2** (▲, long dashed regression lines) and **Ervália** (■, short dashed regression lines). The dotted lines represent the 1:1 relation ($v_{obs} = v_{bin}$).

The dispersion index DI was significantly greater than 1 for all tested quadrat sizes and assessment dates for all plantations, indicating an aggregated pattern of diseased plants. Apart from the last assessments of the plots SG2 and Ervália, the values of DI grew throughout the analysed period in all plots, suggesting an increasing grade of aggregation with escalating disease incidence (Tab. 8, Annex). This is also reflected in the slopes of the linear regression lines (Fig. 31), i.e. the parameter b of the Taylor law (Tab. 3). For the tested quadrat sizes, the relation between $\log(v_{\text{obs}})$ and $\log(v_{\text{bin}})$ was significant ($p < 0.05$, F-test) for all plots. The estimates of the parameters $\log(A)$ and b were significantly above 0 and 1, respectively, for all quadrat sizes for the plots SG1 and Ervália. This suggests a general and significant aggregation, whereby the degree of aggregation is a function of disease incidence. For the plantation SG2, only the parameter $\log(A)$ for the quadrat sizes 1 x 3 and 1 x 8 showed a significant departure from 0, although all DI values and all tested rows in the ordinary runs analysis for all quadrat sizes showed a significant aggregation of diseased plants (Tab. 8, Annex). The cause for this may be the inappropriateness of the linear regression for the data points, especially for the quadrat sizes 1 x 8 and 1 x 10, as the relatively low coefficients of determination R^2 and the relatively high standard errors for $\log(A)$ and b indicate (Tab. 3). In the plots SG1 and SG2, a disease gradient within the fields existed on a broad scale. Higher disease incidences were observed at the inner parts of the plantation, represented by low column numbers, compared to the borders of the plantation (Tab. 9 & 10, Annex).

Tab. 3: Taylor law parameters $\log(A)$ and b and their standard errors (SE) as well as the coefficients of determination R^2 for different quadrat sizes of the plots **SG1** and **SG2** at **São Gotardo** and at **Ervália**.

Plot	Quadrat size	No. of quadrats	Taylor law parameters				R^2
			$\log(A)^a$	SE	b^a	SE	
SG1	1 x 3	3914	0.85***	0.061	1.41**	0.047	0.99
SG2		2000	0.49*	0.202	1.12	0.161	0.87
Ervália		465	0.46***	0.058	1.16***	0.041	0.99
SG1	1 x 5	2318	1.36***	0.111	1.59***	0.073	0.99
SG2		1200	0.72	0.342	1.16	0.232	0.78
Ervália		270	0.63***	0.026	1.20***	0.016	0.99
SG1	1 x 8	1444	1.91***	0.162	1.76***	0.094	0.98
SG2		740	2.81*	1.151	2.31	0.692	0.61
Ervália		165	0.91***	0.023	1.27***	0.013	0.99
SG1	1 x 10	1140	2.22***	0.188	1.86***	0.104	0.98
SG2		600	1.28	0.633	1.32	0.356	0.66
Ervália		135	1.07***	0.030	1.31***	0.016	0.99

^a A significant departure of $\log(A)$ from 0 and of b from 1 is indicated as *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$, T-test.

4.5.2. Spatial pattern of CVC in the State of São Paulo, Brazil

In the citrus plots in the State of São Paulo, different tendencies of the spatial distribution of diseased plants were observed.

For the plot at São Carlos, an aggregation of diseased plants was indicated for 38 to 100 % of the tested rows by the ordinary runs analysis. The dispersion index DI showed a significant aggregation of diseased plants at all assessment dates and tested quadrat sizes. The highest DI values were calculated for dates at the end of the evaluations, at which the highest disease incidence of 3 % occurred. The DI values of the quadrat sizes 5 x 3 and 3 x 5 showed no great differences, indicating the same degree of aggregation within and across the rows (Tab.11, Annex).

The relationship between $\log(v_{obs})$ and $\log(v_{bin})$ was highly significant ($p < 0.001$, F-test) for the three quadrat sizes used in the Taylor law analyses (Tab. 4). The intercept $\log(A)$ and the slope b of the regression lines (Fig. 32, a, b, c) were significantly greater than 0 and 1, respectively, for all tested quadrat sizes, confirming a general aggregation of diseased plants and an increasing degree of aggregation with rising disease incidence.

In the Engenheiro Coelho plot, an aggregated pattern of diseased plants could not be detected by the ordinary runs analysis in any of the tested rows at any assessment date. The dispersion index DI indicated a significant aggregation of diseased plants for the second half of the evaluations for all tested quadrat sizes, apart from the quadrat size 5 x 3 (Tab. 11, Annex). Additionally, some DI values were significantly greater than 1 in the middle of the first half of the assessments for the tested quadrat sizes, except for quadrat size 3 x 5. For the quadrat size 5 x 3, higher DI values were obtained in the first half of the evaluation compared to the size 3 x 5. This relation turned to the opposite in the second half of the evaluation, indicating no clear tendency for a stronger aggregation within or across the rows.

For the three quadrat sizes used in the Taylor law analyses, the relationship between $\log(v_{obs})$ and $\log(v_{bin})$ was highly significant ($p < 0.001$, F-Test). Although the linear regression lines were close to the 1:1 relation and not all of the points had DI values statistically different from 1 (Fig. 32, a, b, c; Tab. 11, Annex), a significant departure of the Taylor law parameters $\log(A)$ and b from 0 and 1, respectively, was detected (Tab. 4), suggesting an increasing degree of aggregation as a function of disease incidence.

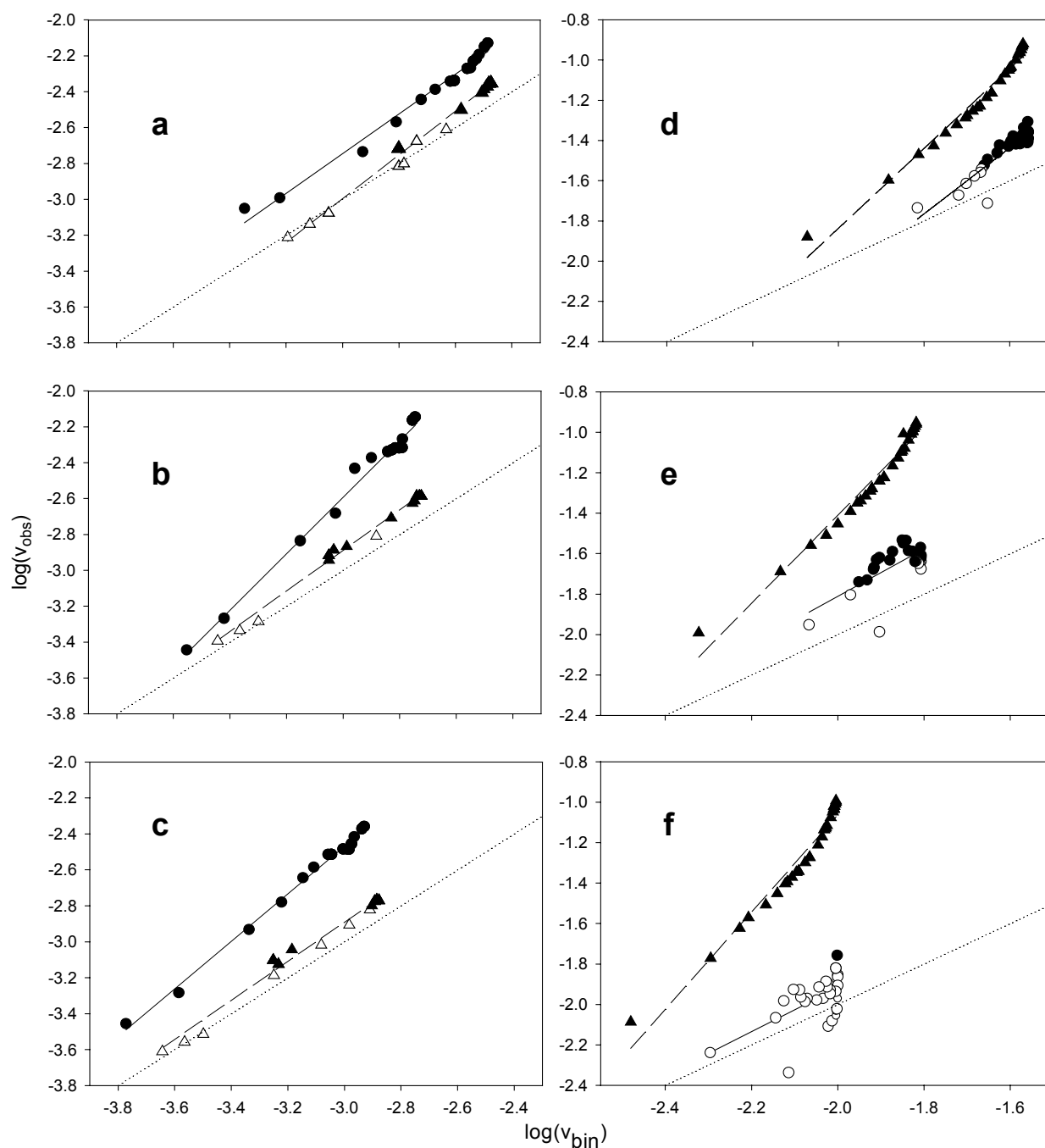


Fig. 32: Relationship between the logarithms of the observed (v_{obs}) and the estimated binomial variance (v_{bin}) in a quadrat based analysis with quadrat sizes of 3x3 (a, d), 4x4 (b, e) and 5x5 (c, f) plants in the plots at **São Carlos** (●, a, b, c, solid regression lines), **Engenheiro Coelho** (▲, a, b, c, long dashed regression lines), **'B untreated'** (●, d, e, f, solid regression lines) and **'B treated'** (▲, d, e, f, long dashed regression lines) at **Bebedouro**. Open dots (○)/ triangles (△) do not significantly differ from 1 ($p > 0.05$, Chi-Square test). The dotted lines represent the 1:1 relation ($v_{obs}=v_{bin}$).

In the plantation 'B untreated' at Bebedouro, the percentage of rows with significantly aggregated diseased plants ranged from 0 to 17 %. Higher values occurred in the first half of the evaluation at disease incidences below 55 % compared to the second half. No clear tendency was obtained by the dispersion index DI (Tab. 11, Annex). An

aggregated pattern of diseased plants for the quadrat size 5 x 3 was indicated for the whole assessment period, apart from the first two values. All values except one for the quadrat sizes 3 x 5 and 5 x 5, showed no departure from randomness. A significant aggregation was suggested for the quadrat size 3 x 3 from the third until the sixth but last assessment. For the quadrat size 4 x 4, aggregation was consistently detected in the second half of the evaluations, except from the last assessment, but only occasionally in the first half.

The relationship between $\log(v_{\text{obs}})$ and $\log(v_{\text{bin}})$ was highly significant ($p < 0.001$, F-test) for the three quadrat sizes used in the Taylor law analyses. For the quadrat size 3 x 3, the parameters $\log(A)$ and b were significantly greater than 0 and 1, respectively (Tab. 4). For the quadrat sizes 4 x 4 and 5 x 5, no significant difference from 0 and 1 could be detected for $\log(A)$ and b . Due to the high variability of the data for these quadrat sizes, very low values of R^2 and high standard errors for $\log(A)$ and b were obtained (Tab. 4, Fig. 32, d, e, f).

Tab. 4: Taylor law parameters $\log(A)$ and b and their standard errors (SE) as well as the coefficients of determination R^2 for different quadrat sizes of the plots at **São Carlos (SC)**, **Engenheiro Coelho (EC)** and **Bebedouro**, plots 'B untreated' and 'B treated'.

Plot	Quadrat size	No. of quadrats	Taylor law parameters				R^2
			$\log(A)^a$	SE	b^a	SE	
SC	3 x 3	165	0.58***	0.079	1.11**	0.030	0.98
EC		192	0.59***	0.084	1.19***	0.031	0.98
B untreated	3 x 3	48	1.19***	0.225	1.64***	0.139	0.83
B treated		384	2.14***	0.112	1.99***	0.067	0.97
SC	4 x 4	82	2.13***	0.109	1.57***	0.038	0.98
EC		108	0.50***	0.062	1.13***	0.021	0.99
B untreated	4 x 4	27	0.54	0.435	1.17	0.233	0.47
B treated		216	2.94***	0.150	2.17***	0.078	0.96
SC	5 x 5	66	1.50***	0.072	1.32***	0.023	0.99
EC		70	0.36***	0.084	1.09**	0.027	0.98
B untreated	5 x 5	14	0.24	0.589	1.08	0.288	0.34
B treated		126	3.75***	0.200	2.40***	0.096	0.96

^a A significant departure of $\log(A)$ from 0 and of b from 1 is indicated as *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$, T-test.

For the plot 'B treated' at Bebedouro, less than 20 % of the rows showed significant aggregation in the ordinary runs analysis during the whole assessment period. On the other hand, the dispersion index DI clearly indicated an aggregated pattern of diseased plants for all tested quadrat sizes at all assessment dates, with increasing DI values throughout the evaluation period. Higher values were obtained for the quadrat size 5 x 3

than for the size 3 x 5, reflecting in this case a higher degree of aggregation across than within the rows (Tab. 11, Annex).

The $\log(v_{\text{obs}}) - \log(v_{\text{bin}})$ relationship was highly significant for all quadrat sizes ($p > 0.001$, F-test). Very high values were obtained for the Taylor law parameters $\log(A)$ and b for all tested quadrat sizes (Tab. 5), confirming the high degree of aggregation based on the dispersion index DI , which escalated with the disease incidence. Moreover, for all quadrat sizes, the points of the last assessments were lying above the linear regression curve, indicating an increase above average (Fig. 32, d, e, f).

4.5.3. Spatial pattern of CVC in the State of Bahia, Brazil

In the irrigated plot in the State of Bahia, the percentage of rows with a significant aggregation of diseased plants ranged from 11 to 44 % (Tab. 12, Annex). The lowest values were obtained for the last assessments with disease incidences above 70 %. The dispersion index DI was statistically different from 1 for all quadrat sizes at all analysed assessment dates. The values increased from the beginning of the evaluation until a disease incidence of 60 % was reached, and then decreased.

The relation between $\log(v_{\text{obs}})$ and $\log(v_{\text{bin}})$ was highly significant ($p < 0.001$, F-test). The linear regression lines for the three quadrat sizes showed a distinct departure from the 1:1 relation (Fig. 33, a, b, c). The Taylor law parameters $\log(A)$ and b were statistically different from the expectation under the assumption of randomness for the three tested quadrat sizes, indicating stronger aggregation with increasing disease incidence (Tab. 5).

Tab. 5: Taylor law parameters $\log(A)$ and b and their standard errors (SE) as well as the coefficients of determination R^2 for different quadrat sizes of the plots **irrigated**, **non-irrigated 1** and **non-irrigated 2** in **Bahia**.

Plot	Quadrat size	No. of quadrats	Taylor law parameters				R^2
			$\log(A)^a$	SE	b^a	SE	
irrigated	3 x 3	95	0.87***	0.138	1.35**	0.083	0.97
non-irrigated 1		31	0.17*	0.080	1.08	0.046	0.98
non-irrigated 2		49	1.11***	0.309	1.43*	0.178	0.79
irrigated	4 x 4	47	1.42***	0.176	1.53***	0.091	0.97
non-irrigated 1		17	0.21	0.151	1.11	0.077	0.94
non-irrigated 2		27	1.88***	0.409	1.72**	0.206	0.80
irrigated	5 x 5	29	1.83***	0.267	1.60***	0.126	0.95
non-irrigated 1		9	0.75*	0.300	1.29	0.140	0.87
non-irrigated 2		17	2.26***	0.377	1.79***	0.174	0.86

^a A significant departure of $\log(A)$ from 0 and of b from 1 is indicated as *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$, T-test.

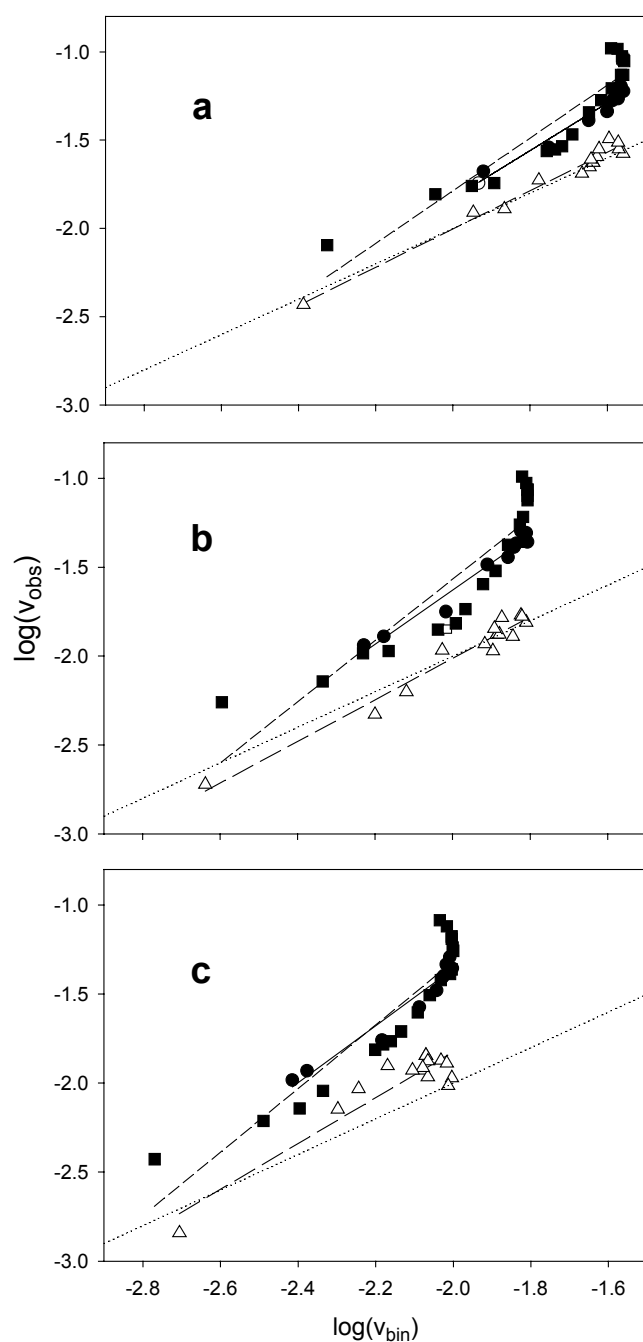


Fig. 33: Relationship between the logarithms of the observed (v_{obs}) and the estimated binomial variance (v_{bin}) in a quadrat based analysis with quadrat sizes of 3x3 (a), 4x4 (b) and 5x5 (c) plants in the plot irrigated (●, solid regression lines), plot non-irrigated 1 (▲, long dashed regression lines), and plot non-irrigated 2 (■, short dashed regression lines) in Bahia. Open dots (○)/ triangles (△)/ squares (□) do not significantly differ from 1 ($p > 0.05$, Chi-Square test). The dotted lines represent the 1:1 relation ($v_{obs}=v_{bin}$).

In the plot non-irrigated 1, the ordinary runs analysis detected an aggregation of diseased plants in less than 20 % of the rows, whereby the highest values were obtained for the first two assessments with disease incidences below 15 % (Tab. 12, Annex). The dispersion index DI showed no departure from randomness for all assessment dates and quadrat sizes, only three values of the quadrat size 5 x 3 were statistically different from 1. For this quadrat size, a bit higher DI values were obtained than for the size 3 x 5, indicating a slightly stronger aggregation within than across the rows.

The relation between $\log(v_{obs})$ and $\log(v_{bin})$ was highly significant ($p < 0.001$, F-test). The linear regression lines were very close to the 1:1 relation (Fig. 33 a, b, c). Consequently, neither $\log(A)$ nor b showed a departure from 0 and 1, respectively (Tab. 5).

In the plot non-irrigated 2, 7 to 33 % of the rows showed significant aggregation of diseased plants, whereby higher values were obtained in the first half of the evaluations with disease incidences below 60 % (Tab. 12, Annex). The dispersion indices DI were significantly different from 1 for

all tested quadrat sizes and assessment dates, except from the first four values for the size 3 x 5 and one value for the quadrat size 4 x 4 at the sixth assessment. At the beginning of the evaluations, higher DI values were obtained for the quadrat size 5 x 3 than for 3 x 5, while for the second half it was the other way round. For all quadrat sizes, the DI values rose overproportionally with increasing disease incidence. The $\log(v_{\text{obs}})$ - $\log(v_{\text{bin}})$ relation was highly significant ($p < 0.001$, F-test), although the relation was not linear, as can be seen in Fig. 33 a, b and c. This led to relatively low R^2 values and relatively high standard errors for the Taylor law parameters $\log(A)$ and b , which were nevertheless significantly greater than 0 and 1, respectively, for all quadrat sizes (Tab. 5).

4.5.4. Spatial pattern of CVC in Province Corrientes, Argentina

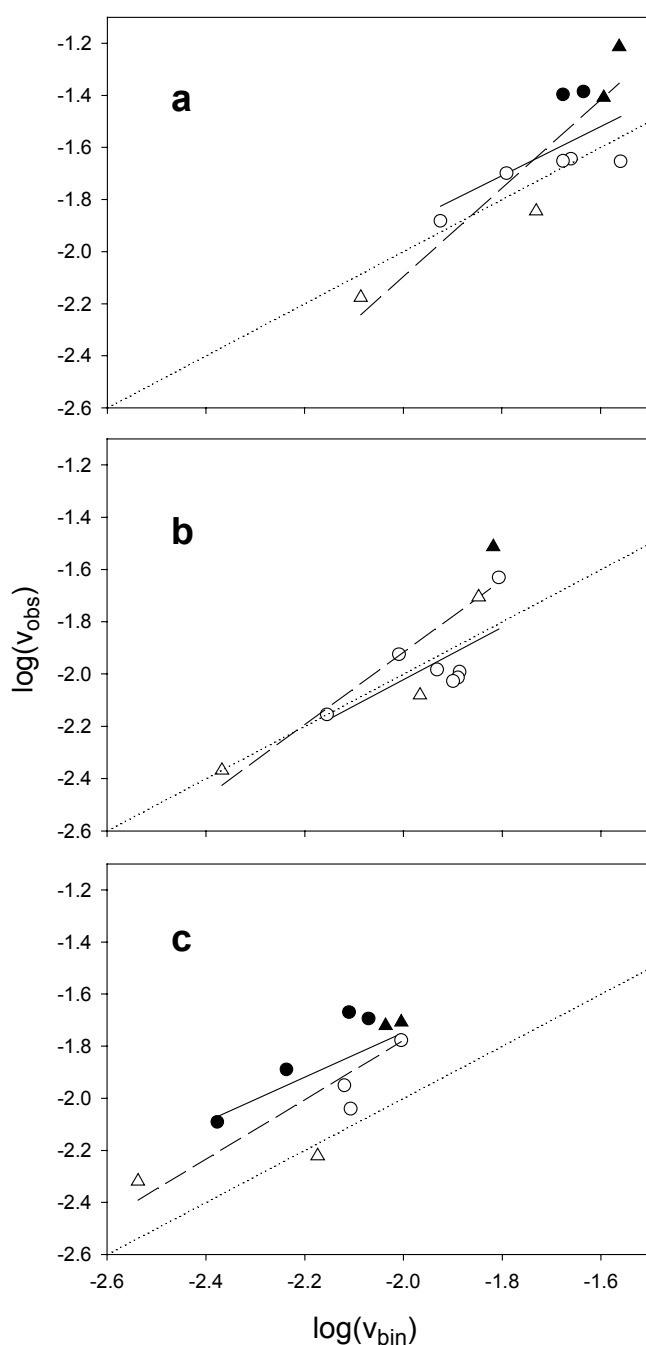


Fig. 34: Relationship between the logarithms of the observed (v_{obs}) and the estimated binomial variance (v_{bin}) in a quadrat based analysis with quadrat sizes of 3x3 (a), 4x4 (b) and 5x5 (c) plants in the plot **A** (●, solid regression lines) and **B** (▲, long dashed regression lines) in the Province **Corrientes**. Open dots (○)/ triangles (△) do not significantly differ from 1 ($p > 0.05$, Chi-Square test). The dotted lines represent the 1:1 relation ($v_{obs} = v_{bin}$).

For the plot A, the ordinary runs analysis indicated aggregation of diseased plants only for 1 out of the 10 rows tested at 5 from 7 assessments (Tab. 13, Annex). The dispersion index DI significantly differed from 1 at the first two evaluations for all tested quadrat sizes, except for the quadrat size 4 x 4. For the remaining data, aggregation was indicated only for the last two assessments for the quadrat size 5 x 5 and for the fifth assessment for the quadrat size 5 x 3. No clear trend was observed for the comparison of the quadrat sizes 3 x 5 and 5 x 3.

Due to the high variability of data, the linear regression lines had R^2 values below 0.5 for all quadrat sizes (Fig. 34, a, b, c; Tab. 6). Consequently, no statistical difference from the random case for the parameters $\log(A)$ and b could be detected.

Only four assessments of the plot B could be considered due to the rapid exceed of a disease incidence of 85%. The maximum percentage of aggregated rows detected by the ordinary runs analysis was 21% at the third assessment date (Tab. 13, Annex). The dispersion index DI indicated an aggregated pattern of diseased plants for all tested quadrat

sizes at the third assessment and for the quadrat sizes 3 x 3, 5 x 5 and 3 x 5 for the second assessment (Tab. 13, Annex). For the tested quadrat sizes, neither $\log(A)$, nor b showed a significant difference to 0 or 1, respectively (Tab. 6). The regression line for the quadrat size 5 x 5 showed a different tendency than for the sizes 3 x 3 and 4 x 4 (Fig. 34, a, b, c).

Tab. 6: Taylor law parameters $\log(A)$ and b and their standard errors (SE) as well as the coefficients of determination R^2 for different quadrat sizes of the plots **A** and **B** in the Province **Corrientes**.

Plot	Quadrat size	No. of quadrats	Taylor law parameters				R^2
			$\log(A)^a$	SE	b^a	SE	
A	3 x 3	30	-0.01	0.854	0.94	0.500	0.41
B		25	1.29	0.769	1.70	0.438	0.88
A	4 x 4	16	-0.02	0.894	1.00	0.460	0.49
B		16	0.85	0.368	1.38	0.429	0.84
A	5 x 5	12	-0.04	1.000	0.85	0.465	0.40
B		9	0.50	1.044	1.14	0.475	0.74

^a A significant departure of $\log(A)$ from 0 and of b from 1 is indicated as *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$, T-test.

4.6. Analysis of foci dynamics and structure

For this analysis, again the cumulative maps with the information of the diseased plants were used. The focus analysis of diseased plants inside a plot aimed to detect whether new appearing symptomatic plants occurred directly adjacent to other diseased plants. This two-dimensional method could not be applied to the maps of the coffee plots as well as to the irrigated and non-irrigated plot in the Province Corrientes, due to the great difference in the spacings of the plants within and across the rows. It was also not applied to the plots A and B, since the number of assessments with a low disease incidence was too small.

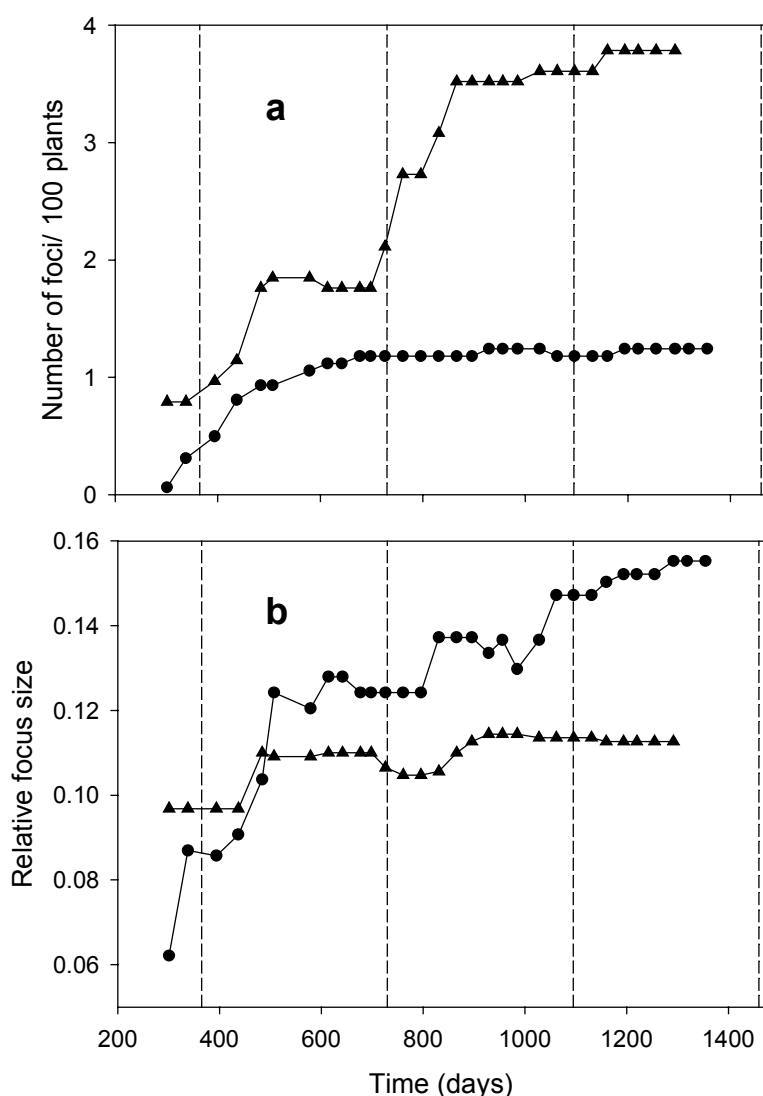


Fig. 35: Number of foci of CVC diseased plants per 100 plants (a) and relative focus size (b) in the plots at **São Carlos** (●) and **Engenheiro Coelho** (▲). Vertical lines represent the 1st January of the years. Day 0 = 1.1.2002

4.6.1. CVC foci in the State of São Paulo, Brazil

For the plot at São Carlos, the number of foci increased only at the beginning of the evaluations until the end of 2003 (day 726) and remained almost constant later (Fig. 35 a). The relative focus size showed an increasing trend over all assessments, but the ascent was stronger until mid of 2003 (day 507) (Fig. 35 b). This suggests for the first part of the evaluations until the end of 2003 an occurrence of new symptomatic plants partly in a distance of at least one plant to the already existing ones and partly directly adjacent. From the beginning of 2004 (day 761) on, the new diseased plants occurred

mainly directly adjacent to other diseased plants.

In the plot at Engenheiro Coelho, a different trend was observed. The number of foci increased from the beginning of the evaluation until mid of 2003 (day 507) and from the end of 2003 (day 726) until May 2004 (day 852), and was nearly constant during the remaining time (Fig. 33 a). The relative focus size increased only slightly from March to April 2003 (days 136 to 183) and remained constant during the rest of the evaluation period (Fig. 35 b). This indicates the occurrence of new symptomatic plants not directly adjacent to other diseased ones.

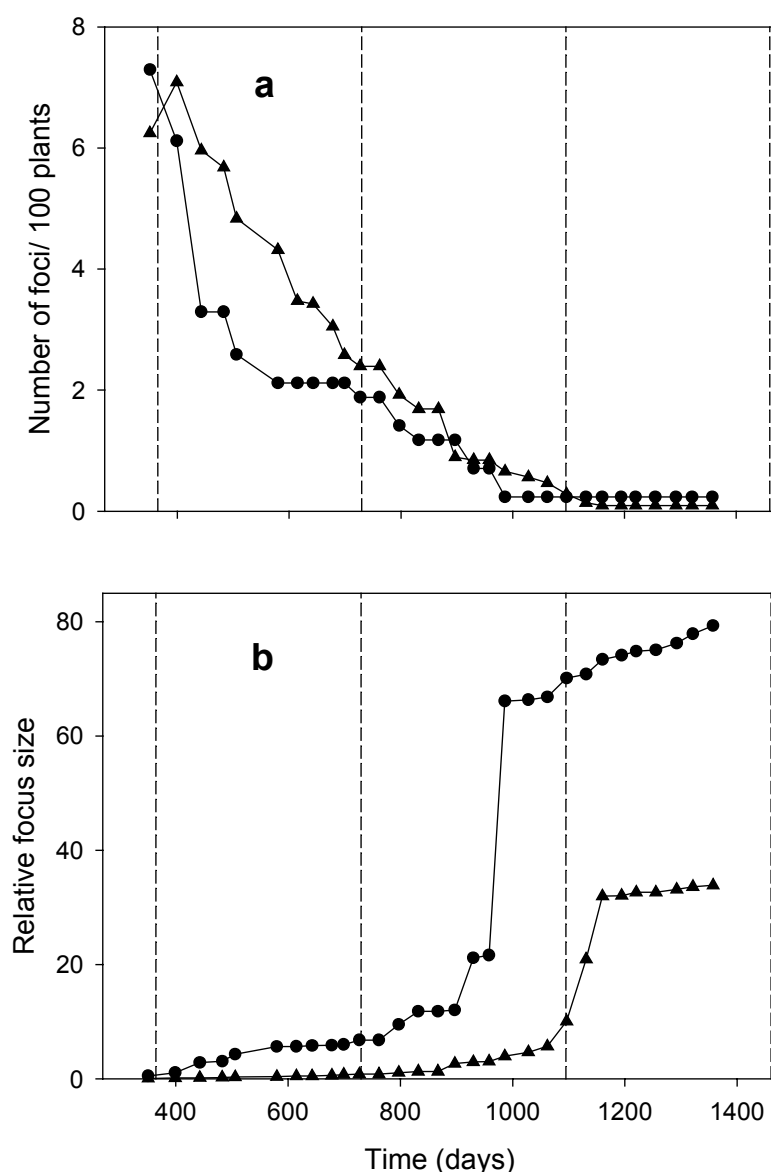


Fig. 36: Number of foci of CVC diseased plants per 100 plants (a) and relative focus size (b) in the plots 'B untreated' (●) and 'B treated' (▲) at Bebedouro. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2002

In the plots at Bebedouro, the incidence of CVC diseased plants had a much higher level than in São Carlos and Engenheiro Coelho. This led to a high number of foci at the beginning of the evaluation, which continuously decreased in both plots during the assessment period (Fig. 36 a). With the declining number of foci, the relative focus size increased. The strong rise in the middle of 2004 in the plot 'B untreated' and at the end of 2004 in the plot 'B treated', respectively, can be explained by the coalescence of single plants to the main focus (Fig. 36 b).

4.6.3 CVC foci in the State of Bahia, Brazil

In the irrigated plot in the State of Bahia, the number of foci per 100 plants decreased steadily from the beginning of the evaluation until day 298 (October 2003), remained on the same level for four months and then continued to decrease. The relative focus size enlarged only slightly in the first evaluation year (2003), but showed a steep increase in the second evaluation year (2004) until it reached the maximum at day 520 (May 2004). Although the number of foci decreased at the beginning, the relative focus size increased only slightly, indicating the coalescence of mainly small foci, formed by a few plants.

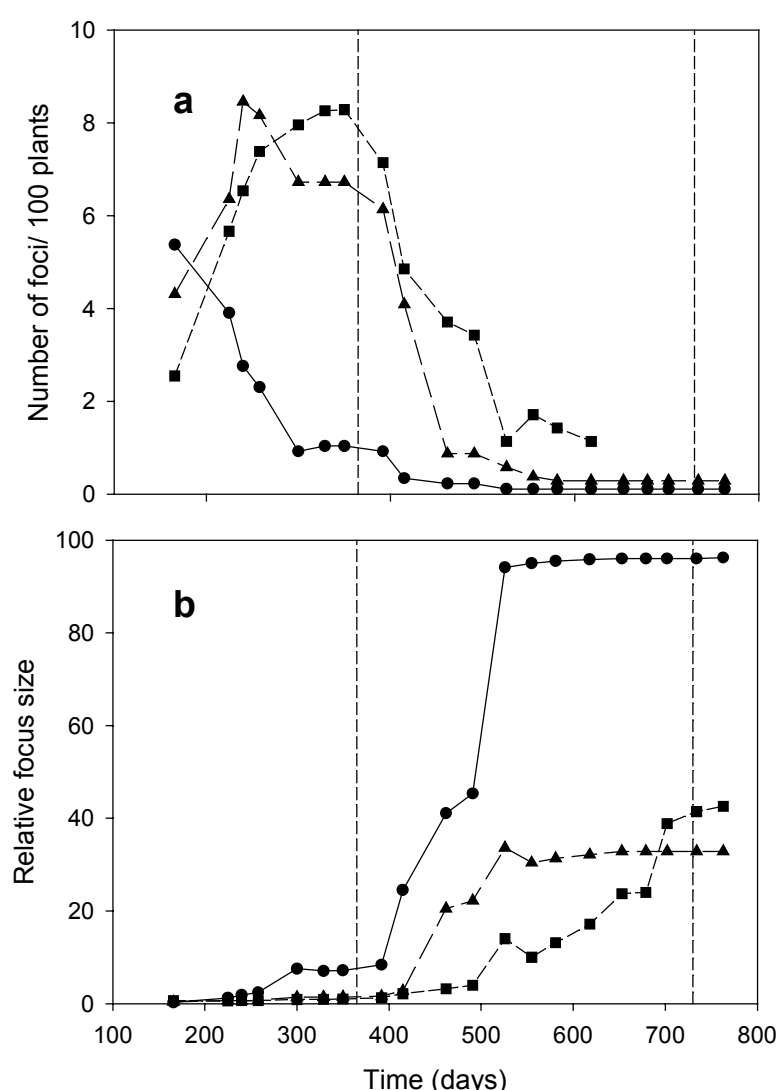


Fig. 37: Number of foci of CVC diseased plants per 100 plants (a) and relative focus size (b), plots irrigated (●), non-irrigated 1 (▲) and non-irrigated 2 (■) in Bahia. Vertical lines represent the 1st January of the years. Day 0 = 1.1.2003

In the non-irrigated plots, the number of foci increased at the first assessments (Fig. 37 a) and the relative focus size remained constant (Fig. 37 b), reflecting the formation of new foci by new symptomatic plants. In the middle of 2003 (non-irrigated 1) and at the end of 2003 (non-irrigated 2), the number of foci reached maximum values of approximately 8 foci per 100 plants. With the following decrease of the foci number, the relative focus size increased, indicating the coalescence of foci.

5. Discussion

5.1. Temporal aspects of the diseases

A disease starts with the infection of a susceptible plant. After the incubation period, symptoms become visible, indicating the presence of the disease. In this context, the visible symptom is either the pathogen itself, as for most fungal diseases, e.g. powdery mildew, or resulting of the host colonisation as for the majority of viral and bacterial diseases, like in the case of *Xylella fastidiosa*.

For CLS, little is known about the infection and incubation period. The vectors found in coffee groves are partly the same species responsible for CVC transmission (PAIÃO ET AL., 2002a; 2003b), but information on the abundance and feeding preferences are rare. For CVC, spring, summer and autumn are assumed to be the critical infection periods for the State of São Paulo, with the highest transmission risk in summer (PEREIRA, 2005; LOPES, personal communication). This conclusion was based on the higher vector population and feeding activity in spring and summer. Additionally, experiments with mechanical inoculations showed a higher probability for the establishment of an infection in summer and autumn than in spring and winter. Inoculations under water deficit and low temperatures were less successful, explaining the lack of new infections in the winter months (PEREIRA, 2005).

Under field conditions, the incubation period lasts between 6 and 14 months (LOPES ET AL., 1996), influenced by plant and environmental aspects. In former CVC assessments, phases of increasing symptom incidence were assumed to correspond to previous infection periods in spring, summer and autumn, while the lack of winter infections led to constant levels (LARANJEIRA, 2002; BERGAMIN FILHO ET AL., 2002). Another possible explanation for peaks in the appearance of symptoms was an influence of the season. Moreover, BERGAMIN FILHO and AMORIM (2002) proposed that the appearance of symptoms was related to the flushing of the citrus trees and thus a function of the host phenology.

5.1.1. Increases of the symptom appearance

The CLS symptoms in coffee are described as shortened internodes, clusters of small, deformed and yellowish leaves at the tip of the branches, similar to zinc deficiency, premature leaf fall, dry or dead twigs, small fruits and sometimes a scorching at the leaf borders (PARADELA FILHO ET AL., 1997; DE LIMA ET AL., 1998; LI ET AL., 2001; QUEIROZ-VOLTAN ET AL., 2003). In the evaluations of this project, mainly small, deformed leaves at

the tip of the branches together with shortened internodes were considered as an indication of the presence of the disease. Other CLS symptoms, like small fruits, premature leaf fall, leaf scorching and dead twigs, were considered as inappropriate for the assessment, since many other biotic or abiotic factors may lead to these symptoms and the differentiation is difficult (DE LIMA ET AL., 1998; TAKATSU ET AL., 2001; BARBOSA ET AL., 2002).

General reports stated a higher presence of symptoms during the winter (June-August), especially under hydric stress (DE LIMA ET AL., 1998). This could not be confirmed in the observations made in this project. Only small, deformed leaves in connection with shortened internodes were evaluated. Further CLS symptoms like leaf scorching, premature leaf fall and dead twigs could have been more prevalent during times of hydric stress.

The evaluations of the coffee plots in the State of Minas Gerais showed a strong seasonality in the appearance of the assessed symptoms. Peaks were observed at the end of spring/ beginning of summer (November/ December). Comparable observations were made by QUEIROZ-VOLTAN ET AL. (2003; 2004a), also in the State of Minas Gerais, who found higher disease incidences and severities in November/ December than at the beginning of autumn (March/ April). However, the authors did not try to explain this phenomenon, they just concentrated on the comparison of varieties.

Taking up the assumptions made for CVC (LARANJEIRA, 2002; BERGAMIN FILHO ET AL., 2002; BERGAMIN FILHO & AMORIM, 2002), a relation between the infection period and the time of the symptom appearance is unlikely, since in the evaluations of this project most of the symptoms disappeared within the rest of the year and returned at the same time in the following year. Consequently, it is rather reasonable, that the season had a great impact.

The appearance of the symptoms could be related to the growth of the plants, which was initiated by the increase of the temperatures in spring. Due to the high hydric deficit in the soil, resulting from the low rainfall during winter, the diseased plants developed symptomatic shoots at the affected branches, because water stress enhances the symptom development of *Xylella* diseases (GOODWIN ET AL., 1988; HOPKINS, 1989; TARGON ET AL., 2004). The restriction of this phenomenon to the first flushing in springtime can be explained by the higher precipitation during summer, which may have enabled most of the plants to develop asymptomatic leaves. During autumn and winter, vegetative growth almost stopped, leading to a lack of new shoots, resulting in a very

low incidence of symptomatic plants. A strong relation between the phenology of the host plant and the appearance of symptoms, as it was proposed for CVC (BERGAMIN FILHO & AMORIM, 2002), would in this case be true for CLS, at least for the considered symptoms.

The number of new flushes was not assessed in the coffee orchards. This could have confirmed the hypothesis of the strong relation between the flushing in spring and the increase of the incidence of symptomatic plants.

For CVC in citrus, no clear seasonal pattern of the appearance of symptoms was observed in the plantation in the State of Bahia as well as in two plots in the Province Corrientes. In the other two plots (irrigated and non-irrigated) in the Province Corrientes, the incidence of symptoms escalated between autumn and beginning of winter (March-June) as well as between end of winter and spring (August-November), periods within or after times of high precipitation.

In the State of São Paulo, the incidence of symptoms increased distinctly in all evaluated plots in all assessment years (2003-2005) at the end of summer (around February/ March), which is characterised by high temperatures and high precipitation. In the mid of winter (around July/ August), the dry and colder period, peaks occurred in three of the four plots in 2005 as well as in all plots in 2004. The observed rise of the incidence of symptoms at the end of summer (around February/ March) agrees with reports from other authors for the State of São Paulo (PALAZZO & CARVALHO, 1992; RODAS, 1994; LARANJEIRA, 1997a; 2002). The favoured symptom appearance in times of high precipitation in the State of São Paulo as well as in the Province Corrientes would confirm the assumption of a connection with the flushing (BERGAMIN FILHO & AMORIM, 2002). However, this theory could not explain the increase frequently observed in the São Paulo orchards at the end of winter (July/ August) as well as the escalation in the plots A and B at the Province Corrientes in autumn and winter (May-July), since no vegetative growth occurred at this time. The possibilities of either the influence of the season or a relation between the favoured infection periods and peaks in the symptom appearance will be discussed in combination with the results of the disease severity (5.1.3.).

5.1.2. Decreases of the symptom appearance

In coffee, declines of the symptoms incidence were measured from summer until winter (January-August). This disappearance of the symptoms may have been caused by the drop of symptomatic leaves, which has also been reported as a consequence of the disease by PARADELA FILHO ET AL. (1995). After the shedding of the symptomatic leaves, dead twigs remained, or, due to the high precipitation in this period, normal leaves developed, even on diseased twigs. Another explanation may be that the non-symptomatic leaves, which were growing in the raining season, have covered the smaller, symptomatic leaves. A 'healing' of the symptoms by a delayed expansion of the internodes and of the small leaves combined with a reverse of the chlorosis is unlikely. Other diseases, which may also have caused a leaf drop, were not observed to a greater extent.

The decline of the symptom incidence for one plot in the plantation at São Gotardo was very steep in 2004. In 2005, the decrease was slow and almost parallel to the other plot, although they had different incidence levels. This rather reflects an influence of external conditions on the disappearance of the symptoms than the incidence level.

A generally neglected possibility, which may have influenced the evaluations, is the 'training effect' of the assessors. In the case of the CLS assessment, the persons had no experience prior to this project. Consequently, the observed higher incidence of symptomatic plants in 2005 compared to 2004 can also be explained by the increased ability of the persons to identify even less obvious symptoms (NUTTER, 1999).

The frequency of a plant in showing symptoms may allow conclusions about the disease severity and thus the time the disease persisted, assuming that severely diseased plants may show symptoms at different branches and probably over a long period of time. On the other hand, it may be that a severely diseased plant sheds the symptomatic leaves earlier than a less affected plant, for instance if the twig dies. If this has happened, a plant would be ranked as asymptomatic, since dead twigs and the general appearance of the plant were not considered as symptoms in the assessments. Under the previously made assumption that the appearance of symptoms is related to the growth of the plants, a slightly diseased plant may develop new shoots with symptoms rather than a severely affected one. Consequently, no conclusions can be made from the frequency a plant shows symptoms on the persistence of the disease.

For CVC in citrus, a reduction of the incidence of symptoms occurred in the orchards to different extents. In the plots in the State of São Paulo as well as in the irrigated and non-irrigated plot in the Province Corrientes, the decreases almost coincided with the dry seasons. In the plantation in the State of Bahia, the disappearance of symptoms occurred throughout the whole assessment period and was not restricted to certain seasons. In the first half of the evaluation, the symptoms disappeared in some plants while at the same time others developed new symptoms. In the second half of the evaluation, the incidence of symptomatic plants decreased almost steadily throughout a long period of time and remained on a very low level afterwards.

Several reasons could have caused this disappearance of CVC symptoms. It may have been due to a shedding of affected leaves, probably as an effect of the disease (ROSETTI, 2001). A covering of the symptomatic leaves, as it was proposed for coffee, is less likely, since the symptoms in citrus mainly occur on the older leaves of the tree, which have a certain distance to the new flushes (ALVES ET AL., 2004). LARANJEIRA (2002) considered a covering of the leaves by dust as a possible reason for the disappearance. This is not a reasonable explanation, since the symptoms of the plants did not suddenly reappear at the start of the raining season and, moreover, even disappeared within wet seasons in the State of Bahia. Another possibility can be that mild symptoms like a slight leaf chlorosis were reversed due to favourable conditions for the plant (TORRES ET AL., 2002). Due to the unfavourable conditions for the plants in the dry period, the latter point can be excluded. Moreover, this would not explain the high degree of the symptom disappearance observed in the plantation in Bahia, which was even higher under dry and consequently worse conditions in the non-irrigated plots.

It can be assumed that a shedding of leaves occurred, probably enhanced if the plants were facing drought stress (GOODWIN ET AL., 1988; MC ELRONE ET AL., 2001). The old leaves, which show the CVC symptoms, are generally the first ones aborted under these conditions. This would also explain the higher level of symptom disappearance in the non-irrigated plots in Bahia compared to the irrigated one. Water stress also served as an explanation for a decrease of the CVC symptom incidence in the same season observed by AYRES ET AL. (2000) in surveys in the State of São Paulo. However, for the irrigated plots in the Province Corrientes as well as in Bahia, this cannot serve as the only explanation, since drought stress of the plants should not have been that prevalent, although in Bahia the disappearance of symptoms was higher in the non-irrigated than in the irrigated plot. Influences of other stress factors like nutrient deficiencies (TORRES

ET AL., 2002) or of other diseases promoting defoliation are possible. For the orchards in the State of São Paulo, an occurrence of other diseases has been assessed additionally to the CVC incidence, but this incidence was very low and a reasonable effect can be excluded. In the plantation in the State of Bahia, the strong and persistent defoliation of leaves, before or shortly after they developed CVC symptoms, specifically in the second part of the evaluations, may have been mainly caused by non-seasonal stimuli, since no seasonal trend was observed. In the plots in the Province Corrientes, seasonal influences were visible, rather reflecting an effect of meteorological conditions. A possibility might be that foliar diseases, which predominantly occur under wet conditions, caused defoliation.

In the orchards in the State of São Paulo, a controversial behaviour was observed in the dry seasons. While in 2003 in all four plots and in 2005 in one plot the incidence of symptoms declined, it rose in 2004 in all plots and in 2005 in three plots. In the dry seasons of 2004 and 2005, the temperature was slightly lower and the precipitation slightly higher than in 2003. An explanation for this observation may be that drought stress generally enhances the symptom expression (GOODWIN ET AL., 1988; HOPKINS, 1989; MC ELRONE ET AL., 2004; TARGON ET AL., 2004), but severe stress leads to the shedding of the leaves (AYRES ET AL., 2000). Surprisingly, in the plot at Engenheiro Coelho and partly also at São Carlos, these plants did not show symptoms again within the next two years. The death of affected twigs is possible (LARANJEIRA, 1997b), but it was not reported whether this could lead to a complete recovering of the plant.

As already considered for the coffee fields, the experience of the evaluators may have influenced the results of the CVC assessments (NUTTER, 1999). For the State of São Paulo, this can be excluded, since the evaluator had a great experience in CVC assessments prior to this project. For the State of Bahia and the Province Corrientes, the skills of the assessors were not advanced, which may partly explain the low levels observed at the beginning of the evaluations followed by strong escalations of the CVC incidence.

5.1.3. Severity of the disease symptoms

The previously made assumption that water stress may lead to an obviously low incidence of symptomatic plants due to the shedding of affected leaves could also be confirmed for the severity of the symptoms for all plots in the Province Corrientes. The severity indices tended to be slightly higher in the wet periods compared to the dry periods. In the orchards in the State of São Paulo, a clear seasonal effect was not reflected in the severity indices.

In the plot at São Carlos, two decreases in the incidence of symptomatic plants were observed. At the first occasion in October 2003, the severity index slightly increased, indicating an overproportional decline of the severity grade 1. At the second point in August 2005, the incidence of the severity grade 3 diminished and of the severity grade 1 slightly increased, while no new symptomatic plants were observed, indicating a shift of the severely affected plants to a lower severity grade.

The severity indices in the plots at Bebedouro rose steadily throughout the assessment period and were positively correlated with the disease incidence, apart from the effect of the pruning of severely affected branches in one of the plots. At the times of higher increase of the incidence of symptomatic plants, the severity index did not change notably. That indicates a simultaneous and proportional increase of all three severity grades. Returning to the two possibilities, that either the season is favourable for the appearance of new symptoms or the peaks in the symptoms incidence are related to the flushing and high vector occurrences of the previous year (LARANJEIRA, 2002; BERGAMIN FILHO ET AL., 2002), this observation supports the first proposal. In the latter case, an overproportional increase of new symptomatic plants with the grade 1 would have caused a decline of the severity index.

The development of the severity indices can be assumed to be a function of the time the disease persisted. The longer a tree was infected, the wider was the pathogen's spread and establishment within the plant, leading to higher severity grades. The low, but also increasing level in the plot at Engenheiro Coelho, where the disease was in an initial stage, supports this assumption. A different situation occurred in the plot at São Carlos. Although the disease incidence was on a comparable low level like at Engenheiro Coelho, the majority of the plants showed severe symptoms (severity grades 2 and 3), which normally occur at a more advanced stage of the disease (FONTANEZZI-HUANG & CHIARADIA, 1998; BERETTA & LEITE, 2000; ROSETTI, 2001). Generally, higher disease severities occur in the Northern and Western parts than in the Central and Southern

parts of the São Paulo State (AYRES ET AL., 2000; FUNDECITRUS, 2004). Hence, São Carlos, located in the South of São Paulo, should have comparable conditions to Engenheiro Coelho (Center). Since genetical differences of the bacterial strains from different regions were not found (QIN ET AL., 2001), other unfavourable conditions for the plant, e.g. nutrient deficiencies, may have led to the high severity in São Carlos (TORRES ET AL., 2002). On the other hand, assuming that the severity is also a function of the duration the disease persisted, most of the plants may have been infected for a long period of time and the amount of new established infections, which would show the severity grade 1, was very low. Multiple infections of the plants by nymphs can be excluded, since citrus is not the primary breeding host of the known vectors, which prohibits a great contribution of nymphs to the within tree spread (LOPES ET AL., 1999; MILANEZ ET AL., 2001). An elevated presence of endophytic bacteria, which enhanced the symptom development (ARAUJO ET AL., 2002; LACAVA ET AL., 2004), may have also been a reason.

In the plantation in the State of Bahia, no clear pattern in the dynamics of the severity indices was obtained, as also observed for the appearance and disappearance of symptoms. As a tendency, low disease severities corresponded to low incidences of symptomatic plants and vice versa. The high degree of the disappearance of symptoms, most probably caused by defoliation, could explain the restriction to the grade 1 in the second part of the evaluation. In the first part of the assessment, the leaves remained longer, allowing the development of higher severity grades. The lack of plants with the severity grade 3, apart from few assessments, may have been due to the early abortion of the fruits at severely affected branches (LARANJEIRA, personal communication). Although the observed severity grades in the irrigated plot were higher than in the non-irrigated plots, a higher damage cannot be implied for the irrigated plot. The defoliation in the non-irrigated plots was much stronger than in the irrigated plots, indicating a worse condition of the plants.

The vector species as well as the number of sharpshooters caught in Argentinean citrus groves correspond to the ones of the São Paulo State (LOPES, 1999; REMES LENICOV ET AL., 1999) and the susceptibility of Valência Late is comparable to Pêra (LARANJEIRA & POMPEU JR., 2002).

5.1.4. Temporal progress of the diseases

Xylella fastidiosa is a systemic pathogen, and a plant, which has once shown symptoms, can be considered as diseased for further assessments. Based on this, cumulative maps of diseased plants were generated, which were also used in former works on the CVC and CLS epidemiology (LARANJEIRA, 1997a; 2002; ROBERTO ET AL., 2002; BARBOSA ET AL., 2004) as a tool to obtain monotonously growing curves, a precondition for the application of most epidemiological models.

As already discussed, the assessment of visible CVC and CLS symptoms is to varying extents an inappropriate tool to measure the actual disease status of a plant, since many factors influence the presence of symptoms. This may lead to erroneous assumptions on the actual progress of the disease, which will be explained in the following part.

For coffee, the incidence of CLS symptomatic or diseased plants increased distinctly at the end of the years (November/ December). As previously discussed, these rises were rather related to the plant phenology than to the time of infections of the plants. Based on this, it can be assumed that the incidence of symptomatic plants measured at the beginning of the evaluations in March/ April did not reflect the actual disease status. Consequently, only limited information can be obtained from the progress curves on the actual disease dissemination. This also explains the failure of the generally used epidemiological models to describe the progress of the measured disease incidence.

Theoretically, it would have been possible to measure the disease increase on a yearly basis, that means from one peak of the symptom incidence to the next one. For the realisation, frequent assessments during spring and summer (September-January), the time of the symptom appearance, should have been carried out. In the evaluations of this project, an extreme change from asymptomatic to symptomatic within a short period of the year was observed. Thus, the real maximum point of the symptom incidence could have been missed, as it most probably occurred in the plot at Ervália. Additionally, evaluations over several years would be required, since the measured peak at the end of each year resulted in only one data point.

Applying this theoretical approach to the data obtained in this project, one to three points were available. The one and only point for the plot at Ervália was at 50 %. The plots in the plantation at São Gotardo showed 64 and 78 % incidence of symptomatic plants at the first peak. One plot reached 88 % disease incidence at the second peak

and the latter one almost 100 %, hence, the third peak of this plot did not yield additional information. At the beginning of the evaluations, the plants had an age of 3 years after stumping. On condition that all plants were healthy after the stumping, the disease incidence levels of 100 and 88 % were obtained after 4 ½ years, indicating a relatively quick dissemination of CLS compared to CVC. Maybe the dissemination rate for CLS is higher than for CVC, since even the nymphs can contribute to the plant to plant spread within the rows, favoured by the narrow planting pattern of the coffee trees. Opposite to citrus, coffee is thought to be a suitable breeding host for two known vectors of *Xylella fastidiosa*, *Bucephalogonia xanthopis* and *Agroconia* sp. (LOPES ET AL., 1999). Moreover, a transmission through root grafts, which had been proven for citrus (HE ET AL., 2000), may also occur in coffee. The narrow planting of the trees would promote this way of transmission. Another factor leading to a higher dissemination rate may be the bacterial titer in the plant's xylem, which is higher in coffee compared to citrus. A higher transmission efficiency of the sharpshooter vectors as a result of the elevated bacterial titer can be assumed (HILL & PURCELL, 1997; LOPES, 1999; ALVES ET AL., 2001).

Another factor, which has to be considered, is that the stumping of the coffee trees may not have eliminated the pathogen. *X. fastidiosa* can be found in the trunk and also in the roots of infected coffee, although the titer is low (DE LIMA ET AL., 1996, TAKATSU ET AL., 2001; QUEIROZ-VOLTAN ET AL., 2005). After a stumping, TAKATSU ET AL. (2001) could detect neither symptoms nor bacteria in the new flushes. However, after two to three years, symptoms were observed again in the plantation. It has to be mentioned that the authors did not exclude new infections by sharpshooters. An absence of symptoms and detectable bacteria in the first years after stumping could be explained by a strong dilution under the detection threshold of the remaining bacteria (MC ELRONE, 2001), caused by the relatively strong growth of the plants after the stumping. Moreover, a well-developed root system provides a relatively small aerial biomass; hence, factors enhancing a symptom development, e.g. drought stress and nutrient deficiencies, are less likely to occur. Consequently, the reappearance of an old infection could be measured in stumped coffee groves few years after the stumping. Starting in a new established plantation with a low disease incidence and evaluating several years, it could be possible to obtain information on the temporal progress of CLS, provided that a seed transmission of CLS can be excluded (YORINORI ET AL., 2003).

Many evaluations of the CVC development have been carried out, producing extremely varying results for the temporal progress of CVC (GOTTWALD ET AL., 1993; LARANJEIRA, 1997a; 2002; LARANJEIRA ET AL., 2002), but little attention has been paid to the symptomatology of the disease.

For the evaluations of Citrus Variegated Chlorosis, three different progress curves of the diseased plants were observed. All cases shared a relatively steep rise at the beginning of the evaluations. For the first type, the strong rise was followed by a slower increase on a low incidence level for the rest of the time with only few occasions at which symptoms disappeared. This occurred in the State of São Paulo in both plots at Bebedouro as well as in the plot at São Carlos. The fourth plot at Engenheiro Coelho showed a different pattern: the incidence of diseased plants increased distinctly in the first and second evaluation year, but not in the third one. The second type was characterised by reaching almost 100 % disease incidence after a short period of time, while almost all plants remained symptomatic after once showing symptoms, as it occurred in the plots A and B in the Province Corrientes. In the third case, also high incidence levels were reached relatively soon, but the majority of the plants were symptomatic just for a limited period of time. This type was observed in all plots in the State of Bahia as well as in the irrigated and non-irrigated plot in the Province Corrientes. Many other reports agree with the steep increase of the disease incidence of the second and third case (LARANJEIRA, 1997a; 2002; ROBERTO ET AL., 2002), and also a slow disease progress has been observed in evaluations in the State of São Paulo (LARANJEIRA, 1997a).

The escalations of the disease incidence at the beginning of the evaluations may not reflect the actual disease spread in the plots, but rather a reappearance of symptoms. In the State of São Paulo, evaluations started in October and December 2002. A shedding of symptomatic leaves, as it was observed in 2003, may have also occurred at the end of the dry season in 2002 (July/ August) (AYRES ET AL., 2000). This is the only reasonable explanation for the high rate in the first six evaluation months, which did not occur again during the next 27 or 29 months, respectively. A restraint of the disease progress due to high disease levels can be excluded at the observed incidences. Additionally, other factors, which may influence the CVC dissemination, like vector abundance and precipitation (AYRES ET AL., 2000; PEREIRA, 2005) can be assumed to have been nearly constant within a natural range.

In the same way, the observed disease progresses in the State of Bahia and in the Province Corrientes may have been rather due to a reappearance of symptoms than to the consequences of new infections of the trees. The constantly high degree of symptom disappearance in most of the plots supports this assumption. For two plots in the Province Corrientes, a disappearance of symptomatic plants was not observed to a large extent. However, the shift in the disease severities also indicated a shedding of symptomatic leaves. Additionally, training effects of the evaluators may have contributed to this observation (NUTTER, 1999). As previously described for coffee, the evaluators in the State of Bahia and in the Province Corrientes had limited experience in the assessment of CVC symptoms. Less obvious symptoms may have been rather missed at the start of the evaluations. A comparable steep escalation of the disease incidence observed by LARANJEIRA (2002) was explained by infections of the trees within the nursery. The high age of the plots (12-14 years at the start of the assessments) rejects this theory.

For most plots, one of the generally used epidemiological models gave a satisfactory fit to the data, however, in some cases non-random patterns of the residuals were observed. For one plot in the State of Bahia as well as for two of the plots in the Province Corrientes, models were found, which produced high coefficients of determination with random patterns of the residuals, indicating a high appropriateness of the model to describe the progress data (CAMPBELL & MADDEN, 1990). However, as previously explained, the 'disease' progress measured at these locations is not related to the actual disease spread. In other epidemiological works on the temporal CVC progress, varying models were found appropriate to describe the disease progress. GOTTWALD ET AL. (1993) suggested the Gompertz model to describe the development of a CVC epidemic. For the development of CVC in eleven groves, LARANJEIRA (1997a) found models of a double sigmoid pattern to be most appropriate. In later assessments in three citrus groves, none of the generally used models gave a satisfactory fit to the data (LARANJEIRA, 2002). These disagreements in finding the best model for the CVC progress may have also been due to the varying patterns produced by the appearance of symptoms, which are not necessarily related to the actual disease progress. The fact that symptoms are less visible at certain seasons was known, but that this may result in erroneous conclusions on the disease incidence when evaluations started in these seasons was not considered (LARANJEIRA, 1997a; 2002). In the evaluations of this project, plots in the same region mainly showed the same tendencies in the appearance

of symptoms. Hence, the agreement of the eleven plots to fit to a model of double sigmoid pattern (LARANJEIRA, 1997a) is not surprising. Furthermore, changes in the incidence of symptoms were much more influenced by the reappearance and disappearance than by an actual disease progress. For the plots in the State of São Paulo, the selection of a model was mainly determined by the high increases at the beginning of the assessments. The rise of the incidence of symptomatic plants, which was eventually due to the actual progress of the disease, resulted in non-random patterns of the residuals.

The suggestion to overcome this problem is similar to the one proposed for coffee: frequent assessments over a long period of time are required to obtain information on the disease progress. Fitting a model to all data points leads to non-random patterns of the residuals due to the seasonal appearance of the symptoms ('waves'). To avoid this, just the points at which an increase has been measured should be considered, i.e. the first maximum point of the 'waves', similar to the peaks for coffee. One has to be aware that other factors like drought stress and other diseases may influence the symptoms appearance, as in the plantation in Bahia and in the plots in the Province Corrientes; thus, great care has to be taken when judging the disease progresses.

In this project, the application of this suggestion is not possible for the data obtained in the plantation in the State of Bahia as well as for the plots in the Province Corrientes. For the plots in the State of São Paulo, the first data points have to be neglected due to the previously discussed reasons. For three of the plots, this would result in linear progress curves. In the plot at São Carlos, the disease progressed very slowly and reached 3 % when the plants had an age of 7 years. This fits to the general low CVC level in the southern region of the State of São Paulo, which was justified by the lower abundance of vectors (AYRES ET AL., 2000). The high symptom severity combined with a low disease incidence observed in this plot strengthens the theory of a very slow disease progression. Most of the plants showed severe symptoms, indicating a longer lasting infection (FONTANEZZI HUANG & CHIARADIA, 1998; BERETTA & LEITE, 2000; ROSETTI, 2001;). Newly infected plants with mild symptoms occurred to a relatively low extent.

For the plots at Bebedouro, a much higher dissemination rate for the disease spread than in the São Carlos plot was obtained. This agrees with other reports, stating a higher CVC incidence in the northern part of the São Paulo State due to elevated vector abundance (AYRES, 2000; FUNDECITRUS, 2004). The disease progress curves increased

linearly, but lacked a restrain, although the disease incidence went far beyond 50 %. At least in one plot a slight decrease of the rate at the end of the evaluations could be detected, although the disease incidence was lower than in the other plot. The reason for this linear increase of the disease incidence is obscure. In epidemiological models, the point of inflection, which reflects a restrain of the increase of an epidemic, lies at disease incidences of 0 % (monomolecular), 37 % (Gompertz) or 50 % (logistic) (CAMPBELL & MADDEN, 1990).

For the plot at Engenheiro Coelho, the higher progression rates in 2003 and 2004 compared to 2005 could be justified by a delayed reappearance of the symptoms; part of the plants in which symptoms had disappeared prior to the first assessment did not develop new symptoms until the second evaluation year (2004). Again, this prohibits conclusions on the actual disease spread in this plot. In general, the low disease incidence and severity agrees with other reports for this region (Center) in the State of São Paulo (AYRES, 2000; FUNDECITRUS, 2004).

5.2. Spatial aspects

The analysis of the spatial distribution of diseased plants within a field aimed to obtain information on the mechanisms of the spread (CAMPBELL & MADDEN, 1990). For the analysis, cumulative data were used, since they should rather reflect the disease status of the plants than the incidence of symptomatic plants. If symptoms disappear during the evaluations, this will not be considered in the cumulative maps. However, if this has happened prior to the first assessment as it was assumed for most of the plots, the obtained information is limited.

For CLS in coffee, a high degree of spatial aggregation of diseased plants was detected for all assessment dates and all plots. According to the previous considerations, that the observed incidence of diseased plants at the first assessments did not reflect the actual incidence, the results for these assessments are of limited value for conclusions. The first observed peak was assumed to be the first point at which all diseased plants showed symptoms. At this point, all plots had reached high disease incidence levels (51, 78 and 64 %). Hence, that information on the spatial distribution of diseased plants is only valid for an advanced stage of the epidemic. BARBOSA ET AL. (2004) also found an aggregated pattern of CLS diseased coffee plants, while new symptomatic plants randomly appeared. It has to be mentioned that only four evaluations with six months intervals in between were carried out.

For both plots in the plantation at São Gotardo, a gradient to the inner part of the plantation existed, low disease incidences occurred at the borders of the plantation. Both sides of the plantation, represented by the two plots, were stumped in different years. The non-stumped part could have served as the main inoculum source for the vectors.

The narrow planting pattern of coffee promotes transmissions through root grafts to adjacent plants (HE ET AL., 2000) and by sharpshooter nymphs with a limited movement range (LOPES ET AL., 1999). This also suggests a higher degree of aggregation within the rows in coffee compared to citrus.

For the citrus plots, similar reasons as for coffee limit conclusions on the spatial disease spread. The detected lack of correlation between the symptom appearance and the actual dissemination of the disease could explain the differences in the results obtained for some of the plots. In the State of Bahia, a constantly high degree of aggregation was

found in the irrigated plot, the plot non-irrigated 2 showed varying tendencies at the beginning of the evaluations and aggregation in the later part. The distribution of diseased plants of the plot non-irrigated 1 showed almost no departure from randomness. The two plots in the Province Corrientes showed no clear tendency of the spatial pattern of diseased plants.

For the State of São Paulo, the results of the spatial analyses rather reflect the actual disease status, apart from the first evaluations, as previously discussed. A continuously high degree of aggregation of the diseased plants was observed for two of the plots and varying results for the other two plots, while each group consisted of one plot with a low and one with a high disease incidence, respectively. Varying results for the plots with the low disease incidence (São Carlos and Engenheiro Coelho) agree with findings from other authors, who detected either a random distribution (ROBERTO ET AL., 2002) or an aggregation of diseased plants at the beginning of a CVC epidemic (LARANJEIRA, 2002; LARANJEIRA ET AL., 2002). The evaluations ran over a long period of time, but the change of the disease incidence in these plots was very low. Consequently, the conclusions are only valid for a very small part of the epidemic.

The plots at Bebedouro showed a greater change of the disease incidence than the plots at São Carlos and Engenheiro Coelho. In one of the plots (treated), the pattern of diseased plants can be described as loosely aggregated, indicated by high dispersion index values and the Taylor law analysis, whereas the number of aggregated rows detected by the ordinary runs test was relatively low. The other plot (untreated) showed a lower degree of aggregation, especially at greater quadrat sizes and the amount of rows with a significant aggregation of diseased plants was lower than in the other plot. Clusters of diseased plants seemed to be small, since the lower quadrat sizes produced significant results for the Taylor law and higher dispersion indices than the bigger ones. It has to be mentioned that this field contained much less plants (450) than the other plot (2130). Since the significance limits of the dispersion index increase with decreasing numbers of plants per plot, this may partly be a reason for the low detection of aggregation. Another reason may be that aggregation on a wide range is difficult to detect in small fields. These findings also agree with LARANJEIRA ET AL. (2002), who found slightly aggregated patterns of CVC diseased plants at later stages of the disease.

5.3. Management of the diseases

5.3.1. Vector control/ roguing of diseased plants

The comparison of a citrus plot, in which chemical insecticides were applied regularly to control the CVC vectors, with an untreated plot aimed to evaluate whether this measure yielded a lower dissemination rate of the disease (GARCIA JR. ET AL., 1995). Unfortunately, an effect of the vector control was difficult to determine due to the roguing of diseased plants at the beginning of the evaluations in the treated plot. Although this measurement should have reduced the disease dissemination (RODAS, 1994; GARCIA JR ET AL., 1995) additionally to the vector control, the slope of the regression line, which can be interpreted as the rate of the disease progress, was even higher in the treated plot than in the untreated plot. This indicates neither an effect of the vector control nor of the roguing. Several researches doubted the benefit of a pruning on a reduction of the disease progression (DE LIMA ET AL., 1996; UESUGI & UENO, 1999; HE ET AL., 2000).

5.3.2. Irrigation

In the State of Bahia as well as in the Province Corrientes, the CVC incidence was assessed in irrigated and non-irrigated plots to evaluate whether irrigation is a recommendable CVC management tool, as suggested by several authors (GOODWIN ET AL., 1988; HOPKINS, 1989; TARGON ET AL., 2004).

Unfortunately, it was not possible to obtain information on the actual disease spread for both locations, as already discussed in 5.1.3. The higher level of symptomatic plants observed in both irrigated plots compared to the non-irrigated ones in the first part of the evaluations may have been due to an actually higher disease incidence. The irrigation could have led to high vector abundance extended into the dry periods (GARCIA JR. ET AL., 1997) and an elevated probability for infections to establish (PEREIRA, 2005). On the other hand, the initial disease foci could have been at the irrigated sites and the high level observed was just caused by the longer time the disease could progress. Considering the disappearance of symptoms, another explanation could be that the irrigated plants were rather able to maintain the diseased leaves, while the plants in the non-irrigated plots shedded the leaves much faster. This was confirmed for the plots in the State of Bahia, but the observed disappearance of symptoms was almost on the same level in the irrigated and non-irrigated plot in the Province Corrientes. However, a more intensive shedding may have occurred in the non-irrigated plot prior to the first

assessment, accounting for the low disease level at the start of the assessments. The progress of the diseased plants in the Province Corrientes support the latter assumption. After eleven evaluation months, both plots had reached the same level in the incidence of symptomatic plants and almost 100 % incidence of diseased plants after fifteen months. Moreover, plants, which have been asymptomatic at the first assessment, have shown severe symptoms (severity grade 4) less than one year later. As mentioned before, severe symptoms normally occur at an advanced stage of the disease (FONTANEZZI HUANG & CHIARADIA, 1998; BERETTA & LEITE, 2000; ROSETTI, 2001). Generally, irrigation should lead to a lower severity of symptoms (MC ELRONE ET AL., 2001). This was only observed for the plots in the Province Corrientes, in Bahia the severity of symptoms was slightly higher in the irrigated plot. However, the disappearance of symptoms in the irrigated plot in Bahia was not as accentuated as in the non-irrigated plots. With the previously discussed background that this was caused by a defoliation of leaves, the plants in the irrigated plot must have been in a better condition compared to the non-irrigated plants. Moreover, the yield in this plot was higher than in the non-irrigated ones (LARANJEIRA, personal communication).

5.4. Outlook

The results of this project pointed out disadvantages of the CLS and CVC symptom assessment to obtain information on the disease status of the plants. At the beginning of the project, this was considered as an appropriate tool to measure the disease incidence of CVC in citrus (LARANJEIRA, 1997b; 2002). For coffee, researchers were rather aware that the symptoms are difficult to detect and distinguish from nutritional disorders (DE LIMA ET AL., 1996; BARBOSA ET AL., 2002). This finding helps to better judge former evaluation results, especially for citrus.

Molecular test could be a better method to identify diseased, but non-symptomatic plants. This would be an expensive and time consuming method, since samples should be taken from different parts of the plants, assuring the detection of initial infections restricted to a single branch. Furthermore, if a strong defoliation occurs, it is unclear whether bacterial titers above the detection threshold can be obtained from the remaining leaves. For citrus, the highest bacterial titers were found in the leaves close to the main stem (ALVES ET AL., 2004), which are the first ones aborted under stress conditions (AYRES ET AL., 2000).

In the case of coffee, molecular tests could help to solve the question, whether the existence of the disease is due to a survival of the pathogen in the trunk after a stumping or to a new infection (TAKATSU ET AL., 2001) by preventing the stumped plants from new sharpshooter infections.

More information on the epidemiology of CVC and CLS could be obtained from newly established plantations excluding an infection of the young plants in the nursery or through seed transmission in the case of coffee.

In citrus, orchards should be selected, which are not heavily affected by other citrus diseases and which do not suffer from nutritional stress. Additionally, evaluations should start at the beginning of the year (for climatic regions similar to the São Paulo State), since symptoms are more obvious at this time.

As a final remark, no absolutely safe visual method exists to evaluate the presence of CVC and CLS, since too many factors influence the appearance of symptoms.

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7. Annex

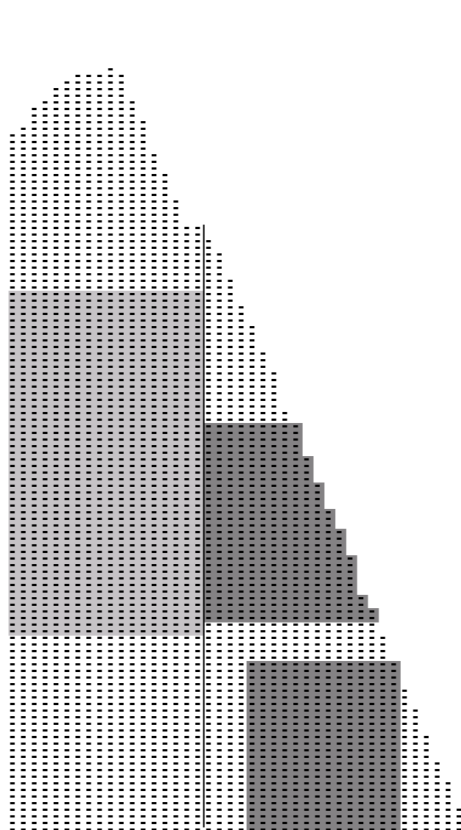


Fig. 38: Assessed citrus plantation in the State of **Bahia** with the **irrigated** plot (bright grey rectangular on the left side) and the plots **non-irrigated 1** (lower grey area on the right side) and **non-irrigated 2** (upper grey area on the right side). Each black point represents a single plant.

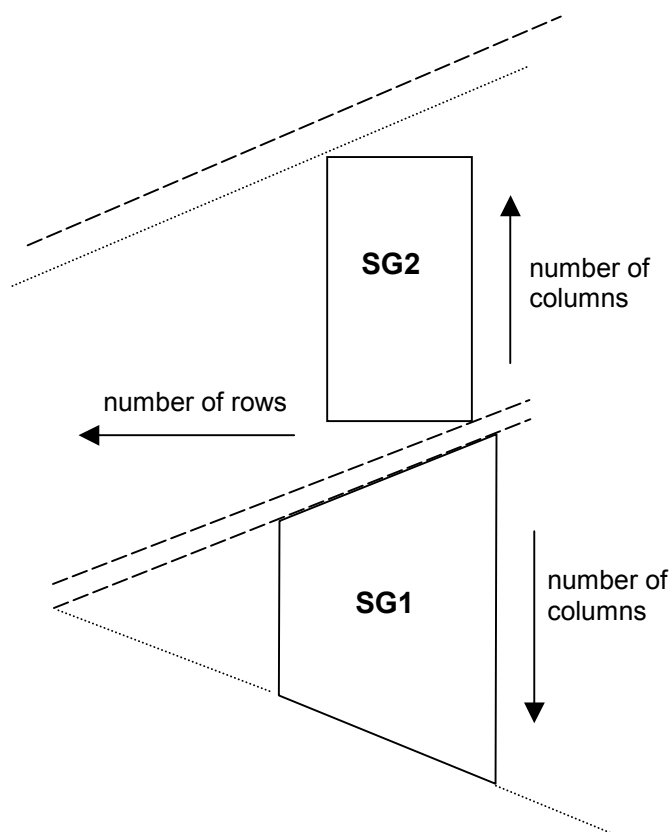


Fig. 39: Assessed coffee plantation in **São Gotardo** with the plots **SG1** and **SG2**. The dotted lines represent the borders of the coffee plantation while the broken lines represent streets/ path.

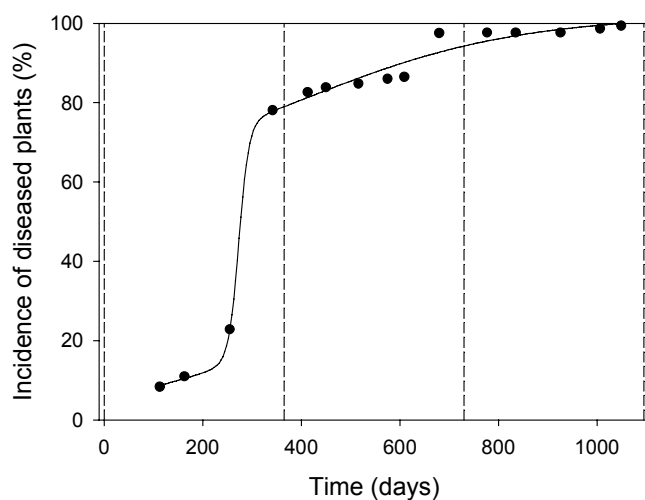


Fig. 40: Incidence of diseased plants and the fitted double sigmoid logistic function ($R^2=0.996$) in the plot **SG1** at **São Gotardo**.

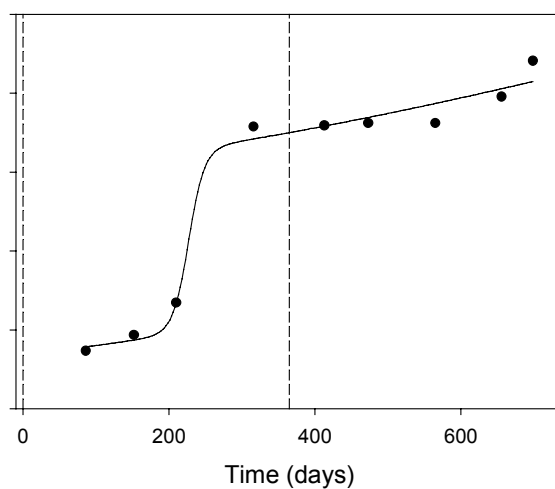


Fig. 41: Incidence of diseased plants and the fitted double sigmoid logistic function ($R^2=0.989$) in the plot **SG2** at **São Gotardo**.

Tab. 7: Background information on the assessed plots.

Plot	Crop, variety	age ^a	spacing (m)	Number of			Assessment		
				plants	rows	plants per row	start	end	duration (months)
SG1	Coffee, 'Catuaí vermelho'	3	0.7 x 3.5	7712	38	98-304	Apr 03	Nov 05	31
SG2	Coffee, 'Catuaí vermelho'	3	0.7 x 3.5	6000	20	300	Mar 04	Nov 05	20
Ervália	Coffee, 'Catuaí vermelho'	2	1 x 2	894	15	14-93	May 03	Jan 05	20
São Carlos	Sweet orange, 'Pera' on rangpur lime	4	3 x 7	1610	10	154-167	Oct 02	Sep 05	35
Engenheiro Coelho	Sweet orange, 'Pera' on rangpur lime	4	4 x 7	1217	38	4-50	Oct 02	Sep 05	35
'B untreated'	Sweet orange, 'Pera' on rangpur lime	4	4 x 7	425	12	34-36	Dec 02	Sep 05	33
'B treated'	Sweet orange, 'Pera' on rangpur lime	4	3.5 x 7	2130	49	1-74	Dec 02	Sep 05	33
Irrigated, Bahia	Sweet orange, 'Pera' on rangpur lime	12	1.5 x 3.5	900	18	50	Jun 03	Oct 05	28
Non-irrigated 1, Bahia	Sweet orange, 'Pera' on rangpur lime	12	1.5 x 3.5	364	14	26	Jun 03	Oct 05	28
Non-irrigated 2, Bahia	Sweet orange, 'Pera' on rangpur lime	12	1.5 x 3.5	362	9-16	30	Jun 03	Oct 05	28
A, Province Corrientes	Sweet orange, 'Valência Late' on rangpur lime	12	3 x 5	320	10	32	Apr 04	Sep 05	17
B, Province Corrientes	Sweet orange, 'Valência Late' on rangpur lime	13	3 x 5.5	289	17	17	Apr 04	Sep 05	17
Irrigated, Province Corrientes	Sweet orange, 'Valência Late' on rangpur lime	12	2,3,4,5 x 4,5,6,7	398	13	12-16	Apr 04	Sep 05	17
Non-Irrigated, Province Corrientes	Sweet orange, 'Valência Late' on rangpur lime	12	2,3,4,5 x 4,5,6,7	398	13	12-16	Apr 04	Sep 05	17

^a age of the plants at the beginning of the assessment

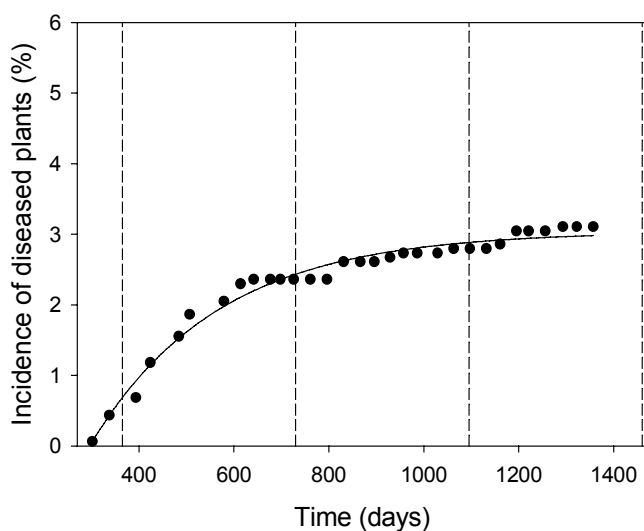


Fig. 42: Incidence of diseased plants and the fitted monomolecular function ($R^2=0.979$) in the plot at **São Carlos**.

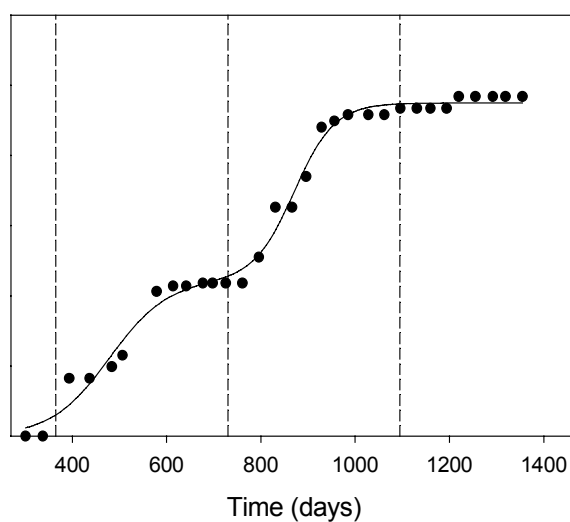


Fig. 43: Incidence of diseased plants and the fitted double sigmoid logistic function ($R^2=0.996$) in the plot at **Engenheiro Coelho**.

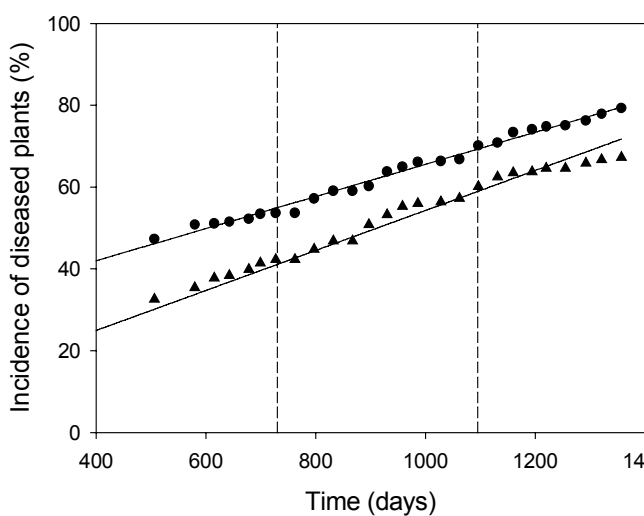


Fig. 44: Incidence of diseased plants and the fitted linear function in the plots '**B treated**' (▲) ($R^2=0.965$) and '**B untreated**' (●) ($R^2=0.989$) at **Bebedouro**. The first four evaluation points were disregarded in this analysis.

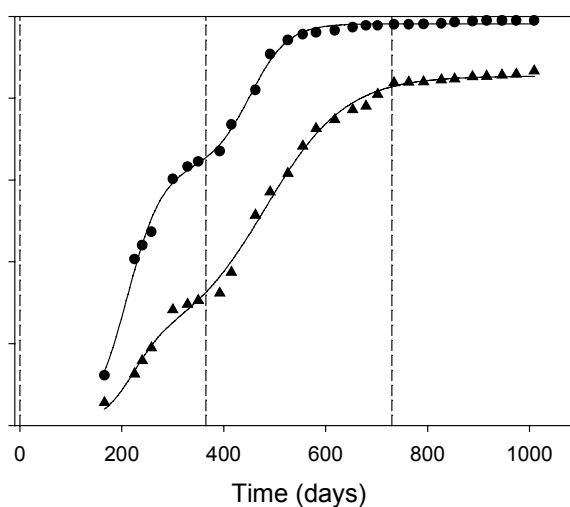


Fig. 45: Incidence of diseased plants and the fitted double sigmoid logistic function in the **irrigated** (●) ($R^2=0.998$) and **non-irrigated** (1 & 2)(▲) ($R^2=0.997$) plots in **Bahia**.

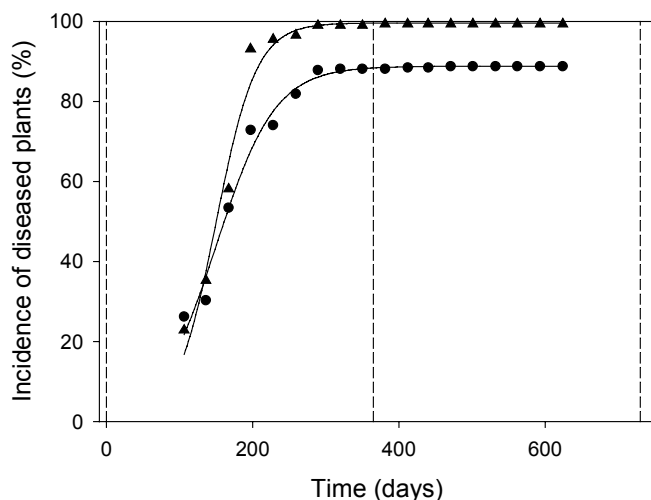


Fig. 46: Incidence of diseased plants and the fitted logistic functions in plot **A** (●) ($R^2=0.992$) and plot **B** (▲) ($R^2=0.983$) in the Province **Corrientes**.

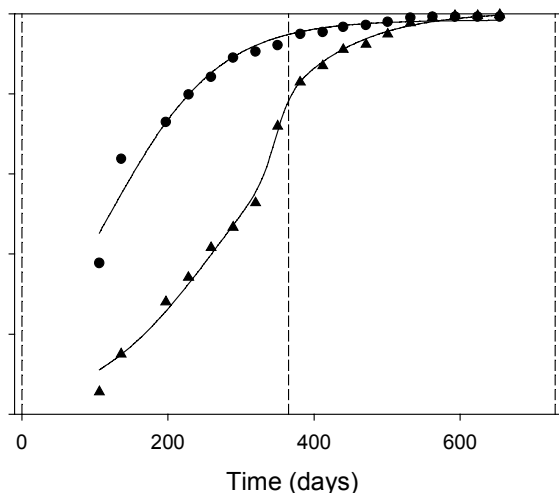


Fig. 47: Incidence of diseased plants and the fitted logistic function in the **irrigated** (●) ($R^2=0.967$) plot and the fitted double sigmoid logistic function in the **non-irrigated** plot (▲) ($R^2=0.997$) in the Province **Corrientes**.

Tab. 8: Disease incidence, ordinary runs and dispersion index analyses of CLS diseased coffee plants in the plots **SG1** and **SG2** at **São Gotardo** and at **Ervália**.

Plot	Date	Cum. inc. (%) ^a	Ordinary runs		Dispersion Index (<i>DI</i>) ^c			
			No. of rows tested	Aggregated rows (%) ^b	Quadrat size 1 x 3	Quadrat size 1 x 5	Quadrat size 1 x 8	Quadrat size 1 x 10
SG1	23.04.03	8.4	32	56.3	1.38***	1.69***	2.03***	2.18***
SG1	12.06.03	11.0	38	73.7	1.49***	1.82***	2.24***	2.39***
SG1	12.09.03	22.9	38	89.5	1.58***	1.98***	2.48***	2.75***
SG1	8.12.03	78.1	36	75.0	2.48***	3.85***	5.79***	7.01***
SG1	17.02.04	82.7	36	72.2	2.53***	3.96***	5.99***	7.26***
SG1	25.03.04	83.9	36	69.4	2.56***	4.01***	6.10***	7.38***
SG1	31.05.04	84.8	36	66.7	2.57***	4.04***	6.13***	7.43***
SG1	29.07.04	86.0	36	69.4	2.60***	4.10***	6.29***	7.55***
SG2	26.03.04	14.7	20	100.0	1.89***	2.57***	3.17***	3.56***
SG2	01.06.04	18.7	20	100.0	1.87***	2.51***	3.06***	3.55***
SG2	29.07.04	26.9	20	100.0	1.86***	2.52***	3.18***	3.75***
SG2	12.11.04	71.5	20	100.0	2.32***	3.46***	4.79***	5.99***
SG2	17.02.05	71.8	20	100.0	2.33***	3.45***	4.79***	5.96***
SG2	18.04.05	72.4	20	100.0	2.33***	3.45***	4.79***	5.93***
SG2	18.07.05	72.4	20	100.0	2.33***	3.45***	4.79***	5.93***
SG2	06.10.05	79.1	20	100.0	2.34***	3.47***	4.82***	6.01***
SG2	18.11.05	88.2	20	100.0	2.29***	3.42***	4.55***	5.61***
Ervália	20.05.03	4.1	8	37.5	1.34***	1.56***	1.70***	1.82***
Ervália	24.09.03	10.0	13	46.2	1.47***	1.74***	2.22***	2.34***
Ervália	13.12.03	42.2	14	50.0	1.74***	2.17***	2.87***	3.20***
Ervália	25.02.04	50.9	14	64.3	1.82***	2.34***	3.13***	3.62***
Ervália	05.05.04	51.0	14	64.3	1.82***	2.34***	3.12***	3.60***
Ervália	18.08.04	51.0	14	64.3	1.82***	2.34***	3.12***	3.60***
Ervália	31.01.05	51.6	14	64.3	1.76***	2.29***	3.47***	3.01***

^a Incidence of diseased plants, generated from the cumulative maps.

^b Values indicate the percentage of rows with a significant aggregation of diseased plants, divided by the number of rows tested.

^c *DI*-values were calculated as the quotient of the observed quadrat variance and the estimated binomial variance. Values significantly greater than 1 are indicated as *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$, Chi-Square test.

Tab. 9: CLS disease incidence in the respective columns of the coffee plot **SG1** at **São Gotardo**. The counting of the columns starts at the path inside the coffee plantation (see Fig. 39).

date	columns				
	1-60	61-120	121-180	181-240	241-300
23.04.03	13.3	7.0	8.7	9.9	4.4
12.06.03	15.3	9.2	10.8	13.7	6.6
12.09.03	42.1	22.4	21.2	23.7	16.7
08.12.03	87.9	79.7	77.6	79.3	64.3
17.02.04	92.5	83.9	81.5	83.8	69.9
25.03.04	93.7	85.3	82.9	85.3	73.9
31.05.04	94.3	86.0	83.7	86.5	74.9
29.07.04	94.5	86.9	85.0	88.2	75.9
mean	66.7	57.6	56.4	58.8	48.3

Tab. 10: CLS disease incidence in the respective columns of the coffee plot **SG2** at **São Gotardo**. The counting of the columns starts at the path inside the coffee plantation (see Fig. 39).

date	columns				
	1-60	61-120	121-180	181-240	241-300
26.03.04	24.8	24.1	12.0	7.8	4.7
01.06.04	29.0	30.6	16.2	11.4	6.3
29.07.04	37.7	41.8	26.3	18.3	10.6
12.11.04	98.1	93.5	75.0	56.4	34.6
17.02.05	98.2	93.5	75.5	56.9	35.3
18.04.05	98.3	93.5	76.1	58.4	35.8
18.07.05	98.3	93.5	76.1	58.5	35.8
06.10.05	99.2	95.7	83.1	70.2	47.5
18.11.05	99.5	99.2	92.0	84.2	66.3
mean	75.9	73.9	59.1	46.9	30.8

Tab. 11: Disease incidence, ordinary runs and dispersion index analyses of CVC diseased citrus plants, plots at **São Carlos**, **Engenheiro Coelho** and **Bebedouro**, plots 'B untreated' and 'B treated' in the State of São Paulo.

Plot	Date	Cum. inc. (%) ^a	Ordinary runs		Dispersion Index (DI) ^c				
			No. of rows tested	Aggregated rows (%) ^b	Quadrat size 3 x 3	Quadrat size 4 x 4	Quadrat size 5 x 5	Quadrat size 3 x 5	Quadrat size 5 x 3
SC ^d	04.12.02	0.4	1	100.0	1.98***	1.28*	2.98***	1.82***	2.31***
SC	29.01.03	0.7	3	66.7	1.72***	1.43**	1.97***	1.84***	1.95***
SC	28.02.03	1.2	5	60.0	1.55***	2.07***	2.57***	2.03***	2.01***
SC	29.04.03	1.6	6	50.0	1.77***	2.22***	2.78***	2.10***	2.47***
SC	22.05.03	1.9	7	42.9	1.89***	3.31***	3.18***	2.53***	2.50***
SC	02.08.03	2.0	7	42.9	1.91***	3.37***	3.28***	2.47***	2.43***
SC	06.09.03	2.3	8	37.5	1.88***	3.19***	3.49***	2.53***	2.63***
SC	04.10.03	2.3	8	37.5	1.88***	3.19***	3.49***	2.53***	2.63***
SC	08.11.03	2.4	8	37.5	1.85***	3.16***	3.39***	2.47***	2.56***

^a Incidence of diseased plants, generated from the cumulative maps.

^b Values indicate the percentage of rows with a significant aggregation of diseased plants, divided by the number of rows tested.

^c DI-values were calculated as the quotient of the observed quadrat variance and the estimated binomial variance. Values significantly greater than 1 are indicated as *=p<0.05; **=p<0.01; ***=p<0.001, Chi-Square test.

^d SC= São Carlos, EC= Engenheiro Coelho, Bu= 'B untreated', Bt= 'B treated'

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Plot	Date	Cum. inc. (%) ^a	Ordinary runs		Dispersion Index (DI) ^c				
			No. of rows tested	Aggregated rows (%) ^b	Quadrat size 3 x 3	Quadrat size 4 x 4	Quadrat size 5 x 5	Quadrat size 3 x 5	Quadrat size 5 x 3
SC	29.11.03	2.4	8	50.0	1.85***	3.16***	3.39***	2.47***	2.56***
SC	27.12.03	2.4	8	50.0	1.85***	3.16***	3.39***	2.47***	2.56***
SC	31.01.04	2.4	8	50.0	1.85***	3.16***	3.39***	2.47***	2.56***
SC	28.02.04	2.4	8	50.0	1.85***	3.16***	3.39***	2.47***	2.56***
SC	30.03.04	2.6	8	62.5	1.94***	3.14***	3.31***	2.46***	2.55***
SC	01.05.04	2.6	8	62.5	1.94***	3.14***	3.31***	2.46***	2.55***
SC	31.05.04	2.6	8	62.5	1.94***	3.14***	3.31***	2.46***	2.55***
SC	03.07.04	2.7	8	62.5	1.90***	3.05***	3.22***	2.41***	2.49***
SC	31.07.04	2.7	8	62.5	2.02***	2.97***	3.14***	2.51***	2.44***
SC	29.08.04	2.7	8	62.5	2.02***	2.97***	3.14***	2.51***	2.44***
SC	11.10.04	2.7	8	62.5	2.02***	3.05***	3.15***	2.51***	2.44***
SC	14.11.04	2.8	8	62.5	2.04***	3.05***	3.30***	2.55***	2.59***
SC	18.12.04	2.8	8	62.5	2.04***	3.05***	3.30***	2.55***	2.59***
SC	22.01.05	2.8	8	62.5	2.04***	3.05***	3.30***	2.55***	2.59***
SC	20.02.05	2.9	8	50.0	2.10***	3.33***	3.54***	2.73***	2.68***
SC	26.03.05	3.2	8	62.5	2.22***	3.91***	3.96***	3.08***	2.57***
SC	21.04.05	3.2	8	62.5	2.22***	3.91***	3.96***	3.08***	2.57***
SC	26.05.05	3.2	8	62.5	2.22***	3.91***	3.96***	3.08***	2.57***
SC	2.07.05	3.2	8	62.5	2.27***	3.97***	3.73***	3.11***	2.65***
SC	31.07.05	3.2	8	62.5	2.27***	3.97***	3.73***	3.11***	2.65***
SC	03.09.05	3.2	8	62.5	2.27***	3.97***	3.73***	3.11***	2.65***
EC ^d	04.10.03	2.1	6	0.0	0.96	1.41**	1.28*	1.02	1.20
EC	08.11.03	2.2	6	0.0	1.21*	1.36**	1.40*	1.21	1.38**
EC	29.11.03	2.2	6	0.0	1.21*	1.36**	1.40*	1.21	1.38**
EC	27.12.03	2.2	6	0.0	1.21*	1.36**	1.40*	1.21	1.38**
EC	31.01.04	2.2	6	0.0	1.21*	1.36**	1.40*	1.21	1.38**
EC	28.02.04	2.6	7	0.0	1.15	1.32**	1.39*	1.19	1.27*
EC	30.03.04	3.3	12	0.0	1.05	1.18	1.16	1.03	1.21
EC	01.05.04	3.3	12	0.0	1.05	1.18	1.16	1.03	1.21
EC	31.05.04	3.7	12	0.0	1.20*	1.32**	1.19	1.19	1.03
EC	03.07.04	4.4	13	0.0	1.26**	1.35**	1.22	1.24*	1.10
EC	31.07.04	4.5	14	0.0	1.29**	1.36**	1.27*	1.30**	1.12
EC	29.08.04	4.6	14	0.0	1.31**	1.42**	1.32*	1.36**	1.14
EC	11.10.04	4.6	14	0.0	1.31**	1.42**	1.32*	1.36**	1.14
EC	14.11.04	4.6	14	0.0	1.31**	1.42**	1.32*	1.36**	1.14
EC	18.12.04	4.7	14	0.0	1.34**	1.39**	1.32*	1.34**	1.16
EC	22.01.05	4.7	14	0.0	1.34**	1.39**	1.32*	1.34**	1.16
EC	20.02.05	4.7	14	0.0	1.34**	1.39**	1.32*	1.34**	1.16
EC	26.03.05	4.7	14	0.0	1.34**	1.39**	1.32*	1.34**	1.16
EC	21.04.05	4.8	14	0.0	1.32**	1.37**	1.28*	1.31**	1.14
EC	26.05.05	4.8	14	0.0	1.32**	1.37**	1.28*	1.31**	1.14
EC	02.07.05	4.8	14	0.0	1.32**	1.37**	1.28*	1.31**	1.14
EC	31.07.05	4.8	14	0.0	1.32**	1.37**	1.28*	1.31**	1.14
EC	03.09.05	4.8	14	0.0	1.32**	1.37**	1.28*	1.31**	1.14

^a Incidence of diseased plants, generated from the cumulative maps.

^b Values indicate the percentage of rows with a significant aggregation of diseased plants, divided by the number of rows tested.

^c DI-values were calculated as the quotient of the observed quadrat variance and the estimated binomial variance. Values significantly greater than 1 are indicated as *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$, Chi-Square test.

^d SC= São Carlos, EC= Engeheiro Coelho, Bu= 'B untreated', Bt= 'B treated'

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Plot	Date	Cum. inc. (%) ^a	Ordinary runs		Dispersion Index (DI) ^c				
			No. of rows tested	Aggregated rows (%) ^b	Quadrat size 3 x 3	Quadrat size 4 x 4	Quadrat size 5 x 5	Quadrat size 3 x 5	Quadrat size 5 x 3
Bu ^d	17.12.02	16.7	12	0.0	1.20	1.30	1.14	1.22	1.08
Bu	03.02.02	28.2	12	0.0	0.87	0.82	0.60	0.66	1.04
Bu	19.03.03	40.2	12	16.7	1.44*	1.73**	1.16	0.88	2.09***
Bu	28.04.03	43.1	12	16.7	1.70**	1.68**	0.83	0.85	2.39***
Bu	21.05.03	47.3	12	8.3	1.78***	1.73**	0.85	0.81	2.57***
Bu	03.08.03	50.8	12	8.3	1.59**	1.47	0.89	0.90	2.14***
Bu	07.09.03	51.1	12	8.3	1.57**	1.44	0.94	0.91	2.11***
Bu	05.10.03	51.5	12	8.3	1.47*	1.35	0.95	0.88	2.13***
Bu	09.11.03	52.2	12	8.3	1.46*	1.43	1.04	0.92	2.05**
Bu	30.11.03	53.4	12	16.7	1.40*	1.57*	1.41	1.07	2.08***
Bu	28.12.03	53.7	12	16.7	1.40*	1.52*	1.37	1.10	2.03**
Bu	01.02.04	53.7	12	16.7	1.42*	1.59*	1.24	0.98	2.08***
Bu	29.02.04	57.2	12	8.3	1.58**	1.47	1.76*	1.41	1.82**
Bu	31.03.04	59.1	12	8.3	1.58**	1.52*	1.53	1.36	1.85**
Bu	02.05.04	59.1	12	8.3	1.58**	1.52*	1.53	1.36	1.85**
Bu	01.06.04	60.2	12	8.3	1.57**	1.52*	1.17	1.07	2.01**
Bu	04.07.04	63.8	12	8.3	1.47*	1.78**	1.22	1.00	2.06**
Bu	01.08.04	64.9	12	8.3	1.63**	2.03**	1.18	1.06	2.21***
Bu	30.08.04	66.1	12	0.0	1.56**	2.04**	1.34	1.19	2.09***
Bu	12.10.04	66.4	12	0.0	1.51*	2.00**	1.29	1.15	2.00**
Bu	15.11.04	66.8	11	0.0	1.49*	2.08**	1.38	1.15	1.97**
Bu	19.12.04	70.1	11	9.1	1.59**	1.92**	1.35	1.36	1.99**
Bu	23.01.05	70.8	11	9.1	1.47*	1.77**	1.18	1.22	1.84**
Bu	21.02.05	73.4	11	0.0	1.44*	1.93**	1.26	1.14	1.99**
Bu	27.03.05	74.1	11	0.0	1.36*	1.90**	1.23	1.02	1.91**
Bu	22.04.05	74.8	11	0.0	1.33	1.77**	1.32	1.05	1.89**
Bu	27.05.05	75.1	11	0.0	1.28	1.74**	1.44	1.06	1.86**
Bu	03.07.05	76.2	11	0.0	1.27	1.59*	1.50	1.00	1.86**
Bu	01.08.05	77.9	11	0.0	1.22	1.63**	1.39	0.99	1.61**
Bu	04.09.05	79.3	11	0.0	1.12	1.47	1.20	0.77	1.48*
Bt ^d	17.12.02	12.3	36	0.0	1.56***	2.14***	2.47***	1.81***	1.90***
Bt	03.02.02	20.7	43	4.7	1.94***	2.79***	3.33***	2.43***	2.46***
Bt	19.03.03	27.0	43	4.7	2.22***	3.18***	4.02***	2.92***	2.98***
Bt	28.04.03	30.0	43	11.6	2.24***	3.30***	4.20***	3.07***	3.03***
Bt	21.05.03	32.6	43	14.0	2.43***	3.52***	4.56***	3.32***	3.33***
Bt	03.08.03	35.5	43	16.3	2.52***	3.81***	4.89***	3.49***	3.48***
Bt	07.09.03	37.8	43	16.3	2.61***	4.03***	5.23***	3.65***	3.70***
Bt	05.10.03	38.5	43	16.3	2.64***	4.07***	5.30***	3.71***	3.75***
Bt	09.11.03	39.9	43	18.6	2.71***	4.18***	5.44***	3.83***	3.86***
Bt	30.11.03	41.6	43	20.9	2.74***	4.32***	5.60***	3.88***	3.97***
Bt	28.12.03	42.5	43	20.9	2.76***	4.38***	5.62***	3.89***	4.00***
Bt	01.02.04	42.5	43	20.9	2.76***	4.38***	5.62***	3.89***	4.00***
Bt	29.02.04	44.9	43	20.9	2.94***	4.60***	5.99***	4.10***	4.28***
Bt	31.03.04	46.9	44	18.2	3.01***	4.66***	6.21***	4.22***	4.42***
Bt	02.05.04	46.9	44	18.2	3.01***	4.66***	6.85***	4.22***	4.42***
Bt	01.06.04	50.8	44	18.2	3.31***	5.09***	6.85***	4.61***	4.85***
Bt	04.07.04	53.2	44	22.7	3.48***	5.44***	7.37***	4.89***	5.18***

^a Incidence of diseased plants, generated from the cumulative maps.

^b Values indicate the percentage of rows with a significant aggregation of diseased plants, divided by the number of rows tested.

^c DI-values were calculated as the quotient of the observed quadrat variance and the estimated binomial variance. Values significantly greater than 1 are indicated as *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$, Chi-Square test.

^d SC= São Carlos, EC= Engenheiro Coelho, Bu= 'B untreated', Bt= 'B treated'

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Plot	Date	Cum. inc. (%) ^a	Ordinary runs		Dispersion Index (DI) ^c				
			No. of rows tested	Aggregated rows (%) ^b	Quadrat size 3 x 3	Quadrat size 4 x 4	Quadrat size 5 x 5	Quadrat size 3 x 5	Quadrat size 5 x 3
Bt	01.08.04	55.3	44	22.7	3.58***	5.68***	7.85***	5.14***	5.39***
Bt	30.08.04	56.0	44	20.5	3.61***	5.73***	7.92***	5.17***	5.46***
Bt	12.10.04	56.4	44	20.5	3.64***	5.78***	8.00***	5.23***	5.50***
Bt	15.11.04	57.2	44	20.5	3.65***	5.85***	8.15***	5.29***	5.56***
Bt	19.12.04	60.1	44	18.2	3.86***	6.27***	8.72***	5.61***	5.97***
Bt	23.01.05	62.4	44	15.9	4.05***	6.61***	9.20***	5.90***	6.27***
Bt	21.02.05	63.5	44	18.2	4.11***	6.76***	9.42***	6.01***	6.38***
Bt	27.03.05	63.7	44	18.2	4.14***	6.81***	9.48***	6.05***	6.42***
Bt	22.04.05	64.6	44	20.5	4.24***	6.98***	9.73***	6.15***	6.60***
Bt	27.05.05	64.6	44	20.5	4.23***	6.98***	9.75***	6.16***	6.60***
Bt	03.07.05	65.8	44	15.9	4.34***	7.12***	9.93***	6.28***	6.78***
Bt	01.08.05	66.7	44	11.4	4.39***	7.25***	10.13***	6.39***	6.88***
Bt	04.09.05	67.3	44	11.4	4.45***	7.35***	10.26***	6.15***	6.94***

^a Incidence of diseased plants, generated from the cumulative maps.

^b Values indicate the percentage of rows with a significant aggregation of diseased plants, divided by the number of rows tested.

^c DI-values were calculated as the quotient of the observed quadrat variance and the estimated binomial variance. Values significantly greater than 1 are indicated as *=p<0.05; **=p<0.01; ***=p<0.001, Chi-Square test.

^d SC= São Carlos, EC= Engeheiro Coelho, Bu= 'B untreated', Bt= 'B treated'

Tab. 12: Disease incidence, ordinary runs and dispersion index analyses of CVC diseased citrus plants, plots irrigated, non-irrigated 1 and non-irrigated 2 in the State of Bahia.

Plot	Date	Cum. inc. (%) ^a	Ordinary runs		Dispersion Index (DI) ^c				
			No. of rows tested	Aggregated rows (%) ^b	Quadrat size 3 x 3	Quadrat size 4 x 4	Quadrat size 5 x 5	Quadrat size 3 x 5	Quadrat size 5 x 3
Irr ^d	15.06.03	12.2	17	35.3	1.74***	1.93***	2.70***	2.16***	2.25***
Irr	13.08.03	40.6	18	44.4	2.03***	2.91***	4.82***	3.06***	3.01***
Irr	28.08.03	44.0	18	44.4	2.33***	3.19***	5.18***	3.26***	3.33***
Irr	15.09.03	47.4	18	27.8	2.16***	2.81***	4.42***	2.84***	3.05***
Irr	27.10.03	60.2	18	33.3	2.35***	3.36***	4.66***	3.05***	3.15***
Irr	25.11.03	63.2	18	27.8	2.11***	2.95***	4.20***	2.72***	2.89***
Irr	16.12.03	64.5	18	27.8	2.04***	2.85***	4.15***	2.65***	2.74***
Irr	27.01.04	67.1	18	33.3	1.83***	2.59***	3.66***	2.48***	2.26***
Irr	19.02.04	73.5	18	11.1	1.81***	2.67***	3.27***	2.38***	2.06***
Irr	06.04.04	82.0	18	11.1	1.63***	1.85***	2.66***	1.98***	1.59**
Irr	05.05.04	90.7	17	11.8	1.54***	1.95***	2.81***	2.29***	1.65**
NI1 ^d	15.06.03	5.5	5	20.0	0.90	0.83	0.73	0.80	1.16
NI1	13.08.03	13.9	11	18.2	1.09	0.74	1.41	1.01	1.46
NI1	28.08.03	17.2	14	7.1	0.95	0.83	1.63	0.98	1.46
NI1	15.09.03	21.0	14	7.1	1.13	1.15	1.85	1.39	1.61
NI1	27.10.03	31.6	14	0.0	0.98	0.84	1.42	1.07	1.12
NI1	25.11.03	32.5	14	0.0	1.02	1.02	1.69	1.19	1.15
NI1	16.12.03	32.5	14	0.0	1.02	1.02	1.69	1.19	1.15

^a Incidence of diseased plants, generated from the cumulative maps.

^b Values indicate the percentage of rows with a significant aggregation of diseased plants, divided by the number of rows tested.

^c DI-values were calculated as the quotient of the observed quadrat variance and the estimated binomial variance. Values significantly greater than 1 are indicated as *=p<0.05; **=p<0.01; ***=p<0.001, Chi-Square test.

^d Irr= plot irrigated, NI1= plot non-irrigated 1, NI2= plot non-irrigated 2

to be continued on next page

Plot	Date	Ordinary runs			Dispersion Index (<i>DI</i>) ^c				
		Cum. inc. (%) ^a	No. of rows tested	Aggregated rows (%) ^b	Quadrat size 3 x 3	Quadrat size 4 x 4	Quadrat size 5 x 5	Quadrat size 3 x 5	Quadrat size 5 x 3
NI1	27.01.04	33.6	14	0.0	1.08	1.00	1.54	1.12	1.29
NI1	19.02.04	38.6	14	0.0	1.27	0.90	1.44	1.08	1.73*
NI1	06.04.04	61.4	14	7.1	0.96	1.00	1.08	1.06	1.62
NI1	05.05.04	66.7	14	7.1	1.04	1.13	0.99	1.03	1.99**
NI1	09.06.04	67.3	14	7.1	1.14	1.11	1.35	1.07	2.10**
NI1	08.07.04	76.9	14	0.0	1.18	1.23	1.25	0.90	1.62
NI1	03.08.04	79.2	14	7.1	1.08	1.12	1.46	0.93	1.72*
NI1	09.09.04	81.3	14	7.1	0.95	0.97	1.50	0.80	1.33
NI1	14.10.04	83.3	14	7.1	0.87	1.07	1.33	0.75	1.04
NI1	09.11.04	83.3	14	7.1	0.87	1.07	1.33	0.75	1.04
NI1	02.12.04	83.6	14	7.1	0.87	1.07	1.33	0.75	1.04
NI2 ^d	15.06.03	5.7	6	16.7	1.71**	2.17***	2.20**	1.44	2.31***
NI2	13.08.03	11.3	9	33.3	1.74**	1.56*	1.89**	1.44	1.89**
NI2	28.08.03	14.5	11	9.1	1.56**	1.78**	1.79**	1.43	1.75**
NI2	15.09.03	17.1	12	16.7	1.41*	1.57*	1.95**	1.23	1.95**
NI2	27.10.03	25.0	13	30.8	1.57**	1.54*	2.45***	1.81**	2.10***
NI2	25.11.03	26.8	13	23.1	1.53**	1.46	2.49***	1.86**	2.08***
NI2	16.12.03	28.6	13	15.4	1.52**	1.50*	2.48***	1.92**	2.08***
NI2	27.01.04	31.1	13	15.4	1.67**	1.69**	2.65***	2.19***	2.24***
NI2	19.02.04	36.3	13	15.4	2.02***	2.12***	3.08***	2.69***	2.53***
NI2	06.04.04	41.4	14	14.3	2.20***	2.34***	3.59***	2.84***	2.86***
NI2	05.05.04	47.4	14	14.3	2.41***	3.04***	4.07***	3.34***	2.96***
NI2	09.06.04	56.0	14	28.6	2.71***	3.68***	4.17***	3.67***	3.03***
NI2	08.07.04	59.7	14	21.4	2.68***	3.98***	4.38***	3.73***	3.12***
NI2	03.08.04	66.0	14	7.1	3.20***	4.84***	5.53***	4.61***	3.61***
NI2	09.09.04	68.3	14	7.1	3.25***	5.13***	5.78***	4.69***	3.66***
NI2	14.10.04	71.1	14	7.1	3.33***	5.51***	6.51***	4.94***	4.01***
NI2	09.11.04	72.8	14	7.1	3.43***	5.55***	6.74***	5.09***	4.16***
NI2	02.12.04	78.3	12	16.7	3.88***	6.08***	7.88***	5.59***	5.00***
NI2	04.01.05	84.0	12	16.7	4.08***	6.76***	8.87***	6.08***	5.45***
NI2	02.02.05	84.3	12	16.7	4.12***	6.87***	8.99***	6.18***	5.50***
NI2	03.03.05	84.6	12	16.7	4.12***	6.87***	8.99***	6.18***	5.50***
NI2	07.04.05	85.4	12	8.33	4.24***	6.99***	9.20***	6.35***	5.65***

^a Incidence of diseased plants, generated from the cumulative maps.

^b Values indicate the percentage of rows with a significant aggregation of diseased plants, divided by the number of rows tested.

^c *DI*-values were calculated as the quotient of the observed quadrat variance and the estimated binomial variance. Values significantly greater than 1 are indicated as *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$, Chi-Square test.

^d Irr= plot irrigated, NI1= plot non-irrigated 1, NI2= plot non-irrigated 2.

Tab. 13: Disease incidence, ordinary runs and dispersion index analyses of CVC diseased citrus plants in the plots **A** and **B** in the Province **Corrientes**.

Plot	Date	Cum. inc. (%) ^a	Ordinary runs		Dispersion Index (DI) ^c				
			No. of rows tested	Aggregated rows (%) ^b	Quadrat size 3 x 3	Quadrat size 4 x 4	Quadrat size 5 x 5	Quadrat size 3 x 5	Quadrat size 5 x 3
A	Apr 04	26.3	10	10.0	1.90**	0.89	2.75**	2.37***	2.24**
A	May 04	30.3	10	10.0	1.78**	0.78	2.39**	1.98**	2.19**
A	Jun 04	53.4	10	0.0	0.80	1.50	1.68	1.16	1.06
A	Jul 04	72.8	10	0.0	1.04	0.75	1.17	0.97	1.09
A	Aug 04	74.1	10	10.0	1.05	0.74	1.47	1.06	1.77*
A	Sep 04	81.9	10	10.0	1.24	1.21	2.22**	1.41	1.19
A	Oct 04	87.8	10	10.0	1.10	1.00	1.93*	1.44	1.06
B	Apr 04	22.8	10	10.0	0.77	0.77	0.89	1.09	0.69
B	May 04	35.3	14	14.3	1.54*	1.38	2.07*	1.98**	1.52
B	Jun 04	58.1	16	21.4	2.24***	2.02**	1.99 *	1.92**	2.85***
B	Jul 04	93.1	17	0.0	0.82	1.00	1.63	1.06	1.19

^a Incidence of diseased plants, generated from the cumulative maps.

^b Values indicate the percentage of rows with a significant aggregation of diseased plants, divided by the number of rows tested.

^c *DI*-values were calculated as the quotient of the observed quadrat variance and the estimated binomial variance. Values significantly greater than 1 are indicated as *=p<0.05; **=p<0.01; ***=p<0.001, Chi-Square test.