REVIEW

Effects of transient soil waterlogging and its importance for rootstock selection

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Under transient waterlogging, a number of transformations in the soil are generated associated with lack of aeration, seriously affecting the root system. Significant progress has been reported on understanding the effects of lack of oxygen on the metabolism of the roots, although few studies have examined changes in the soil. Diverging conclusions about the degree of tolerance exhibited by plants exclude the effects of hypoxia and anoxia on physical-chemical soil properties under plant experiments. This review examines the main changes occurring in soil and roots due to transient soil waterlogging conditions. Parameters such as antioxidant capacity, nutrient uptake dynamics and regeneration and distribution of the root system are relevant for selecting rootstocks tolerant to soil waterlogging.

Key words: Root system, soil aeration, soil waterlogging, tolerance.

INTRODUCTION

The definition of soil waterlogging as a "plant stress" is considered to be the condition where the soil is fully saturated with water. This transient condition may be caused by a hindered flow of water through the soil profile or problems associated with a high water entry rate or a combination of both. In waterlogged soil, the diffusion of gases through soil pores is strongly inhibited by their water content that it fails to match the needs of growing roots. A slowed oxygen influx is the principal cause of injury to roots and the shoots they support (Vartapetian and Jackson, 1997). In some soils maintained at field capacity, the aeration is a limiting parameter for plant growth, and this can be a common problem in irrigation management. Some orchards can be flooded for 24 or 48 h, by excess water, poor irrigation or absence of a proper drainage system, even in soils near field capacity (Ferreyra et al., 2011).

One of the main soil function is to provide a medium for plant growth; thus, modifications of its physical and chemical characteristics have a great impact on the development of the root biomass and consequently on plant vegetative development (Pierret et al., 2007). The soil should have adequate proportions of water and air in the optimal range for the physiological performance of the plants. This balance may be altered by both natural and anthropic factors. Human-related causes include poor irrigation management and soil compaction, while natural flooding are caused by excessive rain (Irfan et al., 2010). In both cases soils with high clay content and/or compaction due to agricultural practices are susceptible to soil waterlogging. These effects contribute to limiting water and air movement in the soil, generating oxygen (O_2) deficiency and carbon dioxide (CO_2) accumulation (Kozlowski, 1997; Núñez-Elisea et al., 1999; Jackson and Colmer, 2005; Yetisir et al., 2006) as well as changes in the soils chemical properties (Unger and Kaspar, 1994). The gas exchange between soil and atmosphere in a well-aerated soil is sufficiently rapid to impede O_2 deficiency and toxicity due to CO_2 excess or other gases. The roots of most plants need a good supply of oxygen in order to satisfy the water and nutrient needs of the shoots, and the soil oxygen concentration should be above $0.1 \ L \ L^{-1}$ (Brady and Weil, 2008) (O_2 atmospheric concentration is $0.21 \ L \ L^{-1}$). A soil O_2 concentration < 10% by volume indicates poor aeration for root growth. However, O_2 concentration per se is an imperfect predictor of plant responses. The composition of other soil gases, such as CO_2, ethylene, methane, etc., can affect the response to a given oxygen concentration (Sojka and Scott, 2000).

Since an oxygen decrease is one of the first effects of soil waterlogging, most published studies have evaluated the effect of hypoxia and anoxia on plants, either via hydroponic trials or using inert substrates (Morard and Silvestre, 1996; Striker, 2008; Arbona et al., 2009; Bai et al., 2013), but few studies have included the soil as a factor in their analyses. The absence of molecular oxygen triggers a sequence of changes in the physical-chemical

properties of the soil. Many of these changes produce accumulation of reduced metal ions, organic and volatile acids, which are potentially damaging to plant roots. Their accumulation to phytotoxic levels requires time; however, the absence of oxygen alone is enough to alter plant metabolism to critical levels (Perata et al., 2011). These changes greatly affect the capacity of a plant to survive such conditions. As response, stomatal resistance increases, photosynthesis and root hydraulic conductivity decline and translocation of photoassimilates is reduced. Plants may adapt to hypoxic/anoxic conditions by changes in biochemical and metabolic processes when oxygen availability begins to be limiting (Dat et al., 2004). This review firstly describes how the main changes are induced under waterlogging stress, as well as parameters measured in studies of soil waterlogging. Secondly, this work discusses the effects of low soil oxygen concentration on plant responses that may be relevant for selecting plants tolerant to transient soil waterlogging.

CHARACTERIZATION OF WATERLOGGED SOILS

The composition of the soil gaseous phase is similar to that of the atmosphere, but differs in the proportions of oxygen, water vapor and CO_2 depending on the depth and aeration conditions of each soil (Armstrong and Beckett, 2011).

Aeration implies soil ventilation by gas movement both to and from the soil, which determines the resulting velocity of gas exchange with the atmosphere, the proportion and composition of the soil air space and the oxidation-reduction potential in the soil environment (Brady and Weil, 2008). The O_2 consumption and the CO_2 production are caused by the respiration of plant roots in the soil, the activity of microorganisms and chemical reactions (Kozlowski, 1997; Núñez-Elisea et al., 1999). In a well-aerated soil, the O_2 entrance and the CO_2 outflow are sufficiently rapid to impede oxygen deficiency and toxicity due to CO_2 excess. Normally, when more than 90% of the porous space in the soil is full of water, lack of aeration is a serious impediment to root growth due to low oxygen availability (Dexter, 1988; Cook and Knight, 2003), since the water blocks the gas diffusion pathways to and from the atmosphere (Kozlowski, 1997; Jackson and Colmer, 2005).

The movement of gases in the soil is a dynamic phenomenon in which gases are transported mainly by diffusion; the main parameter to consider is the gas diffusion coefficient in the soil, which depends on texture, structure, distribution, size and pores connectivity, and tortuosity (Moldrup et al., 2000; 2001). Thus, an increase in pores saturated with water decreases oxygen movement, since diffusion in water is 10000 times slower than in air (Moldrup et al., 2000; Nickum et al., 2010), producing an anaerobic environment in a few hours if the oxygen

is consumed by the activity of microorganisms and roots (Drew, 1992; Nickum et al., 2010).

When the soil pores are saturated with water, there are a series of chemical modifications such as alterations in pH and the accumulation of toxic products for the roots, such as reduced metal ions, organic and volatile acids and reactive oxygen species (ROS), which are important in the response and capture of signals for the subsequent adjustment of the plant (Irfan et al., 2010). Accordingly, the magnitude of waterlogging in the soil can be characterized through the soil porosity, oxygen diffusion rate, redox potential and the composition of gases in the soil.

Total porosity of the soil
The optimal soil composition for plant growth should contain about 50% solid and 50% pore space. Within the pore space, the mixture of air and water should be close to 50:50; hence, 25% of the soil volume should be occupied by air. Gas diffusion is conditioned by the physical properties of the soil, among which soil porosity is one of the most important, especially the fraction with air (Hillel, 1980). In the majority of soils, microbiological activity and plant growth are seriously inhibited when the porosity with air decreases to less than 20% of the pore space, which is related to soil properties such as texture, structure, water content, mineralogy of clays and sodium adsorption relation (Pezeshki et al., 1996; Brady and Weil, 2008; Tete et al., 2015).

Sandy soils have less total porosity than clays, but the pores are larger and well connected in sand. In fine-textured soils, the percentage of space with air tends to decrease when they are poorly structured, since clays have many small, discontinuous pores and retain water more easily than soils with coarser texture. Thus, plants tend to suffer oxygen limitation more frequently in clay soils in spite of their greater total porosity (Sojka and Scott, 2000). When a large amount of water is applied to the soil, greater than its retention capacity, water excess usually drains; however, when the texture is fine with a tendency to compaction or hanging strata that impede drainage, water is retained for a longer time, producing extended saturation of the pore space. In this first stage, the immediate consequence of inundation is the blockage of gas interchange with the atmosphere and extreme slowing of atmospheric interchange with the gases in the water, especially oxygen.

Oxygen diffusion rate
The oxygen diffusion rate is a measure of the mobility of this gas in the soil (Logsdon, 2003) and is of great importance for its supply to the roots. The Oxygen Diffusion Rate (ODR) measurement method consists of quantifying the electrical current necessary to reduce all the available oxygen on the surface of a platinum electrode placed in the soil. Studies on the responses of

plants to excess water indicate a threshold rate of oxygen diffusion rate of 0.2 μg cm^{-2} min^{-1}. Below this value there is growth retardation and considerable damage to the roots of most cultivated plants (Valoras et al., 1964; Stolzy et al., 1967; Jacobs et al., 1997; Ferreyra et al., 2011). Given the same porosity, oxygen diffusion is less in humid soils compared to dry soils, which have a larger percentage of their pores filled with air (Moldrup et al., 2000; Feng et al., 2002). Although a portion of the oxygen necessary for root development may be obtained from the aqueous solution in which it is dissolved, this represents only a small fraction of the total required. This is because most of the oxygen in the root zone must diffuse from the atmosphere to the soil through the pore spaces, and then diffuse through a film of water that surrounds the roots and into the roots to be utilized (Cook and Knight, 2003).

Once the oxygen begins to be used, this measurement loses relevance when the soil is waterlogged, since the majority of the pores are full of water and diffusion approaches zero (Wu et al., 2003). In parallel, changes in the redox potential (E_h) are triggered, which makes this a good alternative to evaluate the aeration state of the soil.

Redox potential

The redox potential (E_h) provides a measure of the tendency of a substance to accept or donate electrons, and is one of the most appropriate indicators of the chemical changes produced during waterlogging of the soil (Pezeshki and DeLaune, 1998). The E_h of a soil depends both on the presence of an electron acceptor (oxygen or another oxidizing agent) and the pH; it is usually measured in volts or millivolts (mV). In a well-drained soil E_h is in the range of +400 to +700 mV, while in waterlogged soils E_h decreases to a range of +400 to -300 mV. Generally, when oxygen is not present soil values of E_h are below +350 mV (DeLaune et al., 1990; Pezeshki, 2001), while in waterlogged soils that are warm and rich in organic material, values may be as low as -300 mV (Brady and Weil, 2008).

Since both E_h and pH are easily measured, it is possible to infer the redox reaction of an element in the soil. For example, in a soil with pH 6, the E_h should be less than +0.5 V to stimulate the reduction of nitrates to nitrites, and approximately +0.2 V to stimulate the reduction of FeOOH to the Fe^{2+} ion. To form methane in a waterlogged soil at this same pH, an E_h equivalent to approximately -0.2 V is needed (Figure 1) (McBride, 1994).

Since O_2 is reduced to water by aerobic microorganisms when the value of E_h is +380 to +320 mV, once the redox potential is below this range there is almost no O_2 in the soil (Pezeshki, 2001). With even lower levels of E_h (anoxia), the only active microorganisms are those that can use other metabolic electron acceptors instead of oxygen. The element that usually follows is N (nitrates, NO_3^-), due to its ease of reduction. If the soil has abundant nitrate, while this is being reduced, E_h will remain in the range of +280
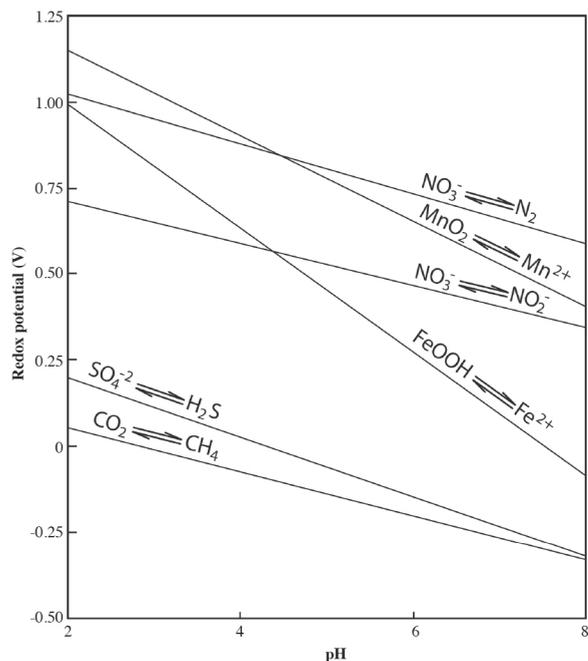
Adapted from McBride (1994).

Figure 1. Effect of pH on the redox potential (Condon and Richards, 1992) at which various oxidation-reduction reactions are generated in soils.

to +220 mV. When almost the entire supply of nitrate has disappeared, E_h will fall even further. At this point organisms capable of reducing Mn will become active, and so on. Thus, as the value of E_h falls, the elements N, Mn, Fe, S (in SO_4^{2-}) and C (in CO_2) accept electrons and are transformed into reduced elements, almost always in this order (McBride, 1994). Accordingly, E_h is not only an indicator of the oxygen level in the soil, but also of the availability and concentration of different plant nutrients indirectly (Pezeshki, 2001). Likewise, the redox condition and slow diffusion of oxygen in the soil accelerate the accumulation of nutrients and/or toxic gases, as well as the production and accumulation of ethylene, which is important for the plant responses.

Composition of gases in the soil

As a result of the aerobic respiration of plant roots and microorganisms, soil atmosphere is characterized by the enrichment in CO_2 and poor in oxygen. However, when the soil is saturated (anaerobic), other gases appear in the soil such as nitrous oxide, gaseous N, and methane as a result of anaerobic bacterial respiration.

Oxygen. The O_2 concentration may be slightly less than 20% in the upper levels of a soil with a stable structure and an abundance of micropores (atmosphere has about 21% O_2). In the inferior horizons of poorly drained soils with few micropores it may fall to less than 5%, or even close to 0 (Brady and Weil, 2008). Low O_2 content is

typical of humid soils, although in well-drained soils after heavy rains air O_2 content may be considerably reduced, especially if it is being rapidly consumed by actively growing plant roots or microorganisms that decompose organic matter. Also, if the soil temperature is high then oxygen is depleted more rapidly due to acceleration of the metabolic activity of microorganisms. When all the soil pores are full of water, microorganisms may remove the majority of the oxygen dissolved in the water; however, this small amount of dissolved O_2 is rapidly depleted. Accordingly, if the excess water is not removed the aerobic activity of microorganisms and plant growth are endangered.

Carbon dioxide. Whereas N_2 content of the soil air is relatively constant, there is generally an inverse relation between the other two principal components, O_2 and CO_2; O_2 decreases as CO_2 increases. Although the absolute differences in the CO_2 quantity may not be considerable, their proportional changes may be very relevant; for example, when soil air only has 0.35% CO_2, this gas is still about 10 fold more concentrated than in the atmosphere (0.035% CO_2). When soil water increases the air space in the pores decreases, which limits the diffusion of CO_2, increasing its accumulation (Bouma and Bryla, 2000). When the CO_2 concentration is as high as 10%, it may be toxic for some plant processes.

Other gases. In waterlogged conditions, the concentrations of some gases that form due to the decomposition of organic material such as methane (CH_4) and hydrogen sulfide (H_2S) are notably greater in the soil atmosphere. Another gas produced by anaerobic microbial metabolism is ethylene (C_2H_4), which is particularly toxic for plant roots even though it is present at concentrations less than 1 μL L^{-1} (0.0001% or 1 ppm). This molecule is indeed a plant hormone capable of influencing processes such as cell expansion, senescence and differentiation at relatively low concentrations (0.01-10 ppm, v/v) (Jackson, 2005). It has been shown that when velocity of gas interchange between soil and atmosphere is too slow, the accumulation of ethylene inhibits the root growth of several plants, but also induces morphological and anatomical changes such as the aerenchyma formation and adventitious roots (Visser and Voesenek, 2005). Similarly, gases such as methane and nitrous oxide are generated or absorbed in the soil depending upon the redox potential under certain aeration conditions. The formation of nitrous oxide is due to processes of de-nitrification in anoxia and nitrification under oxidative soil conditions (Gliński and Stepniewski, 1985), or a combination of both processes under hypoxic conditions (Zschornack et al., 2011). Methane is generated under more intense reduction conditions that occur at values of less than -150 mV (Stępniewski and Stępniewska, 2009).

Factors determining soil aeration and root growth
Soils are normally described based on a set of physical properties, including structure, texture, bulk density, pore distribution, and size and morphological characteristics of the profile. However, it is necessary to separate factors that directly affect plant growth from those that indirectly affect plant growth. Water, oxygen, temperature, and mechanical resistance can impair root growth and directly affect plant growth (Figure 2) as a consequence of interactions with the indirect factors mentioned above and
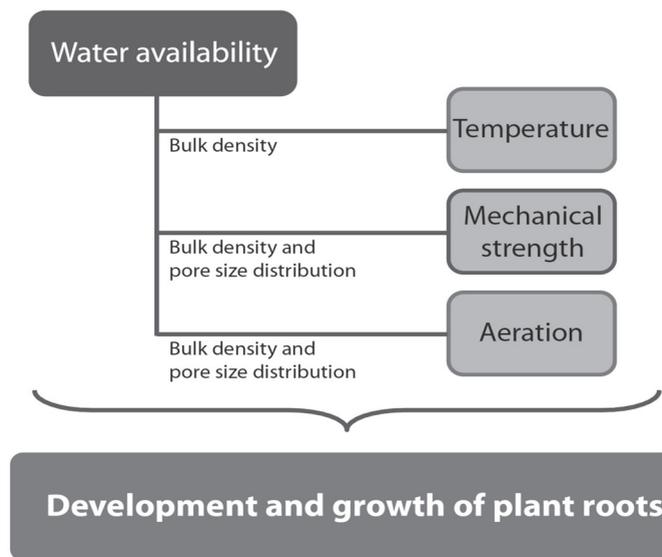
Adapted from Letey (1985).

Figure 2. Relationships between water and other physical soil parameters that directly affect root development and plant productivity.

the management practices used (Letey, 1985; Wu et al., 2003). The soil physical factors are considered interrelated and never independent of each other. Of the four physical conditions directly related to root development, water is the dominant controlling factor. The relationship between water and aeration is opposed to that between water and mechanical resistance. Increasing water content decreases aeration, which is undesirable but in turn decreases mechanical resistance, which is desirable. The effect of water on both these parameters is intensified by increases in bulk density and/or the presence of small pores. The water-temperature *vs*. water-mechanical resistance interrelationship has a similar interpretation (Letey, 1985). Maintaining a relatively high water content at the beginning of spring without affecting gas transport in order to reduce mechanical resistance leads to a colder or harder to heat soil. Relatively low soil temperature, in turn, will produce less metabolic activity in roots.

Modification of these factors also affects the geometry of the pores, producing a decrease in macropores and an increase in micropores (Letey, 1985). Excess water generated by poor irrigation management, soils with low permeability, poor drainage, fine textures and poorly structured soils with discontinuous pores may facilitate the rapid and permanent saturation of soil pores.

The optimum values mentioned by a number of authors (Dexter, 1988, Bengough et al., 2006; Whitmore and Whalley, 2009) are 15% air content in the soil, oxygen diffusion greater than $0.2 \, \mu g \, cm^{-2} \, min^{-1}$ and less than 2 MPa resistance to root penetration for the majority of species. The optimum range of water content for plant growth has generally been established using criteria based on water availability (WA). The upper limit of WA is associated with field capacity (FC) and the lower limit with the permanent wilting point (PWP); under this criterion, irrigation is performed each time the soil exhausts a fraction of the WA (Letey, 1985). Although these thresholds may be useful as a tool for empirical management, they are less useful to identify critical values or soil water tension, which limit plant growth and allow optimization of the soil-plant interaction to maximize yield and quality (Whitmore and Whalley, 2009). Letey (1985) introduced the concept of non-limiting water range (NLWR), which established upper and lower limits of soil water content within which air and mechanical resistance do not affect plant growth. The NLWR may be considered a soil quality index in which a wide range is desirable, since in soils with very low NLWR it is difficult to sustain water content in a range that does not affect the development of the root system, due either to deficient aeration or an increase in soil mechanical resistance (Figure 3).

Da Silva and Kay (1997a) applied the conceptual framework of Letey's NLWR, and renamed it as the least limiting water range (LLWR), demonstrating that apparent density strongly affects the LLWR. These authors indicated that an increase in bulk density decreased the
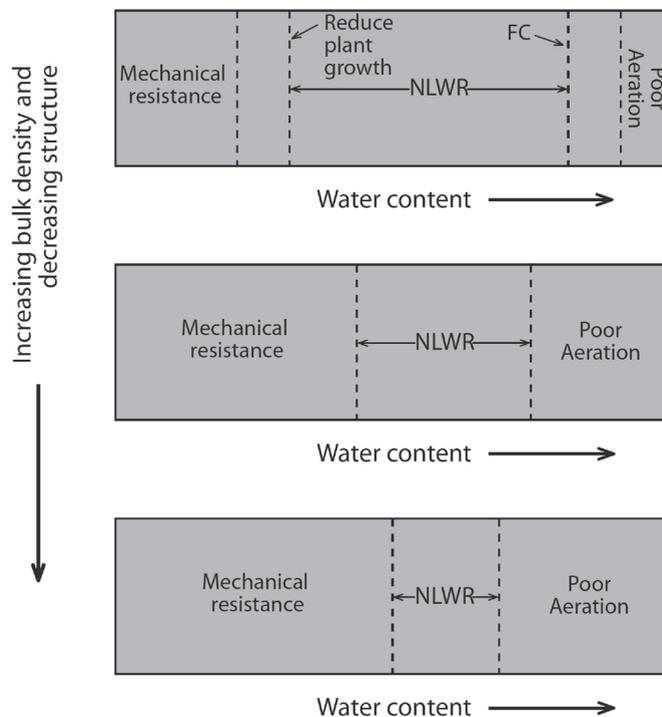
Adapted from Letey (1985).

Figure 3. Non-limiting water range for plants (NLWR).

LLWR, and in soils with equal bulk density this range is smaller when the proportion of clay increases and organic material decreases (da Silva and Kay, 1997a). Under field conditions, given the large temporal variability, there is a very high probability that water content is outside the optimum range (Figure 4) (da Silva and Kay, 1997b).

The soil physical parameters undoubtedly play an important role in problems of soil aeration, due to either lack (mechanical resistance and compaction) or an excess (lack of oxygen and inundation) of water. Jones et al. (1991) investigated root growth in different soil textures and established ranges of relative aeration (air pore volume/total porosity); when this value is less than 38%, relative root growth decreases to less than 80%. For total soil porosity equivalent to 40%, the air space should be greater than 15% so the root growth is not limited, while for a clay soil with a porosity of 50% this limit would be 19% (Figure 5).

Furthermore, Imhoff et al. (2010) established a range of 15% to 20% of soil aeration for maize, so necessarily the air requirements should be about 15% as an upper limit, or even lower for sensitive crops (Bartholomeus et al., 2008; Mohammadi et al., 2010). However, this limit is not an intrinsic, unchanging characteristic of the soil and can fluctuate depending on: 1) The demand for oxygen by roots and soil biota, in turn depends on temperature and root mass; 2) depth at which gas exchange is studied, and 3) the interconnected water-free porous matrix (Pilatti et al., 2012).

## Changes in roots under flooded conditions

As previously mentioned, changes in the soil during an inundation event are relevant to the response of plants and their level of tolerance. Plants differ in their stress tolerance, which is the capacity of a plant to confront an unfavorable environment. If the exposure to stress is greater and tolerance increases, maintaining the plant in a good state, it is called acclimated. The adaptive response, in contrast to acclimation, is related to a level of resistance conferred genetically and acquired after selection processes lasting over many generations. Adaptation and acclimation to stressful environments are a result of integrated events that occur at all levels of organization, from anatomical and morphological to the cellular, biochemical, and molecular levels (Taiz and Zeiger, 2010).

Stress parameters related to plant survival, productivity, and growth (accumulation of biomass), or processes of primary assimilation related to overall growth (CO_2 and nutrient consumption), are used to measure their impact (Taiz and Zeiger, 2010; Pimentel et al., 2014). The immediate consequence of soil waterlogging is a period of hypoxia followed by a strong decrease in oxygen, which leads to anoxic conditions that activate the anaerobic fermentation pathway (Blom and Voesenek, 1996). Thus, when the oxygen supply is insufficient for aerobic respiration, roots begin to ferment pyruvate (formed in glycolysis) to lactate by the action of lactate dehydrogenase (LDH). Lactic fermentation is
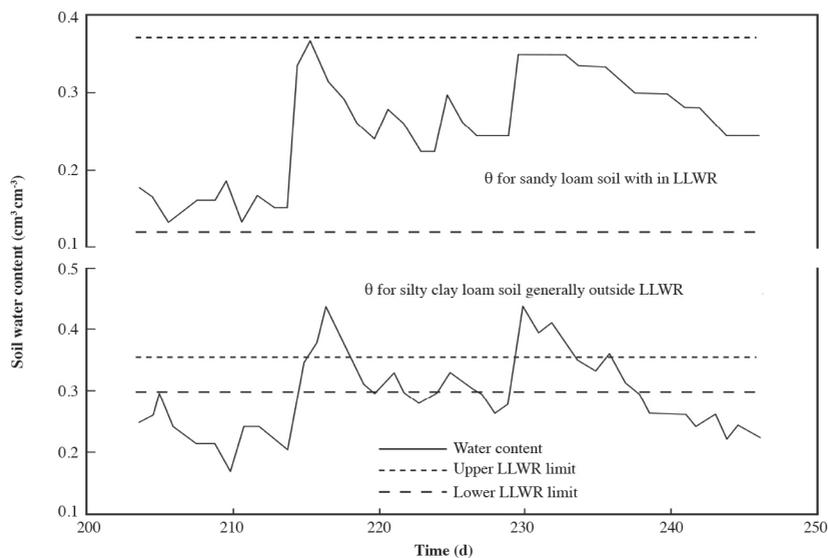
Redrawn from da Silva and Kay (1997).

Figure 4. Least limiting water range (LLWR). Comparison of two soils in relation to water content and time.
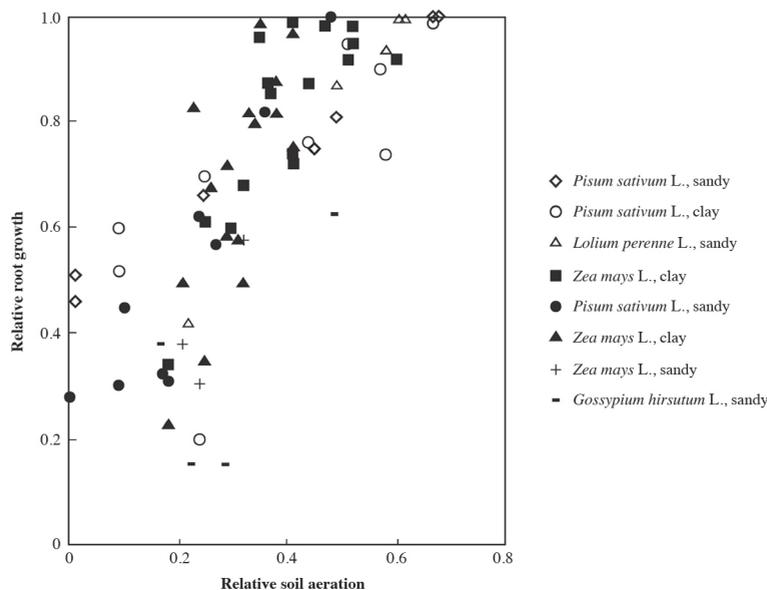
Source: Adapted from Jones et al. (1991).

Figure 5. Relative root growth of various crops on soils with contrasting textures as a function of the relative soil aeration (air pores/total porosity).

transient, because a rapid decrease in intra-cellular pH leads to a switch from lactate fermentation to ethanol fermentation, since the enzymes for these metabolic pathways differ in their optimum pH for functioning. In acid pH, LDH is inhibited and pyruvate decarboxylase is activated. Net ATP production in anaerobic fermentation is only 2 mol ATP per mol of hexose metabolized, compared to 36 mol ATP produced per mol of hexose in aerobic respiration (Taiz and Zeiger, 2010). The attempt to compensate for the reduced ATP production by fermentation accelerates glycolysis, leading to an exhaustion of carbohydrate reserves (Pasteur effect). Species tolerant to soil waterlogging are generally considered those that are capable of maintaining their energetic state by the fermentation pathway (Drew, 1997).

In hypoxic or anoxic roots energy is insufficient to support physiological processes in shoots necessary for their functioning. Experiments have shown that difficulties of wheat and barley in absorbing nutrients and transporting them via xylem rapidly lead to an ion shortage for tissue development and plant expansion. Mature leaves become senescent prematurely due to the relocation of mobile nutrients of the phloem (N, P, and K) to younger leaves. The low permeability of roots to water frequently leads to a decrease in leaf water potential and withering, although this decrease is temporary if the stomata close, preventing further loss of water by transpiration (Taiz and Zeiger, 2010).

Also, the reduced condition of soil and consequent decrease in its capacity for nutrient extraction will tend to limit N and P content of the plant. By contrast, Fe and Mn availability in soil will increase, since they are reduced to more soluble forms. This sudden availability or restriction of various elements will drastically affect the root metabolism (Drew, 1997).

Hypoxia also accelerates production of the ethylene precursor of 1-aminocyclopropane-1-carboxylic acid (ACC) in roots. In tomato ACC travels through the xylem sap to buds, where in contact with oxygen it is converted to ethylene by the action of ACC oxidase. In some species such as peas and tomatoes, waterlogging induces stomatal closure, apparently without detectable changes in the water potential of leaves. The scarcity of oxygen in leaves, as with water deficit and salt accumulation, may stimulate the production of abscisic acid (ABA) and its movement to leaves. However, stomatal closure may be mostly attributed to additional production of ABA by less mature leaves. These leaves wither and export their ABA to turgid young leaves, producing stomatal closure (Zhang and Zhang, 1994).

The slow diffusion of gases in water will accelerate the accumulation of potentially phytotoxic products of anaerobic metabolism such as ethanol, lactic acid, CO_2, N_2, H^+, and methane. These may accumulate within cells and/or be liberated in the soil solution and adversely alter soil chemical properties. Organic acids with low volatility (propionic and butyric acid) may accumulate in waterlogged soils. In a situation of re-aeration after a period of oxygen privation, the remnant ethanol in tissues may be converted to acetaldehyde, which may induce post-anoxic cell damage (Bailey-Serres and Voesenek, 2008). Along with this, alterations in oxygen availability in the soil may induce the proliferation of reactive excited

states such as reactive oxygen species (ROS); these may be superoxide radicals, hydrogen peroxide or oxygen singlets. ROS are produced by normal cell metabolism in a plant, but under stress conditions the relation between their synthesis and elimination is altered (Blokhina et al., 2003).

Transient soil waterlogging and selection of tolerant rootstocks
Most studies of the effects of waterlogged soils on plants have been focused on annual species such as rice, maize, subterranean clover, and alfalfa (Vartapetian and Jackson, 1997) under completely waterlogged conditions. A small number of reports have dealt with the effect produced in woody trees, but there has been little research on fruit trees. Additionally, the type of inundation to which plants are exposed will mostly determine the response mechanisms and adaptations evolved by the species, which are necessary to determine for selecting tolerant plants. The responses and adaptation mechanisms of plants in wetland ecosystems may serve as models, but these species have different mechanisms than plants that have evolved in soils without free water. Most studies have dealt with the effects of continuous waterlogging, not transient situations, in which long-term mechanisms of adaptation or acclimation such as the generation of aerenchyma, hypertrophied lenticels, and adventitious roots become much more important for plant survival.

In Chile, the origin of aeration deficiency in agricultural soils is mainly related to poor management of fine-textured soils, which are subject to compaction. The saturation of the pores produces situations where hours or days pass with little or no oxygen available for the normal development of plants. This problem is more complex when the limits of soil density and structure increase, sometimes reaching reduced values of LLWR, in which small errors in irrigation management inevitably generate hypoxia and/or anoxia.

Current technological tools allow continuous monitoring of water level in soil using water content sensors. These provide information to the depth to which roots are, as well as avoiding excessive water levels by adjusting the application and frequency of irrigation (Schaffer, 1998). One of the main difficulties with the attempt to have a wider vision of the effects of oxygen deficiency in the soil due to the effect of transient conditions of waterlogging is precisely that of including the soil factor in the soil-root interactions. So far there have been significant advances in the detailed study of lack of oxygenation in roots of different species of plant in soilless culture systems (hydroponics and aeroponics), especially in annual species in which it is possible to simulate oxygen deficiency and its effects on nutritional balance (Morard and Silvestre, 1996; Bertholdsson, 2013). However, these methodologies ignore a series of modifications in the soil which may be relevant in field conditions for the adjustment of the plant, such as other gases in the pore spaces (not only oxygen) and the physical structure of the soil mentioned above, which affect root growth (root anatomy and root system architecture) in ways that are impossible to simulate in hydroponic systems. The use of pots with substrate or soil may provide better information with respect to the soil-root interaction for the selection of parameters or mechanisms of adaptation.

In fruit trees, studying the root responses to temporal waterlogging is very relevant, since the development of the aerial part of the plant depends largely on the rootstock employed (Schaffer, 1998). The selection of the scion is thus a relevant and strategic decision for the design and establishment of a fruit tree orchard. From the physiological perspective, tolerance to waterlogging has been defined in studies oriented to the survival or maintenance of growth rates in this condition compared to a control without inundation. The agronomical definition is based on productivity, which refers to the possibility that a cultivar tolerant to waterlogging exhibit a mechanism associated with escape from anaerobic conditions, either due to dormancy or slower growth during the stress and a rapid resume when the stress ends (Setter and Waters, 2003). In rootstock breeding programs yield is not essential, but physiological and morphological traits (or features) of root system that reduce negative effects of waterlogging are important. Accordingly, for the selection of plants tolerant to abiotic stress it is important that the key trait can be selected with precision; in this case it could be related to the root energy balance. Once plants are selected for this characteristic, other features required for establishment at field conditions may be included. In a second stage of tolerant rootstock selection to transient waterlogging, soil factor is essential, especially its relation with dynamics of gas exchange and toxic compounds produced in the soil under waterlogged conditions. In a hydroponic system, N is pumped in to displace the oxygen present, in order to maintain low oxygen content. Although this technique allows the maintenance of precise oxygen concentration in a trial, it removes most of the gases produced close to the root. Under natural conditions, the water block in the upper soil layers does not allow rapid gas exchange and compounds begin to accumulate and may become toxic for the plant or modify its response pathways. Although closed hydroponic systems allow progressive exhaustion of oxygen due to the respiration of the plant itself, resembling natural conditions, results must be carefully validated in a soil system (Morard and Silvestre, 1996).

The plant tolerance to inundation is quite variable and depends upon the species, genotype, age, timing and duration of the stress, and water quality of the waterlogging. Moreover, the susceptibility of a species varies depending on the criterion by which tolerance is evaluated. For example, two species of *Parkia* (*P. pendula* and *P. discolor*) showed similar tolerance levels

in germination, but contrasting responses when seedling survival was used as a criterion of adaptation (Scarano and Crawford, 1992). Due to this filter, the rootstock tolerance classification depends upon factors such as type of waterlogging (gradual or complete), number of days waterlogged (short- and long-term evaluations) (Striegler et al., 1993; Kozlowski, 1997; Schaffer, 1998), with or without soil (Tavakkoli et al., 2010) and with or without recovery (Striker, 2012), all of which make it difficult to have uniform criteria for the selection of tolerant plants and to distinguish plants adapted to waterlogging stress under field conditions (Striegler et al., 1993; Ranney, 1994).

Most studies, however, consider a permanent flooding stress but in field conditions plants are often exposed to intermittent episodes of flooding and recovery. This transient waterlogging is very important because some specific damages, such as oxidative stress, may occur to a higher extent during re-aeration of tissues after a period of stress. Therefore ROS metabolism is an important tolerance criterion for rootstocks. One protection system against ROS is superoxide dismutase (SOD), an enzyme that converts superoxide radicals to hydrogen peroxide, which is reduced to water by peroxidases or catalase (Drew, 1997; Blokhina et al., 2003). In *Citrus* species has been demonstrated that waterlogging generates oxidative damage; however, the damage was reduced and delayed in rootstocks tolerant to waterlogging compared to more sensitive rootstocks. Although SOD activity is directly related to protection from oxidative damage, the activity of ascorbate peroxidase and catalase are strongly increased in tolerant species, avoiding the accumulation of hydrogen peroxide generated by SOD (Arbona et al., 2008); similar results were found in apple (Bai et al., 2010). Accordingly, the cause of the damage to plants in waterlogged soils is frequently questioned. The literature suggests that it is more related to the production of toxic substances than to oxygen deficiency in the roots per se (Setter et al., 2009). In agreement with this, another aspect that may be relevant to consider in rootstock selection is the generation of reduced compounds in the soil such as Fe^{+2}, which change remarkably after an inundation event (Brzezińska et al., 2005). It would be interesting to obtain rootstocks capable of tolerating ferric chlorosis, since in perennial species sensitive to waterlogging such as avocado, one of the most common consequences after stomatal closure is leaf chlorosis (Fassio et al., 2011). In more tolerant species such as grapevine this has not been clearly demonstrated. Another alternative would be to search for genotypes efficient in nutrient use in situations of waterlogging, such as the efficient N use to maintain growth and metabolic activity under stress, although a direct relation between high vigor and tolerance to waterlogging is not clear.

The dynamics of root growth and the ability of trees to recover from transient inundations are significant in evaluating short-term effects of waterlogging (Smith et al., 1990). Species reduce their growth under anoxic conditions, but in contrast to sensitive species, tolerant species can resume growth rapidly once aeration has been restored. Studies in peach showed that the generation of new roots is key for recovery from the damage produced by short-term waterlogging, and can be achieved after 16 d (Nicolás et al., 2005). A similar response was found in almonds (*Prunus dulcis* (Mill.) D.A. Webb) (Sanchez-Blanco et al., 1994), with recovery after 20 d, and also after 29 d in lemon grafted on *Citrus aurantium* L. (Ruiz-Sanchez et al., 1996). It has also been observed in species such as blue oak (*Quercus douglasii* Hook. & Arn.) that root systems with few ramifications or lateral roots are more sensitive to hypoxia due to their reduced ability to absorb oxygen (Jacobs et al., 1997), which has also been reported for other species such as *Eucalyptus* and *Picea* (Philipson and Coutts, 1978; Kozlowski, 1985). Currently, with new tools for observation of roots *in situ* using scanners, these parameters may be evaluated both in potted soil and in the field.

Another feature of rootstocks is susceptibility to diseases, principally fungi of the genus *Phytophthora*, which is strongly associated with waterlogged soils. It has been observed that when this fungus is present in avocados, there is greater damage to photosynthesis and gas interchange (Ploetz and Schaffer, 1989; Schaffer and Ploetz, 1989; Schaffer, 1998); therefore, it is recommended to include rootstock susceptibility to this fungus in breeding programs.

Selection of rootstocks is complex, especially if a multifaceted stress such as transiently waterlogged soils is considered, since factors in the plant interact with soil factors. Hence, information on stress tolerance is not comparable and may appear contradictory. Most stresses do not have a single response pathway, but rather are integrated responses, even more so in the case of transient cycles of oxygen absence. This was corroborated by studies in wheat, where the tolerance of one wheat genotype was variable in two different waterlogged soils (Setter et al., 2009); in the other hand, the response of barley was different when grown in hydroponics or in soil (Tavakkoli et al., 2010), which indicates that we must continue to search for parameters or regulation mechanisms to select and adapt rootstocks to transient inundations, incorporating the soil in the studies.

CONCLUSIONS

Significant advances in the last decade have allowed us to discover responses, mechanisms, and adaptations to stress due to hypoxia/anoxia in plants of different species under controlled trial conditions. To select rootstocks for fruit trees it is necessary to evaluate the trees in semi-controlled conditions including the soil factor, considering large changes generated in soil by excess water. Although

including soil in trials tends to be costly in terms of reducing precision in the treatments and producing a compounding effect of factors, from the agronomic perspective integrating the soil allows a better simulation of the effects of waterlogging stress in plants. Furthermore, the selection of parameters related to events that occur in soil and cannot be captured in closed hydroponic systems may enrich studies associated to rootstocks selection tolerant to waterlogging. In soils with transient waterlogging due to poor management and/or excessive rains, the selection of tolerant rootstocks should consider mechanisms of short-term adaptation, like root mechanisms that respond to changes in the soil such as anti-oxidant capacity and the dynamics of nutrient absorption, as well as the regeneration and distribution of the root system architecture in soils susceptible to waterlogging. Research platforms using soils that can maintain different levels of aeration and monitor water and oxygen content and root growth, as well as measuring the suggested parameters, would be an important advance for rootstock breeding programs in the stage of evaluating waterlogging tolerance and their future field establishment and performance.

ACKNOWLEDGEMENTS

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