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Propagation

Impact and management of diseases in the propagation of fruit plants

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Abstract- Well-formed and healthy propagative material is essential for economic success in fruit growing. The health of the seedlings must be ensured during production to prevent the death of plants and spread of pathogens to new areas, which cause an increase in production costs and reduction in yield, with some pathogens making production unviable in the areas where they are introduced. The most viable strategy for disease control in propagative material is the use of resistant cultivars. However, for many fruit cultivars sources of resistance have not yet been identified and in some cases the resistance is "broken" by the emergence of new races of the pathogen. Other measures are also important and recommended in plant propagation, such as the use of integrated disease management, the use of cultural and biological methods, substrate preparation, irrigation management, grafting, balanced nutrition and use of organic matter, eliminating the initial inoculum, and reducing the disease rate. Among the various procedures in nurseries for ensuring the health of seedlings are the use of pathogen-free seeds and cuttings, handling of substrates in clean facilities, disinfection of hands, tools and containers, maintenance of water quality for irrigation, and the elimination of invasive plants. It is important to clean the nurseries and have a suitable place for the disposal of seedlings, substrates and crop residues. A record and history of the operations in the production of seedlings should be a routine, as well as security and control in access to greenhouses or nurseries. The application of knowledge and the best strategies of integrated disease management for the production of healthy seedlings, guarantees the quality of the productive material and the success of the crop.

Index Terms: Propagative material, diseases, control, management.

Impacto e manejo das doenças na propagação das fruteiras

Resumo-Mudas bem formadas e sadias são o sucesso econômico na fruticultura. A sanidade das mudas deve ser assegurada durante o processo de produção, evitando a morte de plantas e a disseminação de patógenos para novas áreas, tendo como consequência o aumento do custo de produção e a redução da produtividade, podendo determinados patógenos, inviabilizar temporária a cultura nas áreas onde foram introduzidos. As doenças no material propagativo estão entre as principais causas de redução na produtividade agrícola e a estratégia mais viável para o seu controle é o uso de cultivares resistentes. Entretanto, para muitas fruteiras ainda não foram identificadas as fontes de resistência e, em alguns casos a resistência é "quebrada" pelo surgimento de novas raças do patógeno. Outras medidas também são importantes e recomendadas na propagação, como a utilização do manejo integrado, com o uso de métodos culturais e biológicos, preparo do solo, manejo da irrigação, enxertia, nutrição equilibrada e uso da matéria orgânica, eliminando o inóculo inicial e/ou pela redução da taxa da doença. Entre os vários procedimentos nos viveiros visando à sanidade das mudas, estão também a utilização de sementes e estacas isentas de patógenos, a manipulação dos substratos em locais limpos, a assepsia das mãos, ferramentas e recipientes, a utilização de água para irrigação com qualidade e a eliminação de plantas invasoras. É importante a limpeza dos viveiros e ter um local adequado para o descarte de mudas, de substratos ou de restos de cultura. Deve-se manter uma rotina do registro e histórico das operações na produção de mudas, bem como a segurança e o controle no acesso às estufas ou viveiros. A aplicação dos conhecimentos e das melhores estratégias de manejo integrado para a produção de mudas sadias, garante a qualidade do material produtivo e o sucesso da cultura.

Termos para Indexação: Mudas, doenças, controle, manejo.

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Introduction

For the economic success of a culture, the propagative material (seedlings, plantlets, grafts, etc) has a fundamental role in obtaining well-formed and healthy plants. The health of seedlings must be assured during the entire process, preventing premature death of the plants. In addition, there is a risk of introduction of pathogens in uninfested areas, which in most cases occurs through infested propagative material, which leads to the appearance of epidemics and consequent increases in the cost of production. Another important point to highlight is the reduction in the number of plants in the stand in the field, leading to a reduction in production (yield) of the plants, and the temporary unavailability of areas for the culture of desired species and cultivars.

Use of preventative measures in the production of healthy seedlings is one of the most efficient mechanisms of control because once established, a disease is very difficult to control in the field, as well as in the nursery itself. In the production of seedlings, the presence of the pathogens has diverse origins, of which the most important are the seeds, infested stakes, substrates, water (of rain and irrigation), wind (currents of air, for fungi and bacteria), tools (lack of sanitation), host plants (reservoirs of pathogens), substrates and soil (by means of shoes and containers), hands of employees (lack of sanitation), and insect vectors.

The diseases in seedlings may be biotic (infectious) caused by bacteria, fungi, nematodes, phytoplasmas and viruses; or abiotic, caused by non-infectious factors related to the environment, such as stress, humidity, temperature, conditions of planting, etc. Often the symptoms are similar and if there is not adequate control of the environment, one cannot adequately diagnose the etiology, requiring new methods of detection and identification, as well as strategies for control.

In the nurseries, root diseases are among the principle causes of reduction in productivity of fruit plants. They are caused by root pathogens, which inhabit the soil and infect the subterranean organs of plants and include fungi, bacteria, and nematodes (MICHEREFF et al., 2005a).

Among the various items and procedures to be adopted, aiming at health in the production of seedlings, are the utilization of seeds free of pathogens, manipulation of planting substrates in clean locations, disinfestation of hands, tools and containers, utilization of water with quality for irrigation, elimination of weed plants, control of vectors, security and control of entrance of people in the nurseries, cleaning of the nurseries (utilize appropriate flooring of concrete, gravel or rubber to facilitate sanitation), location to discard seedlings, substrates and plant waste, with incineration weekly, as well as maintain, routinely, a record and history of the operations in the production of seedlings. In addition, there must be adequate management of irrigation, because excess water can cause flooding and compromise the respiration and development of the roots, and maintain a balanced nutrition, because the nutrients are part of the mechanism of defense of the plants, acting in their metabolism as activators, regulators, or inhibitors (ZAMBOLIM; VENTURA, 2012). In this review, we present the knowledge and best strategies for integrated management for the health and production of seedlings with quality.

Health of seedlings and seeds

Infected seeds are the origin of diseased seedlings that serve as sources of the initial inoculum of pathogens, introduction of pathogens into areas where they have been absent, contamination of equipment, and increase in the cost for phytosanitary control.

In modern production of seeds and seedlings, health is of fundamental importance, requiring qualified labor to guarantee the quality and certification of the propagative material. Knowledge of the relationships among microorganisms and seeds, with emphasis on pathogens associated with seeds, dynamics and mechanisms of transmission, development of methods of detection, and control of these pathogens, enables the establishment of tolerances for the levels of health established in legislation and guarantees the production of healthy seedlings.

The dissemination of pathogens can occur by water, wind, animals and equipment, but the dissemination via seeds and seedlings is the form of transmission most efficient, since it can occur over long distances, the pathogens remaining viable in the infected tissues of the seedlings, preserving the virulence and enabling the immediate formation of initial inoculum in new areas (Figures 1 and 2).

Figure 1- Plantation of banana plants with high incidence of wilt caused by *Fusarium oxysporum* f.sp. *cubense* race 1, planted with infected seedlings from an area with a history of the disease.

Figure 2 - Pineapple plants infected with *Fusarium guttiforme* via the fruit of the mother plant (A) and planted the in the field, demonstrating characteristic symptoms of the disease (B).

One example of the economic impact of the loss caused by seedlings of fruit trees that carry pathogens to the field is that of the guava with a direct loss caused by the nematode *Meloidogyne enterolobii* Yang & Eisenback, 1983*,* estimated at US$ 61 million dollars in the guava producing areas of the states of Bahia, Ceará, Pernambuco, Rio Grande do Norte and Rio de Janeiro (PEREIRA et al., 2009). Adding to the cost was the loss of jobs for 3,703 full-time rural workers due to the decline and death of the orchards. Because of the great potential of this nematode to cause high losses to national agribusiness, diverse studies for management of areas infested by *M. enterolobii* have been and are being carried out. Preferably, the seedlings should be produced in commercial substrates, without soil, and the containers (sacks or tubes) should not be in direct contact with soil during the time in the nursery.

Knowledge of the life cycle of the pathogens permits a choice of the best form of detection, control, and analytical methods of diagnosis. Control of pathogens in propagative material can be done by means of physical treatment (thermotherapy), chemical and biological methods, or regulating the conditions of production and storage (humidity, temperature, etc.). The certification of seeds and seedlings establishes standards of tolerance by means of tests of field and of health. In diagnosis, the methods of analysis of health should attend basic requisites such as sensitivity, reproducibility, economy, speed, and practicality.

Aspects involved in the health of propagative material

Good Seed and Plant Practices – GSPP is a hygiene protocol for the production of propagative material that guarantees 99.99% efficiency. It is a guarantee of quality, especially, to the producer that needs to use fewer pesticides, which also benefits the consumer and the environment. The seeds and seedlings produced under the GSPP are commercialized with certification, guaranteed by external audit, comparable to ISO 9000 processes, to validate the process.

GSPP is still not being widely used in Brazil because demand does not exist, considering that in the Country most production is in open fields, which have the occurrence of diseases. However, as crops migrate to protected environments, the necessity to guarantee healthy seeds and seedlings will grow and the protocols of GSPP will tend to adapt to the Brazilian reality. The demand has already begun with the culture of crops that require high investments in their implantation and use of grafted seedlings, guaranteeing quality and health of the propagative material.

However, the success of the management of the nurseries for the production of seedlings with quality is based on four basic pillars: Strategic Administration, Operational, Productive Process, and People that work directly in the production of seedlings.

The strategic administrative activities of the financial administration and of the strategic planning of the nursery, establish the quality of the seedlings, quantity/volume, cycles of production, and programming of production (monthly volumes) related to commercial aspects and the market.

The operational part involves the activities, training and attribution of responsibilities to the personnel responsible for the nurseries, as well as the logistical support of supplies for the production of seedlings. Training and capacitation of personnel is very important since in many nurseries professionals that lack formation in plant health is observed, principally in the early diagnosis of the diseases, which is extremely important for the eradication and reduction of the initial inoculum, and for preventing the entrance and dissemination of pathogens in the nurseries.

Management of people should prioritize competence, valuing the professionals involved in the productive process, pursuing results strongly committed to the health of the propagative material.

In the productive process of seedlings there currently exist excellent techniques focused on nutrition, fertigation, climatic management, automation of processes, reduction of the cycle of production, and principally, diagnosis of pathogens, that reduce significantly the risks of infections in the propagative material and the cost of production, but they are neglected and not always utilized.

Quality and technology in the production of propagative material

The seeds and seedlings are the inputs most important of the production process. To guarantee quality and the applied technologies, there are a great variety of products and services available in the market to process the seeds, facilitate their planting by the producer, and conduct quality control, of which stand out are:

- *Upgrading*: the process of physical selection and classification based on weight, density, and size, utilizing techniques such as x-rays, air and color separators, and others.

- *Priming:* a process of pre-germination of the seeds by breaking of dormancy, which is used for only a few fruit species. The influence of temperature on germination enables production in diverse regions, independent of the climate. *Priming* enables more vigor and speed (including in establishment); optimal physiological maturation; uniformity in germination and final stand.

- Disinfestation and sanitation: permit eradication, externally and internally, of pathogens transmitted by seeds, without negatively affecting the speed of emergence and final stand of the plants in the field. Technologies to eradicate pathogens transmitted by seeds are available such as those that utilize humid and hot air under rigorously controlled conditions.

Also, there are available technologies of coating of seeds that currently include three categories:

a)- *Film Coating*: application of a thin layer of adhesive polymer to the seed (film coating), that provides improved performance and secure storage, in that the active principles stay fixed to the seed, preventing their loss and consequent financial loss and environmental impact. This process enables the customization of the seed by color according to a criterion of identification, often solicited by the client.

b)- Encrustation and Pelletization: these are techniques that provide a more uniform and smoother surface to the seed, facilitating mechanized planting. The coating also permits the application of active principles and additives (pesticides, stimulants, biologicals), ease of planting, as enables identification and traceability. The rocess of encrustation generally causes an increase in weight the seed of 1 to 5 times, but does not change the form of the seed, but with pelletization, the increase in weight can be up to 15 times, altering the form of the seed.

Micropropagated Seedlings

Due to the characteristic of totipotentiality of plant cells, it is possible to redirect the morphogenesis of the plants, from a group of cells that can be induced by chemical and physical stimulants (SILVA NETO; ANDRADE, 2011).

Biofactories (tissue culture laboratories) enable a jump in phytosanitary quality and genetics in the seedlings, because tissue culture enables elimination of pests and diseases. However, few laboratories have an organization with a focus on the health of the seedlings produced the. Among the barriers is a lack of specific legislation for the sector, inadequate public policies, difficulty of transport of the seedlings *in vitro*, and scarcity of qualified personnel.

Among some market trends, somatic embryogenesis (synthetic seeds), the automation of *in vitro* production processes and the use of LED lamps, besides providing benefits to the plant, also contribute to the more sustainable use of the energy in the “biofactories”.

In the different processes, among which are conventional micropropagation, temporary immersion bioreactors (TIB), and somatic embryogenesis, one searches for a standard of identity of the propagating material with a guarantee of the health, obtained by screening of pathogens and certification of the seedlings (VENTURA and HINZ, 2002).

In dealing with importation of plant material from other countries it is necessary to carry out an Analysis of Risk of Pests (ARP) required by federal legislation, to prevent the risk of the introduction of pathogens that may economically compromise the crop in the country (Figures 3 and 4). In this sense, we should consider not only the Quarantine Pests of the crop to the country, but also those pathogens that can occur on other plants in those countries that are not present in the Brazil (VENTURA and HINZ, 2002).

Micropropagation by tissue culture permits speed in production and guarantees the health and genetic homogeneity of seedlings, principally when it comes to new varieties, in that it does not require a great volume of seedlings for the formation of commercial crops (CAETANO and VENTURA, 2009). After the period of development in trays of seedlings, pineapple plants, for example, should be transplanted to soil beds, where they will remain until reaching the size for planting in the field.

The protocols for asexual multiplication of fruit trees utilizing tissue culture are available for commercial use for most species; however, for new cultivars research is still necessary to permit their use for attainment of great quantities of certified seedlings, conforming to legal requirements and good practices, principally phytosanitary, by the biofactories.

Figure 3 -Plantlets of banana plants infected by the virus *Cucumber mosaic virus* – CMV (A). Plants obtained by tissue culture *in vitro* and acclimatization in a screened nursery where virus indexation should be made (B).

Figure 4 -Propagative material of strawberry imported from other countries and infected with the bacteria *Xanthomonas fragariae* (A) and with phytoplasmas of the groups 16 SrI and 16SrIII) (B).

Grafted Plants

The cloning of different fruit trees on a commercial scale can also be performed using the techniques of cuttings and grafting. In modern pomology, there is a demand for new resistant rootstocks, which should combine resistance to various pathogens, with desirable agronomic characteristics, including compatibility with grafting, reduction of the size of the plant, in the case of tree species, and tolerance to abiotic stress, such as the water stress.

Grafting of plants is still little used for control of soil pathogens in some fruit trees of economic importance in Brazil, however, its use has been recommended in some production regions of guava, cacao, citrus and cashew. In guava plants a great application of grafting is for the control of the nematode *M. enterolobii*, using the techniques of wedge grafting, made on seedlings in the initial stage of development, or the system using a technique of approach grafting of the seedlings (Figure 5). In this species grafting is only necessary in areas infested with *M. enterolobii*, but, despite the necessity and availability of sources of resistance, the bottleneck of this technique resides in the fact that there is no rootstock validated and recommended and, principally, a technique of grafting that guarantees the vigor of the graft in the phase of nursery, as well as in the longevity of the plants in the field. Therefore, the use of resistant rootstocks has excellent prospects, but still has challenges to overcome, for example the compatibility between the grafts, cost of the grafted plants and availability of the rootstock, and promising material is of a hybrid in which there is no guarantee that characteristics of resistance will be maintained in its descendants.

Figure 5- Guava plant cv Paluma seedling with primary symptoms of *Meloidogyne enterolobii* infection, showing the presence of galls on the roots.

With respect to nematodes, more than 4000 plant parasitic species have been reported, associated with the principle cultures of economic importance in the world, according to production region (NICOL *et al*., 2011). Generally, they live in the soil and feed on the roots of the plants, causing direct damage, by destroying cells and tissues of the roots, as well as indirectly, opening wounds, which serve as entrances for other pathogens.

A list of the "Top 10" nematodes most studied includes the species: (1) nematodes of the galls (*Meloidogyne* spp.); (2) cyst nematodes (*Heterodera* spp. and *Globodera* spp.); (3) lesion nematodes (*Pratylenchus* spp.); (4) the burrowing nematode of banana plants *Radopholus similis*; (5) *Ditylenchus dipsaci*; (6) the pine wilt nematode *Bursaphelenchus xylophilus*; (7) the reniform nematode *Rotylenchulus reniformis*; (8) *Xiphinema* (virus vector); (9) *Nacobbus aberrans*; and (10) *Aphelenchoides besseyi* (JONES et al., 2013).

Currently, the greatest part of the use of grafting in the production of seedlings is carried out in protected culture. It is important to note, however, that the pathogens exhibit genetic variability in the region where the graft will be utilized, making it important to investigate this variability to avoid losses in these areas, and thus use resistant rootstock for each specific region.

In citriculture, rootstocks have been used to protect the plants against pests of the soil for more than 150 years (REISCH et al., 2012). Grafting has been used for the management of *Meloidogyne* sp. and *Xiphinema index* (vector of Grapevine fanleaf virus (GFLV), as well as other genera of nematodes of importance such as *Pratylenchus vulnus*, *Criconemoides xenoplax* (ectoparasite) and *Tylenchulus semipenetrans*. These species were traditionally controlled with chemical products but, with the prohibition of nematicides, the necessity for the use of rootstocks with resistance is greater.

In citriculture, after decades of the research it is possible to confirm the importance of the use of rootstocks for management of diseases. Considering perennial species, it is of fundamental importance to choose the rootstock that presents genes of resistance or tolerance to diseases of the locality of the planting. Therefore, as well as the physical and chemical characteristics of the soil and the climatic conditions, it is necessary to know the history of the incidence of pathogens in the region of planting. This information will help in choosing the best rootstock or that with the lowest risk for the range of pathogens in the area.

For example, among the pathogens that interact with citrus plants, tristeza is limiting to particular combinations of canopy and rootstock. The tragic facts recorded in the decade of 40 of the past century are an example of this affirmation. In that period, the plantings were formed by sweet orange on sour and occurring jointly was the black aphid (*Toxoptera citricida*) an efficient vector of the aggressive virus in the planting conditions.

Currently the use of tolerant rootstocks for cultivars of susceptible crowns is a condition sine qua non for the success of citrus culture, and in general, the trifoliate orange (*Poncirus trifoliata*) and its hybrids are resistant to the infection of tristeza, that is, the virus does not multiply in these plants even when they are grafted. This same rootstock as well as sour orange has low susceptibility to *Phytophthora* sp., the causal agent of gummosis, however FEICHTENBERGER (1990) states that susceptibility is high to very high to *Phytophthora* sp. in true lemon, sweet orange, sour lime, rough lemon and grapefruit.

The lemon 'Cravo' rootstock (*Citrus limonia* Osb.) presents tolerance to the virus of tristeza (COSTA et al., 1954; GRANT et al., 1961) and is susceptible to viroids of xyloporosis and exocortis (MOREIRA, 1938 , 1954, 1955), to decline of citrus (BERETTA et al., 1986a) and sudden death of citrus (MSC) (BASSANEZI et al., 2002). The American hybrid 'Rangpur x Troyer' (RxT), is a result of the cross between lemon Cravo x citrange 'Troyer' {*C. limonia* Osb. x [*P. trifoliata* (L.) Raf. x *C. sinensis* (L.) Osb.] and presents tolerance to tristeza, but is susceptible to exocortis, and xyloporosis, and offers medium resistance to gummosis of *Phytophthora* (CASTLE et al., 1986).

In turn, the tangerine 'Cleopatra' (*Citrus reshni* hort. ex Tan.), another rootstock much utilized in the past, is tolerant to tristeza, exocortis, xyloporosis, decline and MSC (GRANT et al., 1961; BERETTA et al., 1986b, 1994; GIMENES-FERNANDES & BASSANEZI, 2001) and presents medium resistance to gummosis of *Phytophthora* (FEICHTENBERGER et al., 1994). Tolerance to tristeza and xyloporosis also is detected in the tangerine 'Sunki' (*Citrus sunki* hort. ex Tan.); however, this rootstock is intolerant to exocortis (GRANT et al., 1961, OLSON et al., 1962). is susceptible to decline (BERETTA et al., 1986b) and MSC (BASSANEZI et al., 2002).

As for nematodes, note that the trifoliate rootstock (*P. trifoliata*) is resistant to the nematode *Tylenchulus sinipenetrans* Cobb, however not to *Radopholus similis* (O'Bannon & Ford, 1977).

It is noteworthy that the new generation of rootstocks, the citrandarins, aims to bring together the quality of the mandarins, such as tolerance to tristeza, decline, the viroid of exocortis of the trifoliates, among them the immunity to tristeza, and resistance to gummosis. The citrandarins are hybrids of microtangerines (mandarins) with trifoliates.

The use of grafting requires, however, rigor and precision in the processes, especially, in relation to the methods of asepsis, since the greatest risk is sanitary, and standing out the transfer of technology to the field (transplanting and the logistics), the management of the environment, cultural practices, and the differentiated procedures of management (irrigation, fertilization / nutrition) and the lack of qualified labor.

Indexing of seedlings with molecular markers

For the indexation and certification of the propagative material it is important to have methods of identification that are practical, economical, and specific, where the new techniques, principally molecular for the detection of pathogens in fruit trees stand out (ABREU et al., 2012; SANTOS et al., 2016). Modern phytopathology requires multidisciplinary action with emphasis on the great technological innovations that include the molecular structure of DNA, technology of recombinant DNA (cloning), the polymerase chain reactions (PCR, qPCR, etc.) and sequencing of DNA, all being utilized in assisting the production of healthy propagative material.

Biotechnology and genomics are being applied daily in plant health and the protection of plants. The information that genomics generates is used for diagnosis of pathogens, as well as in selection assisted by markers and in the isolation of genes in the systems of transformation or in the selection of cultivars for genetic improvement. However, these technologies do not dispense with the diagnosis and conventional strategies of integrated management of the diseases of plants, both in the field and in the nursery, for the indexation and certification of seedlings.

Care in the Nurseries

It is important to make a careful selection of the seedlings in the nursery, and have special care with the nursery's localization (avoid soils infested with fungi and nematodes). It is also important to know the local where the nursery will be installed (history of the area).

In choice of the area for installation of the nursery, the following characteristics : should be observed:

✓ Availability and quality of water for

irrigation. It is important to observe the salinity, contamination by chemical residues and potential contamination with agents that cause diseases to seedlings;

- Availability of electric power for the pump of the irrigation system;
- Be distant from commercial crops or diseased plants. In this case the objective is to avoid the contamination of the nursery with diseases that occur in the field;
- Easy access of vehicles, but not too close to busy roads to avoid dust accumulation on the plants and/ or screens;
- The nursery soil should be of light texture (sandy or sandy clay) to facilitate the drainage of excess water;
- The nursery soil should also be installed in an area with a slope of 0.5 to 1% to facilitate drainage of excess water;
- Areas infested with *Cyperus rotundus* should be avoided;
- The nursery must be fenced to prevent access to animals that could damage the seedlings;
- Promote staff training.

Health of the plants in the nurseries

The methods of control of root diseases in propagative material consist primarily in the prevention of the introduction of the pathogens in areas where they are absent, avoiding the acquisition of seedlings from regions where the problems have already been found. The association of pathogens with propagative material already has been reported and has long been known, with the irreversible negative implications in the propagative material and in the introduction and dissemination of pathogens, principally those considered to be quarantine pests A1 and A2 for the Country. In addition, infected propagative material is unsuitable for agricultural activity, putting at risk the sustainability of the crops and of the entire region. Therefore, the acquisition of seedlings should be made from professional nurseries registered in the Ministry of Agriculture, Livestock and Supply (MAPA). In areas where the diseases have already occurred, there should be periodical inspections to eliminate diseased plants, reducing therefore the inoculum and dissemination of the diseases.

One of the strategies for integrated management of root diseases in propagative material is sanitation, whereby one reduces, or completely eliminates the initial inoculum responsible for the start of the epidemics. In addition, this prevents the pathogens from being disseminated from one region to another (MICHEREFF et al., 2005a).

The strategies of integrated management that aim at sanitation, never should be restricted to a single strategy to guarantee a quality of the seedlings in the nurseries, but the integration of actions of which stand out:

• Propagative material healthy and certified;

• chemical or heat treatment of seeds and seedlings;

• utilize substrate and soil for the production of seedlings sterilized or treated by solarization. If trays are used, they should be disinfected (chlorine, sodium hypochlorite or quaternary ammonia) and eliminate old and broken trays, that are difficult to disinfect;

• utilize floors covered with plastic, cement or gravel (to prevent drops of water from soil reaching the trays);

• elimination of crop wastes;

• date of planting or production of seedlings to avoid periods of high levels of inoculum;

• realization of roguing of the plants with symptoms of diseases;

• realization pruning or elimination of organs or diseased tissues, principally in perennial hosts;

• elimination of alternative hosts, principally in the case of bacteria and viruses, reducing the presence of inoculum from these plants;

• balanced fertilization;

• use water of good quality for irrigation.

Irrigation

Irrigation should be applied to make available to the plant only the quantity of water necessary for its optimal development, since excess water causes the predisposition to infection by pathogens. The water should be of good quality and without contamination with phytopathogens (ZAMBOLIM et al., 2000). The management of irrigation has a moderating effect on the microclimate within the canopy of plants and interferes in infection and the progress of diseases.

A system of micro-sprinklers should be preferred, and sprinklers that cause great impact of the water against the soil should not be used because they contribute to the dissemination of pathogens, as well as battering the seedlings, which can cause lesions and stress of the plants that facilitates infection. Irrigation should be managed to provide adequate water to the seedlings, but with good drainage so that excess does not occur (Figure 6). It should be noted that the reuse of drainage water in more technologically produced and protected crops, can be characterized as an important factor for preventing pollution of underground and superficial waters with the nutrients utilized. Nevertheless, this reutilization of the water of drainage also includes a huge risk for dissemination of phytopathogens. Thus, when one reuses the water, sanitation of the drainage water of the system is necessary.

The incidence of wilting in seedlings (damping-off) is favored by clayey, poorly drained soils, deficient aeration, and seeding densely and deeply, which predisposes seedlings to the action of the pathogens responsible for wilting (Figure 7). High temperatures and rainy periods contribute to the increase in severity of the disease. The use of mulch also favors significantly the incidence of disease in young plants, independent of the cultivar (ELDER et al., 2000).

Figure 6- Seedbed with pineapple plants ready for transplanting into the field after climatization in a nursery. Observe the construction of the beds, which are higher, facilitating drainage to prevent infection by *Phytophthora* sp.

Various measures should be adopted for control of damping-off of plantlets, beginning with the choice of the location of establishment of the nursery, which should be in a well ventilated location, free of flooding, with good

Figure 7 -Seedlings of papaya plants showing symptoms of damping-off (A) and necrosis of the root tissues (B).

exposure to sun, and away from roads and fields, following recommended techniques for the construction of nurseries.

In the use of plastic sacks (transparent or dark) for seedlings, they should be of proper dimension as recommended for each species, with holes in the bottom to enable the drainage of water. Small sacks are not recommended because they can cause deformation of roots in the sack, posteriorly compromising the development of the plants leading to death.

It is important to make a chemical analysis of the substrate and enrich the mixture with the addition of fertilizer such as superphosphate and potassium chloride. Organic fertilizer should be well weathered and its application should precede the addition of the mineral fertilizer. Barnyard manure presents a risk of contamination with herbicides such as 2,4-D, which is very phytotoxic to seedlings of different species.

Principal Control Measures

The physical, chemical and biological components of the environment of the nursery have a direct impact on the growth of the propagative material and in the development of the plants. Root diseases are generally the results of a disequilibria in the soil. Most of the time, the origin this disequilibrium is in the agricultural systems adopted, that transform the fields into locations of highly simplified ecology, turning them less resistant to perturbations by some agents, among which are the phytopathogens (MICHEREFF et al., 2005b).

Plants well fertilized and with balanced nutrition are more resistant to diseases. Organic fertilizers are good for improving the structure of soil and facilitating the action of antagonists that exert biological control of pathogens (ZAMBOLIM and VENTURA, 2012).

Genetic control of diseases

Methods of control based on chemical, biological, physical, or cultural control have demonstrated limited efficiency in many interactions. Thus, control by genetic resistance constitutes the best alternative for management of these diseases, and with this, can achieve significant increases in productivity. The planting of these cultivars is also in line with the growing pressure of society for a reduction in the use of pesticides and for techniques that lead to a sustainable agriculture.

There are innumerable technologies that contribute to development in this area of knowledge that are already available in the market. Seeds and seedlings can be traced by means of a bar code reading that traces information about the origin, disease resistance, and characteristics of the cultivar. Another novelty is the "Seed Chipper", a device that conducts a genetic analysis of a tissue sample of the seed (obtained through a scraping), enabling the choice to plant only seeds with the desired characteristics selected by the researcher (FRALEY, 2014).

Based on these technological advances, all of the information produced in the field and laboratories can be centralized by means of central servers, available daily to the technicians realizing the phytosanitary strategies most appropriate for each planting, thus enabling recommendations of seedlings of cultivars or varieties based on the edaphoclimatic conditions of the field where they will be planted and the phytosanitary risks, to enable an optimization of productivity.

Cultural control of diseases

Cultural control of diseases consists basically in the manipulation of the conditions of formation of the seedlings, and during the development of the host, to the detriment of the pathogen, aiming at prevention, or interruption of an outbreak, by means that do not include genetic resistance and the use of pesticides. The primary objective of cultural control is to prevent contact between the susceptible host and viable inoculum, in a manner that reduces the infection rate and subsequent progress of diseases (MICHEREFF et al., 2005b).

In general, the methods of cultural control can be considered to aim to avoid the disease or suppress the causal agent, aiming, therefore, for attainment of healthy seedlings. For this, the nutritional state of the seedlings can favor or limit the process of infection and of colonization by root pathogens. The effects of mineral nutrition of plants on diseases have been comprehensively related by (ZAMBOLIM and VENTURA, 2012).

Some nutrients can lead to evasion based on the development and maturity of certain organs. The rapid growth of seedlings can facilitate the evasion of certain nursery diseases. The fungus *R. solani* has preference for young tissues. The resistance in these tissues increases with the content of pectic substances and calcium in the hypocotyl of the plants.

Biological control of diseases in propagative material

In biological control of root diseases of seedlings of fruit trees, fungi are the principle antagonistic agents used in nurseries and in the initial phases of the development of the plants in the field. The mycoparasites *Trichoderma* sp. and *Gliocladium* sp., are the most extensively studied, not only under laboratory conditions, but also in the greenhouse and field. They have been considered to be effective for biocontrol of some phytopathogens, principally those with structures of resistance considered to be difficult to be affected by the microorganisms already present in the substrates, such as spores, sclerotia, chlamydospores and microsclerotia.

As principle mechanisms of action, the species of *Trichoderma* can act by antibiosis, parasitism and competition, alone or jointly. The strategies of biocontrol of diseases of roots are part of integrated management that views the diminution of the population density of the pathogen, not only by the use of antagonistic microorganisms, but associated with other practices, such as utilization of fungicides, solarization, and fumigation, among others.

As strategies to improve the efficiency of the control of particular diseases, including in seedlings and healthy seeds, besides research and development of new molecules, the introduction of biofungicides in programs of management is a great strategy in integrated management. With action distinct from those exerted by chemical products, the biological products stand out by performance in integrated management of diseases. In general, biological products are specific or selective, contributing to greater preservation of natural controls in agroecosystems. When used with great frequency for management of diseases, some fungicides can generate, over time, populations of resistant pathogens, making their control difficult.

Biological products for control of diseases act in distinct manners and may be made utilizing fungi, bacteria, viruses, or plant extracts and oils. These active ingredients can be utilized in programs of control, principally to manage the resistance of populations of pathogens to particular synthetic chemical active ingredients.

One of the agents of biological control that has been widely utilized in the control of diseases in seedlings was developed with the bacteria *Bacillus subtilis*. This is a nonpathogenic bacterium, common in soil and water, that acts as a tool of protection and has as a principal characteristic, inhibition of the development of phytopathogens in management of some diseases.

Physical control of diseases

Physical methods include various forms of physical energy for control of pathogens. Thermal treatment with steam was one of the first to be adopted and, later, solarization was developed, where milder temperatures are attained, causing alterations less drastic to the microbial communities and biota of the soil.

Many research works describe the control of a great variety of pathogens by solarization. The humidity of the soil is important for the efficiency of this treatment, since germination of structures of resistance of the pathogens occurs in humid soil, turning them more sensitive to the action of the temperature and antagonistic microorganisms. Therefore, plastic should be placed on humid soil to be effective.

The area treated with a solarization should be as great as possible and continuous. Solarization of soil in strips is not recommended due to the possibility of re-infestation of the solarized soil with inoculum present in the untreated strip and due to the "border effect" (GHINI et al., 2005).

The disinfestation of substrates for the production of seedlings in containers is a serious problem for many farmers. Infected seedlings and contaminated substrates disseminate pathogens to new areas, as well as foster the emergence of diseases from the start of the crop cycle, which can cause serious losses. The principal treatment utilized is fumigation with methyl bromide, however, the prohibition of this product, which should occur in the coming years, generates a necessity for attainment of alternatives for the disinfestation of substrates (GHINI et al., 2005). Substrates can be disinfested in special chambers, where steam is injected under pressure, as in the case of autoclaves. The advantages and disadvantages of the system are similar to those presented for the use of steam in the field. Some pathogenic inhabitants of the soil, such as fungi, bacteria, and nematodes, can be inactivated in the chamber with some hours of treatment, due to the high temperatures attained (GHINI et al., 2005).

The principal objective is the attainment of plant propagation material free of pathogens. With that purpose, thermotherapy is an efficient method that obtains elimination of pathogens of the tissues of the host, internally as well as externally. The technique has been used to control diseases of sugarcane, cereals, vegetables, ornamentals, and fruit trees; however, it has been limited by empirism and by the lack of utilization of the information published.

The basic principle of thermotherapy resides in the fact that the pathogen is eliminated by treatments in specific time-temperature relationships that produce few deleterious effects in the plant material. In this case, the greater the difference in thermal sensitivity of the host and the pathogen, the greater will be the chance of success of the thermotherapy.

Treatment by heat can be done, basically, in two ways:

a)- by an intense and short exposure, generally used for eradication of microorganisms, or

b)- by a less intense and long exposure to the heat, utilized to reduce the concentration of the pathogen in a plant, generally, associated with a culture of meristems. For this, the material of propagation can be treated with hot water, hot air or steam. In general, treatment with hot water is done with greater temperatures than that with hot air. The association with a chemical treatment, such as fungicides dissolved in the solution, can increase the efficiency of the treatment.

Chemical control of diseases

The improper use of pesticides to control diseases can cause great impacts in the environment, contaminating groundwater, creating disequilibrium in microbial populations in the soil and causing the emergence of new races of pathogens, or the appearance of others that were in equilibrium.

Progress in the development of fungicides for use in the soil has been limited by the fact of that many chemical molecules are degraded rapidly by deterioration of the products in the soil or adsorbed chemical/physically in the soil, especially in soils with high levels of organic matter or clay.

Conclusions

Conclusions about the integrated management of diseases

Integrated management of diseases of propagative material of fruit plants as of any plant can be summarized and organized in seven general principles Whetzel proposed at the start of the past century and that remain valid (ZAMBOLIM et al., 2000): *Evasion* - prevention of disease by planting in periods when the inoculum is inefficient, rare or absent; *exclusion* - prevention of the entrance of a pathogen in an area still not infested; *eradication* - elimination of the pathogen from an area where it was introduced; *protection* - interposition of a protective barrier between the susceptible parts of the plant and the inoculum of the pathogen before deposition occurs; *immunization* - development of resistant or immune plants, or development, by natural or artificial means, of a population of immune or highly resistant plants in the area infested with the pathogen; *therapy* - restoration of the health of a plant with which the pathogen has already established its intimate parasitic relationship; *regulation* - modifications of the environment, making it unfavorable for the pathogen or for the development of the disease.

Therefore, considering the epidemiological approach and the particularities associated with root diseases, principally as to the importance of the initial inoculum as one of the factors most important in the incidence and severity of such diseases, we can emphasize the principle strategies of management of the diseases in seedlings: the choice of resistant varieties, the evasion or exclusion of the inoculum in the nursery, and a reduction of the primary and secondary infection rates. The maintenance of the physical, chemical, or biological conditions of the soil unfavorable to the stages of the life cycle of the pathogen are also fundamental, combined with the cleaning of the nurseries and having a local adequate for safely discarding seedlings, substrates and debris of crops. To enable certification of the quality and plant health, as well as indexation of the propagative material, there should be maintained a routine record and history of the operations in the production of seedlings, as well as security and control of access to greenhouses and nurseries that are of the principle routes of introduction of pathogens.

It is important that knowledge obtained in research be put into practice and that the political commitment, with powers of decision, including in the legislation regarding seeds and seedlings, respect the scientific community.

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