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Measurement of Redox Potential and pH in Plants and their Function in the Mechanism of Plant Resistance and in Plant Physiology

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ABSTRACT: The study of biophysical states in plants was initiated 35 years ago in connection with the looking for the mechanism of plant resistance to parasites. Here the attention was focused on the variable resistance of cereals to obligate parasites such as powdery mildew and rusts. It was necessary to find out a factor which changes during the ontogeny and through the environment and which involves:
1. The disease gradients on plant,
2. The change of susceptibility of organs during the ontogeny and growth,
3. The difference in resistance in individual plant cells,
4. Relatively swift changes of resistance during a couple of hours.
This factor could be found in the biophysical states of plant organs (redox potential (RP) and pH).The method of RP measurement in plant tissues in aerobic conditions and measurement in hypoxia are presented. The redox state changes during the growth and development of organs and is influenced by the outer conditions such as light, temperature, moisture, growth regulators, nutrition, etc. and by the organ senescence.This is not a standard redox potential, as defined in physical chemistry, which is constant, but redox potential in plant is dependent on activity of respiration enzymes and it changes. The actual state can be red from the lower turn point of mV changes on indication instrument (milivoltmetre) with input resistance at least 10 MΩ. A hypothesis was formulated for further investigation:
The principle of resistance consists in the ability of the parasite to gain the energy in the host cell. The parasite uses the terminal oxidase of the host plasma lemma. The specific phenolics are the substrate for this enzyme. The main features of this hypothesis are:
1. There is no free oxygen in the cell plasma. 2. The redox potentials are generated by the respiration of the cells by the sum of activities in cell organelles which produce the electrons and the activity of oxidoreductase
(the terminal oxidase) in the plasma lemma. The electron carriers can permeate from the cell to the environment and they are soluble in water. 3. The redox potential is the basis of electric gradients in the plant which plays the
main role in its integrity as well as for the life of the parasite. 4. The parasite respires through the terminal oxidase of the host. 5. The environmental conditions influence the enzyme activity of host and parasite cells differently by which different redox potentials may appear in the host and parasite cells resulting in unspecific oxidation or reduction of electron carriers. 6. The acidity of the host cells determinates the formation of conidia or cleistothecia
(in powdery mildew) or formation of redia or telia (in rusts). 7. Depend on redox potential it is possible to explain some correlations in plants. Without knowledge of RP gradients it is not possible to explain the integrity of
plants and related phenomena.

KEYWORDS: Aerobic conditions, Biophysical states, Correlations, disease gradient, Hypoxia, Investigation history, linen, Nature of plant resistance, Peas, Plant integrity, Powdery mildew, Pre-polarisation, Redox potential measurement, Rusts

I.INTRODUCTION

In spite of the attention paid to looking for the principle of disease resistance by plant pathologists over many years (see the review by Fuchs (1976), there is still missing general explanation of its nature (Heitefuss 1992, Hartleb, Heitefuss, Hoppe 1997). The basic role is ascribed to the recognition of the host by the parasite and to the exploitation of the host as a substrate without declaring what the real mechanism is. Unconscious generalisation of results obtained with special objects lead to theories which were not generally applicable. Fuchs (l.c.) concluded that the problem
was necessary to recede to new, unknown horizons. The older data on host-parasite relationships were summarized in compendia such as those by Heitefuss & Williams (1976), Vanderplank (1982) and Horsfall and Cowling (1980). In the

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last 20 years more success has been expected from molecular biology and molecular genetics (Heitefuss 1992) and summarized e.g. by Newton and Andrivon (1995). At the beginning there was investigation of variable resistance (VR) to obligate parasites such as powdery mildew of cereals and rusts (Benada 1964b, c). To limited extent other host-parasite couples were studied (Benada 1967a, 1974). For elucidation the VR mechanism the attention was focused on redox potential (RP) and pH (Benada 1966a, 1967c, 1968a). The reasons for the selection of this direction
of investigation are mentioned in the cited literature. |
In this article will be presented most author`s investigations on variable resistance as a starting point for considering the nature of disease resistance, some selected data of redox potential and pH and its application on plant physiology.
The variable resistance (sometimes termed field resistance, race non-specific resistance,partial resistance, horizontal resistance, general resistance) designates a plant resistance which changes during the ontogeny of the host and under the environment and which involves:
1. The disease gradients on a plant,
2. The change of susceptibility of organs during the ontogeny and growth,
3. The difference in resistance in individual cells of a plant and relatively swift
changes of its resistance during a couple of hours.
The aim of this article is to present the method of redox potential investigation in plants and to formulate a hypothesis why the biophysical states may play such an important role in resistance and in plant physiology in general.

II. REDOX POTENTIAL AND pH

2.1. Methods

2.1.1. Reading device: The redox potential (Eh) was measured with mV reading device Digital Multimeter Metex M3650CR (10x106 Ohm input resistance). Other instruments with the same or higher input resistance can be used, eg. various pH meters. It is convenient when the Multimeter has a max/min data hold function.
2.1.2. Electrodes: Standard reference electrode – the saturated calomel elecrode. Ag/AgCl reference electrode can be used as well.
2.1.3. Experimental halfcell: the bright platinum electrode 5 x 5 mm sheet or needleform. Pre-polarisation: before measurement the Pt electrode was dipped for a moment into the solution of kalium-ferricyanide (1 g in 1000 ml H2 O) and then rinsed with distiled water. Later on the prepolarisation with kalium-ferricyanid was replaced by repeating electrode insertion into the different places of a plant tissue to be measured.Both electrodes were separated, the combined electrodes are not convenient, especially for measuring in aerobic conditions.
2.1.4. Measurement in aerobic conditions: Filter paper was inserted to the bottom of a glass dish and sufficiently wetted with 0, 1% solution of KCl or tape water . The calomel electrode was attached. The plant leaf was rolled lenghtwise and the Pt electrode was run through the leaf tissue so that the whole surface of the electrode was covered with it. As far as one leaf was too small to cover the electrode, then it was necessary to take two adjacent leaves for measurement. In the case of solid organs such as potato tuber, the Pt electrode could be pricked directly. The Pt electrode with plant tissue was then put on the filter paper. The electric potential value in most cases began to sink, then was fixed for some time and thereafter began to rise. This lower-turn point was written as the redox potential of the plant organ. More details of measurement method ca be obtained in particular papers, mainly Benada (1966a). There are gradients of redox potential in plants. To simplify the RP interpretation the shown data do not regard the potential of saturated calomel electrode (+244 mV). Generally 10 leaves or other organs were taken for one series of measurement and the results were expressed as mean and SEM (standard error of mean). In some plants upper-turn RP are common. It is recommended to omit the first reading when we begin measurement with new sample of plants. For measurement in rolled leaf it is recommended to repeat two times the piercing into the selected tissue. Then the turn point can be obtained earlier.
The above electrode couples were used for RP measurement in roots exudates (Benada 1995) similarly to that used in the soil by other authors, e.g. Flessa and Fischer (1992), Králová (1992, Husson et al.2016.). For measurement in the soil the measurement„ in situ“was prefered. Measurement in hypoxia. The non-invasive method for RP measurement can be used especially under conditions of substantial decrease in air access to plant tissues by their submergence in water. In following experiments, current tap water was used. In this water no redox systems are present which could react with redox systems of the cell. In tap as well as distilled water the air oxygen is dissolved that could react with a Pt electrode. The concentration of this redox system is however low. The values of RP system diffusing from the cell are dependent on enzyme systems coupled with respiration. The electron carriers of cells do not react with

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oxygen directly, only by means of respiration enzymes. Also in hypoxia RP was measured by a bright Pt electrode and a comparative standard reference electrode. The standard electrode was placed freely beside the plant parts submerged in water. The lower turn point was looked similarly as in the case of measurement in aerobic condition. More details can be found in Benada (2009). Using this method the evidence was obtained that redox potential in plant tissue is very close to the value of surrounding solution and that electron carriers diffuse from plant tissue and they
are soluble in water. pH was settled in disingrated plant tisues using chinhydrone Pt or glas electrode (Benada 1965, 1966b, 1967a), but other methods were tried too.

2.2. Properties of RP

2.2.1. Influence of environment: The redox state changes during the growth and development of organs and
it is influenced by outer conditions such as light, temperature, moisture, growth regulators, nutrition, etc.(Benada 1966c, 1967e, Benada and Váňová 1972). Light is the most important factor in influencing the RP values in leaves. Low values of RP were obtained under intensive light in the field only. Those parts of leaves, which were covered by other parts, had high RP, e. g. basal parts of leaf blades covered by the sheaths during their development. The influence of the temperature is complicated by the fact that RP is simultaneously dependent on the light. In the laboratory trials the increased temperature caused the increase in RP and the lower temperature had a reverse effect. On the contrary, the temperature below +5 oC resulted in the RP increase. It was found that the wilting of young plant leaves in the glasshouse which had the high RP values at the beginning of experiment caused an increased RP. On the contrary, in leaves of plants growing in the field with initial low RP the wilting resulted in the decrease in RP values (Benada 1967e). The first leaves of cereals growing in the hydroponic culture with full nutrition had lower RP than plants growing in pure water. It was assessed that RP state was very sensitive to aerobic conditions of measured organs (Benada 1965, 1966a, 1967d, 1968a, c). The most expressive effect of anaerobic conditions was observed in the inundated roots of cereals where in the root exudates RP sank to -560 mV after 40 hrs or within 30 minutes only in dependence of temperature and plant material, whereas in the aerobic conditions it was +160 mV (Benada 1995).The addition of KNO3 solution prevented the decrease of RP. It is explained that nitrate with combination with nitratereductase supplies oxygen for respiration. Some plant organs (e.g. sunflower hypocotyls) are very sensitive to anaerobic conditions, too. During one hour, RP sank from +200 mV to +60 mV in them. The cereal leaves are not so sensitive.

2.2.2. Influence of ontogeny and senescence: In cereal leaves growing in the field RP sank from approx. +50 mV to -100 mV or lower during the ontogenetic development. In dicotyledon plants such as the sunflower RPs in cotyledons were approx. +260 mV, in the leaves on the stem it sank to the area of -20 mV. The decrease in RP seems to be of general validity during the ontogeny in all plants.

2.2.3. Gradients in plant: There are gradients of RP in the whole plant. In cereal leaves during the stem elongation the lowest RP value was in the second upper leaf, whereas when the ear appeared, then in wheat the lowest value was in the top leaf (Benada 1967c). The RP gradients were not so regular in some cases.

There are differences of RP in leaves on the main and side tillers , the main tiller having lower values (Benada 1966a). There are gradients in other plants,too (Benada 1967b) as well as gradients of pH.: young organs being acidic, older ones having pH near the neutral point. In dependence on pH values the symptoms of powdery mildew as well as production of conidia and cleistothecia in powdery mildew or uredia and telia of rusts were changed (Benada 1965, 1966b, 1967a, 1970a). The formation of conidial stage of powdery mildew or uredial stage of rusts was linked with tissues of pH value 6 and lower, the cleistothecia of powdery mildew and telia of rusts were formed at tissue pH around 7 and higher.

2.2.4. Other electric measurement in plants: Measurement of the RP difference between the plant and soil were performed by Keppel et al. (1997). When there are RP gradients in the plant, then the RP difference may be examined. Similarly the electric current (in □A) was measured by Rajda (1992,1996). Generally when the RP difference between tree and soil is low or when there is low electric current, the health state of the tree or other plants is bad.

Volkov A.G.(ed) in the textbook Plant electrophysiology. Methods and cell electrophysiology. Springer Heidelberg 2012 have shown different measurement method of electric potential and current, membrane potential, electric signals, action potential etc.

2.3. Discussion of RP

From above and other experiments it may came to the conclusion that RP is generated by the respiration of plants, by the production of electrons by dehydrogenases and by the activity of terminal oxidoreductases. It is supposed that the

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activity of these enzyme groups is changing and so different RPs may be created. The increase of RP value during longer time of measurement may be due to electrolysis of water and development of oxygen on the electrode. The analysis of free O2 in the cell is difficult and up to this time the importance of such investigations has not been put in foreground [e.g. Linskens and Jackson (1990)]. Most plant physiologist presume that O2 is present in the cell. Nevertheless, when the RP in plant tissues should be dependent directly on O2 pressure, then there would not be any gradient of RP. But the gradients of RP within different plant organs do exist.

III. FUNCTION OF RP IN THE MECHANISM OF RESISTANCE TO PLANT PATHOGENS

3.1. Variable resistance

The variable resistance in some characteristics is close to race non-specific resistance. The meaning of race non-specific resistance (called field resistance, general, nonspecific, partial resistance) have been formulated by many authors and sometimes with different contents. A recent example is given by Jorgensen (1987): 1. it reduces the amount of powdery mildew , irrespective of the pathogen genotype, 2. it is governed by apparently many additive acting genes, and 3. it is expressed (and measured) in quantitative terms. The differences in the definition of different kinds of resistance reflects the present insufficient knowledge of the mechanism of it.

The experiments with VR have advantage in investigation of resistance nature because we can use one variety only, we can take different organs and parts of it and in this way to eliminate the chemical differences given by varieties.

In experiments and observations of plants grown in the field it was noticed that during the stem elongation of cereals the new leaves were temporarily fully resistant to powdery mildew and behaved similarly to those in the case of non-host resistance (Benada 1966a) in respect to haustoria formation.

3.2. Disease gradients

The lower leaves developed on the cereal plants during the stem elongation are susceptible to powdery mildew, the upper ones are resistant for some time (Benada 1966a). This is called the infection gradient (Tapke 1953 who reviewed the literature on this topic). The outer side of coleoptiles and the young leaves in them are resistant, which was demonstrated by an inoculation test on isolated organs (Benada 1964a, c). Distinct differences in resistance were found within one leaf blade, between its top and base, between leaf sheaths and the stem.

Quantitative differences in resistance were found even in neighbouring individual epidermis cells (Benada 1969, 1970a, b, c, 1971) and it was found that the resistance could change within few hours, which could be demonstrated by haustoria formation.

The dependence of resistance on the ontogeny and on the environment is known for a long time in many parasite-host couples and was shown in the case of powdery mildew (Benada 1966a where some fundamental papers are reviewed).

3.3. Resistance and RP

Already earlier investigations (Benada 1966a) suggested that the biophysical states in plants could accomplish the requirements for expressing the VR: 1. the disease gradients on a plant, 2. the change of susceptibility of organs during the ontogeny and growth, 3. the difference in resistance in individual cells of a plant, 4. relatively swift changes of resistance during a couple of hours.

In special experiments on detached leaves of cereals (Benada 1971) we tried to find exact limits of RP and pH values for resistance or susceptibility against powdery mildew. Because measurement of RP and pH in individual cells was not possible such exact limits could not be found. Nevertheless, organs with a low RP (lower than 0 mV) were resistant against powdery mildew, organs with a higher value were susceptible (Benada, 1971). In the case of tomatoes the resistant leaves and fruits against Phytophthora infestans had RP higher than +100 mV, the susceptible ones had lower RP. So, the leaves of cucumber were resistant against Pseudoperonospora cubensis at RP higher than 0 mV. The RP of older leaves on the stem was falling under 0 mV and they grew susceptible. In potato tubers the inner part of it having RP bellow +100 mV is susceptible to Phytophthora infestans, the outer part having RP above +100 mV is resistant.

3.4. Attempt to formulate the theory of resistance on the basis of RP

It was shown that the resistance in examined plants was linked with areas of distinct RP and that powdery mildew of cereals was a very suitable object of resistance investigation because the development of haustoria could be relatively simply observed. The strategy of looking for the mechanism of resistance on the basis of molecular biology in a substance seems to be very complicated, because there is a great number of substances at stake. Moreover, the valid mechanism of resistance must be applicable in the other areas of plant physiology. During 30 years of investigation it

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has been shown that the RP values could be measured and that under similar conditions the respective organs had rather the same RP. Up to that time biophysical states have not included in the consideration the mechanism of plant resistance at least as it will be formulated in the next part. The electric potential in plants is not a redox potential in a strict sense of physical chemistry because it has no steady state and it only reflects the oxidative and reductive processes in plant cells. Therefore, the measurement of RP in plant is not accepted by the researchers in physical chemistry and it is not mentioned in Physical methods in plant sciences by Linskens and Jackson (1990).

3.5. Why RP has decisive function in the mechanism of resistance

3.5.1 Electron transport: The hypothesis has been formulated that there is no free oxygen in the cytoplasma and the transport of electrons among cell organelles is mediated by a system of "phenolics" forming redox couples in combination with highly specific oxido-reductases being fixed in cell wall (plasmalemma) and cell organelle wall. The specificity consists in oxidation or reduction of OH/O groups of "phenolics" in distinct positions only. The electron exchange must take place in the same position by the enzyme in the walls of inner organelles: chloroplasts, mitochondria, nucleus, etc. I suppose it is the same enzyme as that in outer cell wall which can work as oxidase or reductase in dependence on the supply or demand of oxygen/electrons. The specificity is implemented by this mechanism. The parasite in host cell cannot use its own enzyme (terminal oxidase, oxido-reductase), because there is no free oxygen in the cell cytoplasma, it must use the enzyme in outer membrane of the host cell. The oxidation of its "phenolics" must be so specific that the reductase of inner membrane of its own organelles converts the "phenolics" to the original state. In other cases non-specifically oxidized "phenolics" accumulate in the host cell and the parasite dies for the lack of energy. Because these "phenolics" of the parasite are "strange" for the enzyme of the host, the specific oxidation is very sensitive to redox and pH stage of the host. The analysis of free O2 in the cell is difficult and up to this time the importance of such investigations has not been put in foreground (e.g. Linskens and Jackson (1990).

3.6. Phenolics

There are many redox systems that operate in the plant cell. With respect to the method of measurement and that the redox system exudates from roots (Benada 1995, 2009) and from other plant organs the attention was concentrated on phenolic compounds (Vaugham and Ord 1991). Moreover, the phenols are supposed to play an important role in resistance. The exudation of phenol compounds takes place from both powdery mildew conidia (Vizarová and Janitor 1968) and uredospores of rust (Summere et al. 1957). Nevertheless, it is supposed that the target redox systems are not only phenols but other aromatic (heterocyclic) hydroxy compounds, too. The generally present ascorbic acid redox system is not specific enough for electron exchange (see later) with the exception that it would be combined with phenols. Further on the designation "phenolics" will be used for all these electron carriers.

Expected properties of "phenolics" as carriers of electrons in respiration:

1. Solubility in water.
2. Formation of the redox system in cytoplasma connecting all cell organelles in demand and supply of electrons.
3. Absence of oxidation by the air oxygen at all or of specific oxidation. The same is valid for hydrogen peroxide and other oxidizing factors.
4. Formation of exudates.
5. The phenolics can be obtained in oxidized or reduced states by inducing aerobic or anaerobic conditions.

Point 1, 3-5. Could be demonstrated by growing the cereal plants in hydroponic culture (Benada 1995,2009). The exudation of phenolics and other substances from roots is known (Přikryl and Vančura 1990).

3.7. Concentration of electron carriers

The exact concentration of electron carriers is not known. It can be only estimated e.g. from the experiments with self-inhibition of rust spore germination (Macko et al. 1971). The concentration of respective phenol substances is approximatelly 10-8 till 10 -10 molar solution. They are therefore amongst the most highly active biological agents, equally active or more so than the sex hormones (Allen 1976). When there is interferention with the electrone cariers, then this effect can be understood.

3.8. Redox values

Having found that the plant organs are resistant/susceptible within certain limits of RP and pH, it was necessary to ask a question why RP played such an important role. RP was supposed to play a significant role in respiration of both host and parasite and in the ability of the parasite to gain the energy in the host (Benada 1964a, 1991). The influence of the

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environment on the activity of reducing enzymes in cell organelles and on the activity of terminal oxidase in cell wall (plasmalemma) is expected to be different, therefore low or high RP results.
It remains to explain why the parasite cannot live in the cell at a high, or more often, low RP. It is known that the redox system with a high RP oxidizes other redox systems with a lower RP and vice versa. If the parasite produces its own redox system in the cell which is oxidized by the host at a proper site, but this system interferes with the redox system of the host with much lower RP, then non-specific reduction takes place which cannot be oxidized by the terminal oxidase. It is similar at a high RP of the host cell, too. Thus, the importance of RP of the host cell or tissue consists in the fact that the redox system of the host and parasite interfere mutually through oxidation or reduction, but non-specifically, i.e. in non-correct sites of OH/O groups. Also, reverse reaction would be possible. Here the amount of the redox system of the parasite would surpass the amount of the redox system of the host. However, the host cell would die.Up to now,it is missing the explanation of the pH function for the transformation of the conidium stage to ascomycete one, and similarly uredia to telia. This change may be also associated with the oxidation of phenolic substances as carriers of electrons. On the other hand, an appropriate value of pH is necessary for correct function or oxidation-reduction enzymes.

3.9. Obligate parasites

An obligate parasite without the host has not its terminal oxidase (phenol oxido-reductase) active enough to ensure its energy requirement for the growth and multiplication. For example, the conidia of powdery mildew germinate on leaf surface and the infection hypha grow mainly above the septa of cells where the highest accumulation of host oxidase may be expected (Benada 1970b). Haustoria of powdery mildew are formed in the host epidermal cell only when its redox and pH stage are suitable for the specific oxidation/reduction and in this way satisfying enough energy by respiration. Moreover, IAA (indole acetic acid) is translocated to mycelium areas with high RP and therefore the mycelium grows in the direction of the highest oxidation.The above principle should be the same for the race-specific and race non-specific resistance (Benada 1997). Each race has its own substrate and oxidation/reduction enzyme. When the enzyme of the host is not suitable, then there is no energy supply and no growth of the parasite. This fits with the "gene-for-gene" theory formulated by Flor (1942) very well. The host for mono- and dicaryotic states of some rusts are different. Therefore, the respiratory substrate must be different at these two stages and the parasite grows in host with suitable oxidase for the specific substrate only.Since cereal powdery mildew infects epidermal cells only and rust does mesophyl cells, therefore the different oxidation-reduction enzymes are expected in them. It is not clear if there are different substrates for them, too, or if there is the same substrate, but only the oxidation and reduction go in other places of the same molecule. What may be the fate of this substrate when it comes to the epidermal cell and vice versa is open for further investigation?

3.10. Support of hypothesis

The above-mentioned hypothesis is supported by some previous findings of other authors (cited from Heitefuss and Williams 1976): Control of spore germination and infection structure (Allen, p.78):...endogenous inhibitors are diffusible, readily reversible dormancy agents, small molecular compounds, the target of their action is the spore wall and they are characterized by their mobility from cell to cell.
Protein metabolism (Uritani, p.521): "The injury or death of parasitized cells often induce oxidation of polyphenols or the formation of lignin in the infected cells and in the non-infected cells adjacent to the infected cells." Endogenous auxins in healthy and diseased plants (Pegg, p.575): "Particular interest centres around the role of phenolic compounds and auxin metabolism. The production of phenolic compounds and phenol oxidases and hydroxylases is an almost universal feature of disease involving facultative pathogens." Oxidative enzymes (Frič, p.623): "Increased phenolase activity in diseased or wounded areas of plant tissues is generally accompanied by increased concentration of phenolic substances."
Phytoalexins (Kuc, p.646): "It appears that the key to the timing of the response is determined by the plant`s ability to react to components in or produced by the infection agent (recognition). It is suggested a surface phenomenon based on components of cell walls or membranes." Increased respiration connected with infection (Daly, p.541). My own explanation: when the "phenolics" of the parasite is not specifically oxidized, the product cannot be used for energy gain of the parasite. Also, the "phenolics" from the parasite enter the host cell, they cause damage by competition with indigenous phenolic substances and increased respiration results. The observed different susceptibility of neighbouring epidermal cells to powdery mildew and rapid changes of susceptibility and resistance (Benada 1970b) can be understood by different biophysical states in them.

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3.11. The role of pH

The telia of cereal rusts can scarcely be found on cereal seedlings grown in the greenhouse and they occur in uredial stage mostly on younger tissues in the field, too. Similarly, the powdery mildew on the seedlings in the greenhouse occurs only in the conidial form. The cleistothecia can be found abundantly on the mature plants in the field. This phenomenon is generally known and several attempts were made in the past to explain it (Benada 1966b).

Up to this time it is not clear why pH of host tissue plays such an important role in the change of the conidial or uredial stage of parasites. It is very conspicuous that simultaneously symptoms caused by the parasite in the host change , too (e.g. brown patches or chlorotic spots developing in connection with powdery mildew infection) (Benada 1969).

IV. FUNCTION OF RP IN PLANT INTEGRITY

All deductions originate in the findings that transport of IAA (probably other hormone regulators too) depends on the electric gradient in the plant (Benada 1968b) as was shown on examples on peas and flax.

4.1. Peas and flax

Peas and flax are two typical plants which were used for studying correlations and apical dominances. Basic data achieved in this study are reported by Dostál (1959) and later in several other contributions (Šebánek et al. 1983, Procházka et al. 1997, and others).

Conclusions were as follows: epigeic cotyledons of flax stimulate the growth of their axils. By contrast, hypogeic cotyledons of peas suppress their growth. Until now, explanations of these correlations have consisted in interferences of various growth substances.

The situation from the RP point of view is as follows: RP in cotyledon leaves of flax which grew in the light was on the average +237 mV. In cotyledons of pea, decreasing values from -27 to -140 mV with further decrease were measured. As far as roots are concerned, my technique enabled only to measure in part RP in roots (particularly in cereals) because common metal sheet electrodes were used which are too rough to measure RP. However, based on experiments with root exudates, I suppose that a very high RP (more than +100 mV) is in roots under aerobic conditions. Similarly to flax, cotyledons of sunflower also exhibit high RP. The experiments with sunflower showed that IAA was translocated in the field of high RP. A high (supraoptimum) concentration of auxin which inhibits growth is assumed in axile buds. The concentration is lowered by auxin transfer in the field of high RP. By contrast, organs with low RP increase the concentration in axile buds.

4.2. Cereals

The presented examples of correlations in dicotyledonous plants are completed with correlations in cereals: tillering plants show high RP in leaves in the beginning. Under these conditions, buds are formed in axils. (A precondition: again supraoptimum concentration of IAA; it decreases by its translocation in young blades with high RP). As the leaf grows up, RP goes down and causes reverse reaction, IAA is translocated to the bud that grows to a tiller (shoot). Why do cereals not branch higher on the stem: Blades on the stem show low RP relatively soon, which induces translocation of IAA in an elongation zone of the internodium with high RP, and internodia are elongated. In the case of low RP in blades during stem extension of elongating plants the bud is not formed, so it cannot be elongated in the branch. Generally, I incline to the opinion that the bud is not formed at all in cotyledon axils with low RP in pea from the beginning, but it is formed later, after increasing RP. The development of buds should be studied with regard to dynamics of changes in RP.

It remains to explain a common phenomenon why plants in the glasshouse growing in the insufficient light and higher temperatures have long thin leaves, whereas plants in the field with the same number of leaves have shorter and wider leaves. Plants in the glasshouse have high RP, IAA is transferred in the upper part of blades which lengthen. In the field, RP is considerably lower, IAA is pressed in the growth part of blade bases, therefore wider and shorter leaves are formed.

In the plant physiology manuals auxin is considered to be produced in the top of coleptiles of cereals and transported to the base. In other case it is produced in the root and stem tips. From the root tips it is transported acropetally, from the stem tips basipetally. The explanation of these differences is lacking.

In my opinion IAA is produced in every case in dividing cells (tissues) which have low RP (compare the reaction with triphenyltetrazolium). From these parts it is transported to tissues with higher RP, because there are RP gradients. In the

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tip of coleoptile there is no IAA production, only the secondary accumulation of it due to high RP It is the oldest part of the coleoptile. The dividing tissue in the case of coleoptile are on the basis of it similarly as in any leaf.
Without knowledge of RP gradients it is not possible to explain the integrity of plants and related phenomena.

V.CONCLUSION

1. The principle of resistance is the ability of the parasite to gain the energy in the host cell.
2. The parasite must use the oxido-reductase in the cell wall (plasma lemma) of the host, because there is no free oxygen in the cell plasma. The specific phenolics are the substrate for this enzyme.
3. The function of this enzyme is dependent on redox potential and pH of the host cell, mainly in the case of "strange" (non-specific) phenolic substances of the parasite.
4. The redox potentials are generated by the respiration of the cells by the sum of activities in cell organells which produce the electrons and the activity of oxidoreductase (the terminal oxidase) in the plasmalemma.
5. The redox potential is the basis of electric gradients in the plant which plays the main role in its integrity as well as for the life of the parasite.
6. The environmental conditions influence the enzyme activity of host and parasite cells differently by which different redox potentials may appear in the host and parasite cells resulting in unspecific oxidation or reduction. Increased respiration results.

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66. Remark: Most Benada's contributions can be red in www.vukrom.cz/contacts/Benada