

fertilizers and tip burn in lettuce on certain high lime peat soils. It is necessary to have liberal amounts of potash to produce a satisfactory lettuce head, but if excessive amounts are added there is great danger of tip burn developing and this may result in a loss of the entire crop.

Selective or differential absorption of nutrients by organisms is probably largely determined by the oxidation potential of the various ions. The electromotive series and oxidation potentials are probably the key to the interpretation of the important works on antagonism and selective absorption by W. M. Bayliss, C. M. Child, G. W. Crile, D. R. Hoagland, J. Loeb, M. M. McCool, W. J. V. Osterhout, W. Stiles and numerous other investigators.

The bimodal growth or production curve so frequently met with in plant and animal physiology is probably closely correlated with the electromotive series and oxidation potentials. The hydrogen ion with an ionic velocity nearly five times greater than any other common nutrient cation very probably determines the mode on the acid side of the neutral point, and the hydroxyl ion with an ionic velocity nearly three times greater than any other common nutrient anion determines the mode on the alkaline side of the neutral point. These two high velocity ions greatly influence the absorption of other ions, and are thus very important factors in regulating the growth or development of organisms.

This paper is an attempt to outline briefly the significance of the correlation between the electromotive series and the oxidation potentials, and the nutrition of plants and animals. A more comprehensive statement of the whole subject will be presented in a later paper. It is very clear from the preliminary correlations which have been made that the electromotive series and the oxidation potentials afford a new and an important approach to the whole field of biology. Electrochemistry has illuminated the subjects of chemistry and physics. It will do likewise in the field of biology, when the biologist begins to appreciate more fully the relationship between electrochemistry and vital phenomenon.

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INHIBITION OF ENZYMATIC ACTION AS A POSSIBLE FACTOR IN THE RESIS- TANCE OF PLANTS TO DISEASE¹

SPECULATIONS and investigations on the nature of disease resistance in plants have occupied the minds

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and efforts of plant pathologists since the inception of the science of phytopathology in the classic work of de Bary.² Fragmentary as is the evidence for the correlation of specific factors with specific internal resistance of certain species or varieties to particular parasites, it is sufficient to indicate that ultimate elucidation will probably be found in the domain of biochemistry.

During the course of an investigation which seeks to throw some light on possible bases for the resistance of sour orange (*Citrus aurantium* L.) and for the susceptibility of lemon (*Citrus limonia* Osbeck) to the bark diseases known as *Pythiacystis gummosis* and *decorticosis*, it has been found that the trunk bark of sour orange has a much greater inhibitory or paralyzing influence on the action of certain enzymes found in the dried mycelial powder of the causal fungi than does the trunk bark of lemon. This suggests the possibility that resistance to the invasion of the pathogens may be due to the inhibition of one or more of the enzymes of the fungi by some cellular product of the host, and that a sufficient decrease in this paralyzing power might permit the hyphae to progress rapidly, as they do in the bark of the susceptible lemon, and successfully parasitize the host.

Table 1 shows that the hydrolytic action of the diastase and invertase found in the dried mycelium of both *Pythiacystis citrophthora* and *Phomopsis californica* was inhibited more by sour orange bark than by lemon bark. Bark of tangelo, a hybrid of pummelo and tangerine, which has been found by inoculation tests to be very resistant to *Pythiacystis*, showed about the same degree of inhibition of fungus diastase and ptyalin as sour orange did. It is not to be expected that all enzymes would be similarly affected. Urease in fact was not thus inhibited. Other enzymes are being tried.

The "cultures" were made by placing in a 200 ml. Erlenmeyer flask 20 ml. of the substratum, 500 mgm. of the bark and 250 mgm. or 5 ml. of the enzym source. One ml. of toluol was added as a preservative, the flasks tightly stoppered, and the "cultures" incubated in the dark for 36 to 48 hours at 40 degrees C. At the end of the incubation period the "cultures" were filtered and a 10-ml. portion of the filtrate placed in 25 ml. of solution A of Fehling's reagent to stop enzymic action. Reducing sugars were determined by the Shaffer and Hartmann iodometric method³ and the results calculated as milli-

² Bary, A. de, "Ueber einige Sclerotinien und Sclerotienkrankheiten," *Bot. Ztg.* 44: 377-381, 1 fig.; 393-404; 409-426, 433-441, 449-461, 465-474, 1886.

³ Shaffer, P. A., and Hartmann, A. F., "The Iodometric Determination of Copper and its Use in Sugar Analysis," *Jour. Biol. Chem.* 45: 349-390, 1920.

TABLE I
INHIBITION OF ENZYMIC ACTION BY CITRUS BARK

No.	Substratus	Source of enzym	Inhibition due to:		
			Sour-orange bark mgm. Cu.	Lemon bark mgm. Cu.	Tangelo bark mgm. Cu.
1	10 per cent. Sucrose	Pythiacystis	3.6482	-.1664	
2	1 per cent. Sucrose	Pythiacystis	6.9488	3.8184	
3	1 per cent. Lintner's starch	Pythiacystis	14.2406	11.6272	
4	1 per cent. Lintner's starch	Malt diastase	9.9416	6.4328	
5	1 per cent. Lintner's starch	Phomopsis	16.2636	13.0835	
6	1 per cent. Lintner's starch	Pythiacystis	12.1254	8.4568	12.0162
7	1 per cent. Lintner's starch	Saliva	14.4352	10.8264	14.7475

grams of copper. The intrinsic reducing power of both the active and autoclaved bark and enzym materials in water was used in all the calculations. To illustrate:

$$\left(\begin{array}{c} \text{Reduction} \\ \text{by enzym} \\ + \text{substrate} \end{array} \right) - \left(\begin{array}{c} \text{Reduction} \\ \text{by enzym} \\ + \text{water} \end{array} \right) - \left(\begin{array}{c} \text{Reduction by} \\ \text{autoclaved} \\ \text{enzym + substrate} \end{array} \right) + \left(\begin{array}{c} \text{Reduction by} \\ \text{autoclaved} \\ \text{enzym + water} \end{array} \right) =$$

Reduction due to the hydrolytic products of enzymic action.

The necessity for such a method of calculation was pointed out by Klotz⁴ and is here illustrated farther.

Suppose

	Mg. Cu.
A. Starch solution plus active enzyme gave a reduction	25
and B. Active enzym alone	5
and C. Starch solution plus autoclaved enzym	2
and D. Autoclaved enzym alone	1
and E. Starch solution alone	0

then it is seen that the inactivated enzyme in the presence of the substrate (starch) produces some substance capable of reducing Fehling's solution; that is, there is present a catalytic effect other than the enzymic effect. It is assumed that this property also resides in the active unheated enzyme. Therefore, the reduction due to hydrolyzed starch, that is, the reduction due to truly enzymic action is evidently not A minus C or 25 minus 2 equals 23, but A minus B minus C plus D or 25 minus 5 minus 2 plus 1 equals 19. The value of D (equals 1) must be added because the intrinsic reducing power of the autoclaved enzyme was present also in C.

The figures in Table 1, it should be noted, do not

represent actual copper reduced, but inhibition expressed in milligrams of copper per milliliter of filtrate. They were obtained by subtracting the reduction value of the system, enzym + bark + substrate, from the sum of the values of the two systems, enzym + substrate and bark + substrate; that is, the figures represent loss in reducing power due to the inhibiting effect of the bark on the fungus or *vice versa*. Each horizontal row of results represents a series of cultures. The bark of the first five series was obtained from twelve-year-old lemon trees growing on sour-orange stocks, approximately equal quantities of bark above and below the bud union being taken at the same time. The bark material of lemon and tangelo reported in series 6 and 7 was from twelve-year-old lemon trees in another orchard and the sour-orange bark from a seedling tree near the tangelo. Inoculation tests have indicated that seedling trees of sour orange may be slightly more resistant to *Pythiacystis gummosis* than sour orange used as a stock.

The above data are offered as a suggestion for a possibly new line of attack. So far as is known this inhibiting or paralyzing effect of the plant tissues themselves upon certain fungal enzymes has not been suggested or tested as a possible basis for disease resistance in plants. The paralyzing effect on enzymes of some of the end-products of enzymic reactions, as hydrocyanic acid, benzaldehyde and hydroquinone, and also of the salts of heavy metals, is well known. It is suggested that any one or more of several cellular products might behave similarly. In work of this kind the necessity for check determinations on all materials used is here stressed again. The extension and test of the idea with other hosts, pathogens and enzymes are being continued at this station and it is hoped that others may see fit to test it.

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⁴ Klotz, L. J., "The Enzymes of *Pythiacystis citrophthora* Sm. and Sm.," Hilgardia 3: 27-40, 1927.