MANAGEMENT OF CITRUS HUANGLONGBING
IN THE STATE OF SÃO PAULO, BRAZIL

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ABSTRACT

In São Paulo State (SPS), the occurrence of several citrus diseases and pests has largely increased fruit production costs. Huanglongbing (HLB), caused by two Candidatus Liberibacter species, is currently the most important citrus disease in the country. First reported in Brazil in 2004, HLB is already present in 268 municipalities affecting 24% of the more than 96 thousand citrus blocks. Control measures include elimination of symptomatic trees, insecticide application against the vector of the disease, Diaphorina citri, and planting healthy trees produced in screened nurseries. Tree elimination is mandatory but has not been adopted in all farms, where HLB incidence has increased considerably. This situation affected the effectiveness of disease control in their neighbors. Important research questions to improve disease management also need answers, despite the wide perception that only the increase in citrus resistance to liberibacter or D. citri will bring long-term solution to the HLB problem.

Key words: Huanglongbing, citrus production, São Paulo, Brazil

THE BRAZILIAN CITRICULTURE

In Brazil, citrus is commercially explored in all geographic regions more importantly in the States of São Paulo (SPS), Bahia, Sergipe, Minas Gerais and Paraná located in the southeastern and northern regions of the country. The total citrus area was estimated at 900,000 ha in 2007, of which 70% is planted in SPS (Anonymous 2008), where most of the estimated 200 million trees are of ‘Valência’, ‘Natal’, ‘Pêra’ and ‘Hamlin’ sweet oranges. Close to 80% of all harvested fruits are destined to four major industries for the production of 1.35 million tons of concentrated orange juice exported mainly to European, North American and Asian countries, while the remaining 20% are sold in natura mainly in the local markets (Boteon and Neves 2005). In 2007, the country was responsible for 40% of all citrus fruits and 60% of all orange juice produced in the world (Neves et al. 2007). The citrus business has generated 5.6 billion dollars earnings yearly and more than 400,000 jobs.

MAIN CITRUS DISEASES AND PESTS

The large spatial and long temporal continuities of susceptible tissues, and the low genetic variability, have made the SPS citriculture vulnerable to new diseases and pests and occurrence of severe epidemics. The necessity to implement disease-preventing and contention measures, such as the production of new young trees in screened nurseries to avoid insect vectored diseases, frequent orchard inspections for identification and elimination of disease affected trees, and constant insecticide and fungicide applications, has increased production costs considerably.

Among the diseases that have caused severe losses historically are: 1) citrus tristeza, caused by the Citrus Tristeza Virus, which led to the elimination of more than 9 million trees in the 1940s and replacement of the sour orange rootstock with Rangpur lime; 2) citrus leprosis, caused by the Citrus Leprosis Virus, which was first reported in 1933 and is today responsible for constant applications of costly miticides against the vector of the
pathogen; 3) citrus canker, caused by the bacterium \textit{Xanthomonas citri} subsp. \textit{citri}, which was first detected in 1957 and led to the elimination of more than 7 million trees in the last 9 years; 4) citrus leafminer \textit{Phyllocnistis citrella}, first reported in 1996 and responsible for leaf damage and for favoring infection by the canker bacterium; 5) citrus black spot, caused by the fungus \textit{Guignardia citricarpa}, and today present in several locations where it induces early fruit drop and fruit depreciation; 6) citrus variegated chlorosis, reported in 1987 and today affecting around 80 million trees only in SPS; 7) citrus sudden death, a disease of unknown etiology, which has led to the elimination of 4 million trees since 2001, when it was first reported; and 8) huanglongbing (HLB), caused by \textit{Candidatus Liberibacter americanus} and \textit{Ca. L. asiaticus}, and responsible for the elimination of estimated 5-6 million trees since it was first reported in 2004.

**Huanglongbing**

\textit{Two genetically similar but biologically distinct liberibacter species.} Huanglongbing was first reported in Brazil in March 2004 in two farms in two municipalities in the geographic center of SPS. Currently, the disease has already been detected in municipalities of São Paulo (244), Minas Gerais (1) and Paraná (23) States (see Fig. 1 for disease distribution in SPS in 2008). The affected plants found at the beginning showed the characteristic HLB symptoms observed in other countries, namely, yellow shoots, blotchy-mottled leaves associated in most cases with strong zinc deficiencies, lopsided fruit with aborted seeds, and shoot dieback. Plants with different symptom intensities and blocks with different disease incidences were found in those farms. Initially, the use of PCR with primers specific for \textit{Ca. L. asiaticus} and \textit{Ca. L. africanus} gave negative results (Teixeira et al. 2005). Soon it was shown that the reason was the occurrence of a distinct liberibacter, brought later to species level and named \textit{Ca. L. americanus}, which contains DNA sequence changes in the 16SDNA gene not fully recognized by the primer pairs used for the other two species. With the description of the new species, primers were developed for routine pathogen detection and disease diagnostics, crucial for the field surveys and researches that have been carried out since 2004.

Soon after the discovery of \textit{Ca. L. americanus}, the species \textit{Ca. L. asiaticus}, known to occur for over 100 years in China, was also found in SPS (Colleta et al. 2004) (Fig. 1), but at a much lower frequency than \textit{Ca. L. americanus}. \textit{Ca. L. asiaticus} frequency has dramatically increased over the years. Data analysis of the Fundecitrus’ diagnostic laboratory has shown an increase in \textit{Ca. L. asiaticus} from around 2% in 2004 to around 80% in 2008 (Lopes et al. 2008).

![Fig. 1. Map of the State of São Paulo showing the municipalities affected by \textit{Ca. L. americanus}, \textit{Ca. L. asiaticus}, or both liberibacters, from March 2004 to July 2008.](image-url)
Concurrently, Ca. L. americanus proportion decreased from 98% in 2004 to 20% in 2008. Farms that were initially affected almost exclusively by Ca. L. americanus, are today affected almost exclusively by Ca. L. asiaticus. Experimental data strongly suggest that this shift in Liberibacter prevalence is associated with the different abilities of these liberibacters in multiplying in citrus trees, where Ca. L. asiaticus reaches higher bacterial titers than Ca. L. americanus (Lopes et al. 2009a). Higher titers would increase the chances of pathogen acquisition and transmission by the insect vector, as has been described, for example, for the xylem-limited bacterium Xylella fastidiosa in grapevines by sharpshooters (Hill and Purcell 1997). In fact, in graft transmission experiments, in singly and co-inoculated trees, Ca. L. asiaticus has invariably been transmitted at higher percentages than Ca. L. americanus. Higher frequencies of Ca. L. asiaticus acquisition in relation to Ca. L. americanus also have been observed in D. citri transmission experiments (P.T. Yamamoto, unpublished data).

Ca. L. americanus and Ca. L. asiaticus also differ in their sensitivities to high temperature (Lopes et al. 2009b). When affected trees were maintained for 60 days at daily cycles of 27°C to 32°C, or for 90 days at 24°C to 35°C, a complete symptom remission in the new vegetative flushes was observed in trees affected by Ca. L. americanus, but not in those affected by Ca. L. asiaticus. Contrary to the Ca. L. asiaticus-affectted trees, the pathogen was not detected in any tree previously affected by Ca. L. americanus. Ca. L. asiaticus was however affected by the 24°C to 38°C daily cycle, a temperature condition totally detrimental to Ca. L. americanus. Liberibacter thermal sensitiveness might explain the irregular spatial progress of HLB in SPS, which has moved to a less extent towards the north and northwest regions of the state. These regions are characterized by prevalence of hotter summer days than the center and south of SPS, where HLB occurs at high incidence levels.

**Liberibacter transmission.** Both liberibacters that occur in Brazil are transmitted by the psyllid Diaphorina citri, shown early in 1967 for Ca. L. asiaticus (Capoor et al. 1967), and recently for Ca. L. americanus (Yamamoto et al. 2006). Known to occur in Brazil since at least 1942 but not causing any visible damage to citrus trees, D. citri remained practically unnoticed in citrus orchards until 2004. Today, it is the most unwelcome insect in citrus farms with thousands of dollars spent yearly on the use of insecticides to keep its populations as low as possible. In the presence of new young flushes that develop during the wet spring and summer months, or at any time of the year in irrigated areas, D. citri may reach high populations. Literature reviews show that under favorable conditions, a female may lay up to 800 eggs during the three- to four-month life span (Halbert and Manjunath 2004). It takes 15-30 days to become an adult after passing through five nymphal stages. The duration of the egg and nymphal stages of D. citri in Brazil varied from 2.6 to 7.7 days and from 9.4 to 35.8 days, respectively, at temperatures from 18°C to 32°C (Nava et al. 2007). Egg viability was higher than 81.6% at the 18°C to 32°C. Nymph viability was higher than 70% at the 18°C to 30°C but dramatically reduced at 32°C. The lower temperature development threshold (TT) and thermal constant (K) values were 12.0°C and 52.6 degree-days (DD) for eggs, 13.9°C and 156.9 DD for nymphs, and 13.5°C and 210.9 DD for the complete biological cycle (egg to adult). Minimum periods for pathogen acquisition and transmission are under investigation.

The use of contaminated buds during the process of young nursery tree production could be another means of pathogen transmission. In two greenhouse experiments, in which buds of symptomatic and asymptomatic shoots were grafted on the stems of the Rangpur lime rootstock, Ca. L. americanus was perpetuated in about 2% of the shoots that grew from buds removed from symptomatic shoots, but not in any plant that grew from buds removed from asymptomatic shoots (Lopes and Frare 2009b). When the duration of the egg and nymphal stages of D. citri in Brazil varied from 2.6 to 7.7 days and from 9.4 to 35.8 days, respectively, at temperatures from 18°C to 32°C (Nava et al. 2007). Egg viability was higher than 81.6% at the 18°C to 32°C. Nymph viability was higher than 70% at the 18°C to 30°C but dramatically reduced at 32°C. The lower temperature development threshold (TT) and thermal constant (K) values were 12.0°C and 52.6 degree-days (DD) for eggs, 13.9°C and 156.9 DD for nymphs, and 13.5°C and 210.9 DD for the complete biological cycle (egg to adult). Minimum periods for pathogen acquisition and transmission are under investigation.

In another experiment conducted in the same greenhouse and using identical procedures, Ca. L. asiaticus was perpetuated in all shoots that grew from buds removed from symptomatic shoots, but not in any plant that grew from buds removed from asymptomatic shoots (S.A. Lopes, unpublished data). This indicates that the chance of disseminating Ca. L. americanus in young nursery plants grown by grafting asymptomatic buds onto
Rangpur lime rootstocks is low. In SPS, this kind of dissemination is, however, unlikely to occur today since all nursery citrus buds and rootstocks are produced under screen. Nevertheless, it cannot be discarded that, in the past, when young nursery trees were produced in open areas, some plants might have been infested by psyllids, which then disseminated the pathogen over long distances.

**Murraya paniculata as an alternative host of liberibacter.** In Brazil, hosts of liberibacter include all commercial citrus species and cultivars, with sweet oranges and mandarins showing higher susceptibility levels than limes and lemons, and orange jasmine (*Murraya paniculata*), known in Brazil as ‘murta’, a very popular ornamental tree (Lopes et al. 2005 and 2006). The first *Murraya* tree suspected to host liberibacter was found in October 2004, in the citrus farm most affected by HLB at that time. The tree was showing yellow leaves and shoot dieback throughout the canopy. Leaves were collected and adults of *D. citri* captured from two parts of the plant. The pathogen (*Ca. L. americanus*) was PCR-detected in both leaf samples and in one of seven lots of 10 psyllids. On trees in urban areas, *Ca. L. americanus* was detected in 11.4% and *Ca. L. asiaticus* in 0.5% of the 550 symptomatic trees sampled in 17 SPS municipalities. Like citrus, affected trees show yellow leaves and shoot dieback in one or more sectors of the canopy. Contrary to citrus, the lack of characteristic blotchy mottling on the leaves complicates the identification of affected *Murraya*. However, *Citrus* and *Murraya* showed similar patterns of pathogen distribution in the canopies. In a few tested trees, PCR-positive results were only obtained from leaf samples collected from symptomatic shoots.

**HLB spatial-temporal progress.** The spatio-temporal progress of HLB epidemics in Brazil seems to be similar to the disease progress observed in other countries. Usually, HLB epidemics take some years to reach high incidence. The temporal progress is dependent on: 1) the amount of vector population; 2) the extent of the inoculum reservoir; and 3) the age of the grove at the first infection. Disease progression in an orchard can be relatively fast, reaching more than 95% of incidence in 3-13 years after detecting the first symptom (Gottwald et al. 2007, 2007a). In young groves (up to 3 years old), disease incidence reached asymptotic levels in 3-5 years, while in older groves more than 5 years were needed. Symptom evolution can also be very fast. Severe symptoms were observed 1-5 years after the onset of the first symptoms, which depended not only on the age of the tree at the moment of infection, but also on the number of multiple infections (Aubert 1992). As the disease severity increases, the yield is reduced which makes the orchard economically unfeasible in 7-10 years after planting (Roistacher 1996).

Liberibacter transmission is probably greatest when new flushes are available, increasing psyllid populations and migrations. Symptom expression is usually more easily recognized during the autumn and winter. Thus, trees infected at different times may express the onset of symptom expression at the same time. Due to the delay between vector transmission and symptom expression, it is likely that when one symptomatic tree is found in the field, many other trees are also infected but, due to a low bacterial titer, are not showing any visible symptoms. Presently, an infection cannot be detected soon after vector transmission. In orchards in Florida, Irey et al. (2006) found nearly the same number of visually symptomatic and asymptomatic but real-time PCR-positive trees. So, when a given visual inspection reveals $n$ percentage of symptomatic trees, it can be assumed that there are in fact $\sim 2n$ percentage of infected trees plus an additional population of infected trees that remain subclinical relative to real-time PCR assay.

The spatial dynamics of HLB has been recently studied in SPS (Bassanezi et al. 2005) and the results were quite similar to previous studies conducted in Réunion Island and China (Gottwald et al. 1989; Gottwald et al. 1991). Usually, higher concentrations of symptomatic trees are initially found at the borders of the citrus blocks. Some evidence of clustering among immediately adjacent diseased trees was demonstrated. In many cases, within-row aggregation was slightly stronger than across-row aggregation. Aggregation of diseased trees within groups was found in all plots at all locations and quadrat sizes except when disease incidence was extremely low or extremely high. Core clusters of HLB-infected trees were associated
with secondary clusters (reflected clusters), as far as 25 to 50 meters apart. Thus, in a local-scale, apparently, the vector generally moves from one tree to the next as well as to trees at 25-50 m distance, initiating new foci of infection. Also, there are strong indications of regional, large-scale, spread of HLB (Gottwald et al. 2007a). A continuous relationship among HLB-diseased trees over a broad range of spatial distances up to 3.5 km was observed. The most common distance estimated between pairs of HLB-infected trees ranged from 0.88 to 1.61 km with a median of 1.58 km. From a regional point of view, 1.58 km could be the average distance of psyllid dispersion. In conclusion, HLB seems to spread through an incessant mixture and alternation of: 1) random primary infections, resulting from infective psyllids that periodically emigrate from HLB sources outside the plots; and 2) secondary infections that occur over short distances from psyllids that become infective within the plots.

**HLB MANAGEMENT PRACTICES AND DISEASE SUPPRESSION PROGRAM**

HLB control in SPS can be divided in two complementary actions: 1) adoption of a package of practices for disease management in affected and non-affected areas; and 2) implementation of a HLB Suppression Program, headed by the Ministry of Agriculture and Secretary of Agriculture of SPS.

**HLB Management Practices**

The HLB management strategies include: 1) elimination of symptomatic trees to reduce inoculum sources; 2) application of insecticides to reduce vector populations so as to avoid pathogen dissemination; and 3) production and planting healthy nursery trees.

Shortly after the confirmation of the presence of HLB in Brazil, it was believed that removing symptomatic shoots of affected trees would be enough to remove the contaminated tissues and cure the diseased tree. With this objective several trees were empirically pruned by growers, but failed. A field experiment was conducted with 592, 3- to 16-year-old healthy or Ca. L. americanus-affected sweet orange trees (Lopes et al. 2007). The trees were pruned by removing only the symptomatic shoots (by cutting them at the trunk level), or by removing the entire canopy (decapitation) (by cutting the trunk 15-20 cm above the graft line). The experiment confirmed the ineffectiveness of pruning found by the growers. Mottled leaves reappeared on most symptomatic (69.2%) or appeared on some asymptomatic (7.6%) pruned trees, regardless of age, variety, and pruning procedure. The ineffectiveness of pruning reinforced the importance of symptomatic tree elimination in HLB management.

The elimination of symptomatic trees is probably the most important measure. HLB symptom manifestation can be confused with those induced by other pathogens or mineral deficiencies. As such, it is critical to constantly keep in the farms, motivated and well-trained inspectors who are able to find most HLB affected trees in the field. The use of platforms attached to tractors (Fig. 2), where the inspectors can better look at the tree canopies, also improves the quality and effectiveness of the inspection process. In experimental comparisons of groups of two to four well-trained inspectors, the percentage of symptomatic trees detections increased from average 48% for ground inspections to 60% for platform with four inspectors.

The apparently long and variable incubation period makes it difficult to find all infected trees, hence all blocks should be inspected at least six times a year, but more frequently during the fall and winter months, when leaf and fruit symptoms become more obvious. Even though crucial information on vector transmission and dispersal rates are, at this moment, not available with regard to Murraya, which would allow a more precise evaluation of its importance as source of inoculum to citrus trees, all Murraya trees should also be eliminated from citrus farms. Even if they are not infected with the HLB pathogen, they may contribute to disease spread by serving as ideal rearing places of psyllids. A campaign is also underway to convince the mayors to stop planting, and to substitute the Murraya trees in urban areas by other ornamental native species.

Reduction of vector populations from internal and external sources is the second
parallel measure. It is costly, as it requires frequent applications of insecticides, mainly during the spring and summer months, when the insect usually reaches high populations. To determine the exact time for chemical applications, the presence of psyllids in the orchard should be monitored visually (from three to five young shoots of 1% randomly chosen trees per block) and with the use of yellow sticky traps placed in the border of the farms or blocks to monitor vector movement. If present, the grower can choose one of the several insecticides, systemic or contact, available in the market. The systemic ones are the most effective, with residual periods of 60-80 days. They can be applied via soil, trunk or drenching. They are also the most expensive and, for this reason, are applied in young nursery trees (before leaving the nursery) and in field trees up to three years of age. Product absorption and translocation are more effective during the rainy seasons. The contact insecticides are cheaper but less effective, with residual periods of no longer than 20 days. They are sprayed on trees older than three years, as well as on younger trees during the dry seasons. To reduce costs, some growers apply contact insecticides combined with other chemicals (miticides, fungicides) or micronutrients.

The third HLB control measure is the use of certified healthy young trees for planting. In SPS, there is a law since 2003 that obligates that all trees must be produced in screened nurseries protected from the access of *D. citri*, aphids and sharpshooters, vectors of HLB, citrus-tristeza and citrus variegated chlorosis' pathogens.

Due to HLB's long incubation period and seasonal variation of symptom expression, a positive result of any applied strategy to control the disease will appear only after at least two years of continuous and rigorous operation. Additionally, due to the long distance of pathogen dissemination, the control strategies have to be applied on a large spatial scale, involving, in some cases, several adjacent small citrus farms.

**HLB Suppression Program**

The HLB Suppression Program was initiated shortly after the first report of the disease in SPS. It was designed with the objective of eliminating all symptomatic trees in the affected areas, to avoid disease spread to

![Fig. 2. Inspectors on a platform-tractor structure examining citrus trees for the presence of the characteristic HLB symptoms.](image-url)
other regions of SPS and to other Brazilian States. Initially, the program involved talks and presentations to call growers' attention to the importance of HLB, how to recognize disease symptoms, and the value of eliminating inoculum sources and vector populations. Experimental demonstration that pruning is not effective in re-establishing the citrus health and the demonstration that *M. paniculata* also hosts liberibacter, led to the promulgation of a federal normative instruction (IN10) in 2005. This makes mandatory the elimination of all affected citrus trees as well as of *M. paniculata* present in citrus farms. The identification of the symptomatic citrus trees to be eliminated was based on at least two official inspections per year, in all farms of the municipalities where HLB was present. With the constant increase in the number of affected farms and, consequently, in the number of official inspectors required to cover all affected areas, a second federal normative instruction (IN32) was published. IN32 transferred to the grower all the responsibility of finding and eliminating the symptomatic trees. Fundecitrus has also participated in the HLB Suppression Program by: 1) carrying out free laboratory diagnostic tests to help the growers and assess the quality of their inspectors; 2) disseminating research information and updates on HLB progress in SPS; and 3) providing practical training for HLB symptom recognition and management.

**PERSPECTIVES OF THE BRAZILIAN CITRICULTURE IN THE PRESENCE OF HUANGLONGBING**

In SPS, a survey carried out in April 2009 revealed that 24.02% of the 96.2 thousand citrus blocks contained at least one affected tree, and that the center and south of SPS are the most affected regions, where presumably both liberibacters were first introduced. The high disease incidence in SPS has alarmed growers from other regions in Brazil and growers from neighboring countries like Argentina, Uruguay and Paraguay, which are apparently still free of HLB. There is no reason to believe that HLB will be confined to the locations where it occurs today. Free transit of people and lack of barriers are factors that favor disease dissemination to short or long distances. Indeed, in these four years, HLB has spread to a large geographic area within SPS, as well as to the neighboring Minas Gerais and Parana States. The importance of the disease in the new citrus areas still free of HLB will depend, ultimately, on how fast and how rigorously the removal of the first affected trees will take place.

Despite the enormous efforts in calling attention to the importance of the disease, many growers have not followed the recommended measures. As a consequence, HLB incidence has increased dramatically in the last year, mainly in orchards with trees up to around 6 years old. In some farms, all citrus trees were replaced by sugarcane but in others, for economic reasons and/or lack of fiscapulation, affected trees continue to be explored, especially the older trees in which HLB symptoms seem to progress at lower rates. Most importantly, however, these farms have served as source of inoculum, and it became very challenging and costly to control the disease in their closed neighbors. For example, in one farm of 240,000 four-year old irrigated trees, in some blocks bordering a highly affected farm where no control measure were applied, the disease has increased from 0.03% in 2005 to 7.63% in 2008. This is in spite of constant removal of affected trees and of around 20 insecticide applications per year.

In the HLB control package, frequent elimination of symptomatic trees is considered the first and most important practice. Tree elimination is based almost exclusively on HLB symptom recognition that, in many cases, is not clear or is confused with those with other causes (mineral deficiencies or other diseases). Frequent inspection shows that the disease incubation period is long and variable. Therefore, at any given time in an infected orchard, there will be: 1) trees not infected by the pathogen; 2) trees infected but not showing any symptoms; 3) infected symptomatic trees but not detected during inspections; and 4) infected trees showing unequivocal and obvious symptoms, which are detected and immediately eliminated during field inspection. Due to the relatively high value of an individual tree, especially bearing ones, those showing doubtful symptoms are not eliminated but maintained in the field until future inspections, or until the result of a laboratory PCR test is available which, in any case, may take several weeks. It should also
be considered that the regular PCR usually does not allow pathogen detection from asymptomatic leaves and may give a false negative result. These field- and laboratory-misdiagnosed trees may be contributing to HLB progress and complicating disease control.

*M. paniculata*, commonly found in urban areas, is a host of both liberibacters present in Brazil, and a preferred host of *D. citri*. It can therefore serve as a source of inoculum for transmission of the pathogens to citrus trees. Although no information on these aspects is yet available, a campaign is underway aiming to avoid new plantings of *M. paniculata*, and to substitute the existing trees with native ornamental species. In Parana State, a resolution of the State Secretary prohibits the production and commercialization of *Murraya*. In SPS, as well as in Parana, some local municipalities, worried with the impact of HLB to their local economies, also started eliminating *Murraya* trees from urban areas.

Parallel to the official program to suppress HLB in Brazil and to the major efforts made by many growers in managing the disease in their farms, important aspects of the HLB problem are being investigated in universities, governmental institutions and Fundecitrus. Some aspects under investigation are: 1) the minimal dose and frequency of chemical applications in psyllid control and preventing infections; 2) effectiveness of the psyllid control in relation to symptomatic tree elimination; 3) role of asymptomatic citrus and *Murraya* trees as source of inoculum; and 4) the influence of temperature on pathogen acquisition, latency and transmission. The information generated from such research might help to improve the strategies currently used in disease management, as well as to reduce their impact on the environment. However, only changes in the citrus host, making it more resistant to liberibacter infection and multiplication, or less attractive or repellent to *D. citri*, might bring the most promising solution to the HLB problem in Brazil and other countries.

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