

From the ancient times of the agriculture to the biological control in plants: a little of the history

Dos primórdios da agricultura ao controle biológico em plantas: um pouco de história

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- REVIEW -

ABSTRACT

The advancement of technology in agriculture driven by the need to increase efficiency in production transformed agriculture in an eminently anti-ecological activity, with large-scale use of industrialized products. Human beings still live the paradigm of agriculture based on the use of chemical inputs, which often brings harmful consequences to the environment. As an alternative to the use of pesticides to control pests and diseases, biological control is a practice that has been increasingly encouraged. With it, it is possible to think on changes that promote the conservation of natural resources and of the planet humans live in, contributing to strength the new era in biotechnology education.

Key words: agriculture, *Trichoderma* spp., *Clonostachys rosea*, biocontrol.

RESUMO

O avanço da tecnologia na área agrícola, induzido pela necessidade de aumento da eficiência na produção, transformou a agricultura numa atividade eminentemente anti-ecológica, com uso em larga escala de insumos industrializados. Vivemos ainda o paradigma da agricultura baseada no uso de insumos químicos, a qual muitas vezes traz consequências danosas ao meio ambiente. Como alternativa ao uso de agrotóxicos para o controle de pragas e doenças, o controle biológico é uma prática que vem sendo cada vez mais incentivada. Com ele, pode-se pensar em transformações que, visando a conservação de recursos naturais e do planeta em que vivemos, contribuam para o fortalecimento da nova era biotecnológica em formação.

Palavras-chave: agricultura, *Trichoderma* spp., *Clonostachys rosea*, Biocontrole.

INTRODUCTION

Before the 1500s the dominant world vision in Europe like in the most of civilizations was agriculture based on organic origin. The scientific structure of this vision was due to the authority of the church and Aristotle, when the medieval scientist investigations aimed to various natural *phenomenon*, always considered of the highest meaning, like questions (MELO, 1997). This organic vision radically changed in the centuries XVI and XVII, because it suffered an epistemological rupture which was replaced by the notion of the machine world with revolutionary changes caused by the scientific revolutionary ideas of Copernicus, Galileo, Newton and Einstein (CAPRA, 1982).

Around 1798 the humanity reached the peak of one billion of people. At this time the world's population growth was in geometrical progression, while the food growth rose arithmetically which cause in a long time a negative debt in food, determining hunger (HENRIQUES, 2007).

The vision now is eminently sedentary and agriculture for mass production, mainly in Europe. The first big cycle, the Agricultural, took a little more than 900 years. The second, the industrial, which occurred in about three centuries, and now, in a little more than 40 years, a new revolution has been happening, by the constant and fast development of technologies. For many experts this time of the industrial revolution, should mark a new geological period: The human era

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or anthropocentric period. Since then, the human action over the planet has been extreme and of accelerated development, which effects had extended this model until around 1955 (MELO, 1997).

The Green Revolution which occurred between the years 1940s to 1970s with rural mechanization, irrigation, use of fertilizers and pesticides as well as the selection of more productive seeds, grew up three times the grains production in the developed countries. The impact of humanity over the planet, which started an accelerated growth ten thousand years ago with the discovery of agricultural procedures and the raising of grains production were regulated by the high mortality rate on epidemics and wars. It was the era of antibiotics started by Fleming's discoveries in 1927, which raised the expectancy of life, accelerating the humanity's growth. According to the United Nations, in October 2011 the world population should be 7 billion people, this takes to the old Malthus question: Will there be enough food for everybody? Despite all the previsions of Malthus, this era has being of relatively abundance by the developing of agricultural technologies, which have followed the population growth boom (MOON, 2011).

Today humans live in the rest of the agricultural era and in the plenitude of the motive era, where the model starts presenting signs of extinction, like which occurs in the great deforestation and degradation of the environment, although the conscious of preserving natural resources which begins in 1970, starts generating movements which want to use the land resources coherently, "the profile and formation of the researchers is still conventional". Many projects keep on being presented with the same research logic by consecrated researchers who keep the epistemological bunds and conventional methods, making difficult the transition to the research (GOMES, 1999) that could permit the biological or integrated control. But it can't be denied the many achieved objectives by the current paradigm, for instance, the role of microorganisms on nitrogen recycling (DELÉAGE, 1993).

DEVELOPMENT

The comprehension of many biological phenomenon occurs in the field of physiology, biochemistry and microbiology which has potential to give the biotechnological conditions to the transition which is intended to, begging with the control of the transition on agricultural sickness based on chemicals for an integrated control with the list of residual percentage of contaminants in the soil and in the plants.

Conventional control of diseases in plants

The conventional control of diseases in plants have being made by fungicides in large scale since the discover of Bordaleza Calda (hydrated sulfate of copper and lime) by Milardete in 1982 in France, being the main fungicide used for 50 years followed by mercury organ (1914) and dithio carbamates (decade of 1930). All them have in common the supply of protection on the surface and not penetration on tissues, which could be phytotoxic, because it inhibit vital processes. At the end of the World War II, was marked by the development of fungicides which penetrated in the plants eradicating or protecting them against pathogens, being initially accepted the benomyl and carboxin on large expected followed other systemic fungicides more selective than benzimidazols (GHINI & KIMATI, 2002).

The way of action of some systemic fungicides are known in reasonable way and among them we can mention the benzimidazole, oxatins, inhibitors of ergosterol biosynthesis which derives of phenylamine, but the exact action of many others is still not clarified, like the anilopriminidinas, which inhibit the extracellular protein synthesis of some pathogens like the *Botrytis cinerea* (GHINI & KIMATI, 2002).

Even with this little information the modern agriculture has been traditionally dependent on synthetic pesticides which present themselves as an alternative rather attracting by its simplicity on application with satisfactory results in a short term and by not demanding knowledge on the basic process of the ecological agrosystem (TALAMINI & STADINIK, 2004). On the other hand they bring as a consequence serious problems in an environmental order MELO & AZEVEDO, 1998), like the microflora suppression favoring the appearing of other pathogens and/or resistant line to the active element of these products (DUBOS et al., 1989; CAPIEAU et al., 2004), like the resistance of *B. cinerea* in ciclames observed in 1971 by BOLLEN & SCHOLTEN in the Netherlands, after two years of benzimidazols use and in Italy altogether with dicarboxins in multiple cultures (GULLINO & GARIBALDI, 1983). In Brazil it was observed by many researchers resistance of this pathogen in studies made with strawberry, eucalyptus, rose, eggplant, chrysanthemum, potato, violet, begonia, pepper, apple and grape (GHINI & KIMATI, 2002).

For GHINI & KIMATI (2002), the resistance is one the most serious problems of chemical control of diseases in plants nowadays, it can be disastrous for many segments of the productive chain. Besides, the inappropriate use of pesticides applied during the cycle of different cultures is a

high risk to the man's health, rising the production costs (VALDEBENITO-SANHUEZA et al., 1997a) by the intensification of reapplications which are more and more used by the producers who didn't get the expected results, making it more expensive and accumulating residues damaging the society as a whole (GHINI & KIMATI, 2002).

Biological control of diseases in plants

The world needs an eminent change which guarantees food and preserve the soil's health (productive potential of the soil), shaken by the rests of the Industrial Era. The concerns with chemical products usage and its relation to the preservation of the soil, resistance of pathogens to used fungicides, the raise on asking and demand by the consumers for food which is free of residuals left by the application of agrochemicals, encourages strategies and alternative methods for the control of diseases in multiple cultures, which among them includes the biological control (REDMON et al., 1987; PENG & SUTON, 1991; LOPES, 2009; MORANDI, 2009).

Biological control of diseases in plants basically consists in selecting microorganisms to be used in control or suppression of pathogens that cause diseases in plants. For BETTIOL (1991) these opposed microorganisms must be searched in areas where a disease is caused by a pathogen doesn't occur or is diminished, but for BACKER & COOK (1974), the opposed effect can be found in any place, however, the study of this places is associated to the fungi which is intended to be found. In the case of entophytes, they can be isolated by many parts of asinthomatic plants and, after purified, used as antagonistic (AZEVEDO, 2003; SOUZA et al., 2004). The main characteristics searched in this isolated are the presentation of proprieties which facilitate its application in the plants surface or soil, with fast colonization besides having more than one opposed mechanism, like a competition, parasitism and antibiosis must not be phytopathogenics (BETTIOL, 1991a) to the plants which are going to be applied.

Even though the knowledge of microorganisms like agents of plant diseases controlling, were from hundreds of years ago this actions about phytopathogens was not well known even in the early of 1920s of the 20th century. In the first studies about opposed action of some microorganisms which reduced root diseases, the involved mechanisms was not known. The first written record published about biological controlling in Brazil was in 1950 by FOSTER, in a research with relation to smoking mosaic, controlled by the fungi *Trichoderma*. The first

products with conceptual and scientific bases using microorganisms in the world came up only in the 70s (BETTIOL & MORANDI, 2009), but the organization in this area in Brazil were only made between 1986/87 in Piracicaba, SP, after what, was available by the Centro Nacional de Pesquisa de Fruteiras de Clima Temperado, from EMBRAPA, by Valdebenito-Sanhueza, the commercial product *Trichoderma viride* for controlling of *Phytophthora cactorum* in apple trees (BETTIOL & MORANDI, 2009).

In 1992 was created the first discipline about Biological Controlling of diseases in plants by Wagner Bettiol in a post-graduation course at UNESP/Botucatu, SP. In the same year it was created the first company specialized in production and trading of *Trichoderma*, the first biological fungicide having the *Trichoderma harzianum* for biocontrolling was only registered in 2008. In 2009 CNPQ approves the project for determination of methodologies and evaluation of the quality of the biological products for controlling diseases in plants (BETTIOL & MORANDI, 2009).

Nowadays in Brazil it can be mentioned studies in nematode controlling in guava tree by weevils (*Conotrachelus psidii*); pre immunization against citrus sadness by weak origins of the virus (BOECHAD & BETTIOL, 2009); the control of grey mold in multiple cultures by the fungi *Clonostachys rosea* (ZOU et al., 2010), besides e fungi known as pathogenic which can be opposed to other pathogens which produces metabolites and inhibits enzymes, and/or tissues destruction like the mycotoxins of *Penicillium* sp and *Aspergillus* sp (ESPOSITO & AZEVEDO, 2004) and glucanases and eniatinas of *Fusarium* sp. (POHANKA, 2004).

In the trading many biological products are available and among them it can be find the virus PRSV-W pre immunizing against the zucchini mosaic; *Acremonium* for the lixa of coconut tree, *Bacillus subtilis* for controlling multiple diseases; *Trichoderma* sp for controlling of the soil pathogens substratum and aerial part of multiple cultures (BOECHAD & BETTIOL, 2009).

The manipulation done by e man, however, must go beyond application of opposed microorganisms in the right moment. It's necessary to give condition for its developing keeping the biological balance that exists in the soil. The more complex is for a microbial community of the soil, the more is its stable (REIS, 1991). Chemical methods tend to produce fluctuation in the level of the disease while the biological tends to stable, decreasing the level of the disease (BAKER & COOK, 1974). In

this optical, the management of cultural practices can or can't be favorable to microbial activities. The soil fungi mainly depend on the crop rotation. These reduce pathogens hosts with few survival structures for pathogens like *B. cinerea* (VALDEBENITO-SANHUEZA, 1991).

In the biocontrol of *B. cinerea* it must occur the colonization of phyloplan by the antagonistic agent, before the installation of the pathogen which diminishes the sporulation of this and retards the dissemination and the production of surviving structures. The strategy of preventing treatment recommended and used in the application of inoculus on the field was suggested by DUBOS & BULLIT (1979), BLAKEMAN & FOKEMA (1982) e VALDEBENITO-SANHUEZA et al. (2007). According to these authors the previous installation and colonization of the antagonistic in the fruit depend on making the treatments with the antagonistic from the floration, giving the antagonistic the opportunity to colonize the nutritive base from the senescent floral parts, before the pathogen arrival.

The possibility of new studies and the current results in the biocontrol area, as well as the consumer market demanding, mainly the exportation for high quality products and with no toxicological residues of chemical products, encourages the Brazilian producer to search for raise in exportation investing on biotechnological practices and searching for quality mainly (RETZ et al., 2007).

The fungus *Clonostachys rosea* and *Trichoderma* spp: History of the Bio controlling

Among the agents of the biological control, the filamentous *Clonostachys rosea* was observed as an efficient and versatile antagonistic in studies in the end of the 80s (SUTTON et al., 1997). This agent was isolated at the Guelph University, Canada, in June, 1993 of strawberry leaves in works of selection of antagonism to *B. cinerea* (PENG & SUTTON, 1991). Both of the fungi invade the strawberry leaf of strawberry and progressively colonize the folicle tissues, when the leaf becomes senescent (SUTTON, 1994), there is spore only on the dead leaves. It's common to find *C. rosea* in strawberry leaves without causing sickness symptoms (SUTTON, 1993).

The microparasite necrotrophic *C. rosea* is being quoted like antagonistic *B. cinerea* in studies of several cultures (DUBUS, 1989; SUTTON, 1994; MORANDI et al., 2003; YAHALEM, 2003; YAHALEM et al., 2004; CAPIEAU et al., 2004; ZAMBONI-PINOTTI et al, 2005). It belongs to the Deuteromycetes class, sub-class of the Hifomicetos

Gloisporai (CAPIEAU et al., 2004), being harmless to the man when colonizes fruits efficiently (Sutton quoted by VALDEBENITO-SANHUEZA et al., 1997b).

Natural inhabitants of the soil *C. rosea* can live saprophyte or parasitic other fungus like *B. cinerea* (MORANDI et al., 2003), acting by competition by substrate with pathogens, stopping or reducing its colonization (VALDEBENITO-SANHUEZA et al., 1997a), infecting conidium and germinal tubes through direct penetration by the hyphae without the formation of a pre-ressorium. The rescue and rupture of the walls of the cell by the penetration of the hyphae in conidium and germ tubes parasitized of *B. cinerea* which present signs of cytoplasmic disintegration can be seen in electronic microscopies (LI et al., 2002).

The deuteromycota Fungi, *Trichoderma* spp. is found in the majority of the soil, including on humus forest and presents itself under several species considered effective as agents of bio controlling of phytopathogenic fungi of plants of economic importance (CASSIOLATO & SOUZA, 2000).

The first publishing on the use of *Trichoderma* spp as an agent of biological control of diseases in plants, in Brazil, was in 1950 by Foster, but only in 1987 a product with these fungi were used commercially in the country against the *Phytophthora cactorum*, the causer of rotten of roots (VALDEBENITO-SANHUEZA, 1991). Several studies were conducted with this antagonistic since then, being currently the most used in Brazil and other countries of the Latin America (BETTIOL & MORANDI, 2009).

In April 2008, 13 companies which produced Trichodermas were identified, in formulation of colonized grains, wet powder, concentrated suspension, dispersible granules, emulsifiable oil, and dry spores. The target pathogens *Pythium*, *Rhizoctonia*, *Macrophomina*, some species of *Fusarium*, *Sclerotinia*, *Sclerotium*, *Crinipellis* and *Botrytis* for beans, soya, cotton, tobacco, strawberry, tomato, garlic, onion, ornamental plants and cocoa culture are also being recommended for seeds treatment (BETTIOL & MORANDI, 2009) and composts for plants growth (SILVA-RIBEIRO, 2001; GUIMARÃES, et al., 2008).

Well known as active mycoparasites of phytopathogens, *Trichoderma* spp. and *C. rosea* highlighted the antagonistic interactions and can act through an association of definite mechanism: 1. Antibiosis: It includes the secretion of secondary toxic metabolites to phytopathogens (MELO, 1996). Among these metabolites, studies have shown the production of antibiotics by *Trichoderma* spp

(BELANGER et al., 1995; GRAUME- COOK & FAUL, 1999; HOWELLI, 2006); 2. Competition: It's the negative relation between two populations, in which both are affected by its growth, in relation to the space or limiting nutrients; 3. Micro parasitism: The micro parasitism involves the location, contact and penetration with consequent achieving of nutrients (MELO, 1996). After the contact, *Trichoderma* and *C. rosea* remain on growing on the host hyphaes, generally rolling itself in its electronic extension (MELO, 1996; VALDEBENITO-SANHUEZA, 1997b).

Another studies show that recognized pathogens like *Penicillium* spp, *Fusarium* spp. (ZAZZERINI & TOSI, 1985) and *Fusarium solani* (ILLIPRONTI & MACHADO, 1993) have been used in the control of *S. sclerotiorum*. *Fusarium oxysporum* not pathogenic is pointed by FRAVEL (2008) as a bio controller of pathogens transmitted to the soil with a suppressor effect.

Several fungus produce fungal pigments which are active against bacteria, yeast, fungi and protozoa that diffuse through the Agar type of culture and could vary color depending on the pH (SEIFERT, 2001). For BLANC (1998), *F. oxysporum* produces a fungal pigment of quinona type which correspond to a wide variety of colors like: yellow, orange, pink and red according to ceto group position, metabolites which don't gave clear function of the cellular growth, though are of bio thecnology and agricultural importance. ESPOSITO & AZEVEDO (2004) concludes that the most important produced pigments by *F. oxysporum* are capable to permeable the plasmatic membrane, rising the concentration of phosphorus in the insular mean (MENDONZA et al., 2005).

Other metabolites which are reported in bio control derived from filamentous fungus (GULINO, 2000; THINES, 2004). Some of these composts like the produced eniatinas for *Fusarium* sp can inhibit the germination of the conidies (POHANKA, 2004), et enzymes of agro ecological importance produced by different ceps *Trichoderma* and *Fusarium*, like celulases lise walls, mycelia and conidies of pathogens (ATLAS & BARTH).

Parasit of plants or saprofitic in decomposition of plants (BARNET & HUNTER, 1972), *F. oxysporum* can form inside each specie a subdivision, *formae* specials, which consist in a certain specific host plants as well as by the vascular tissue of these plants not manifesting in other cultures (EGGERT et al., 2010), It can still survive saprofitically for a long time in the organic (EDEL et al., 1997).

As it can be see, the ecosystems detailed are showing that the majority of the relations

between the living beings are essentially cooperative with a coexistence of character and symbiotic interdependence in several degrees (CAPRA, 1982).

CONCLUSION

Despite the current knowledge about this cooperative character observed in studies, there's an infinite possibility of finding microorganisms of agronomical interest, unknown or pathogenic with antagonistic effect, with using potential in bio control still without knowing. The certainty of this fact opens a range variety of possibilities which can't be ignored taking to new studies not attached to the current paradigm and more attached to the real views of the world.

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