Polyphenoloxidase Activity in Healthy Maize Plants and in those Infected by the Maize Rough Dwarf Virus (MRDV)[1]

Introduction. The role of phenol-oxidizing enzymes in the defence mechanisms of plants against their pathogens in general has been comprehensively reviewed by FARKAS and KIRALY[2]. The significance of these enzymes, as regards localization of infection by plant viruses in particular, was discussed by FARKAS et al.[3]. However, in all instances referred to by these authors, none of the plant viruses involved was of the propagative leafhopper-borne group, which differ in many respects from other viruses[4]. It was therefore deemed worthwhile to test the polyphenoloxidase activity following infection by a virus such as the one causing the rough dwarf disease of maize. Unlike other plant viruses studied in this respect, MRDV is not mechanically transmissible, yet it spreads fairly rapidly in young maize seedlings following inoculation by the natural vector, the Araeopid planthopper *Delphacodes striatella* (Fallén)[5].

Materials and Methods. The maize plants serving for these tests were all of the hybrid variety 'Laguna Elite', which is highly susceptible to MRDV. They were grown in a field under sprinkler irrigation. Samples for enzyme tests, consisting of 6 healthy plants and 6 non-infected ones, were harvested at certain dates following vector inoculation (Table), which took place in the field 4–5 days after germination. Non-infected plants were chosen on the basis of absence of any visible symptoms. All tested plants were free of any detectable fungus or pest injuries. The sample plants were stripped of their leaves and sheaths, and 1-mm-thick transverse sections were sliced out of every internode. These disk-shaped slices were macerated for 60 sec in a Waring Blendor with 60 ml of acetate buffer pH 4.8. The homogenates were then centrifuged at 2500 rpm for 10 min at 4°C. The supernatant was filtered and kept in the cold for the next step. Determination of polyphenoloxidase activity: the method adapted for this purpose in the present work was based upon the reports of KEILIN and MANN[6] and of FARKAS and LEDINGHAM[7]. Pyrogallol, freshly prepared for each reaction, served as a substrate, and the light absorbancy of the purpurogallin, formed by this reaction, was read at 430 mμ in a Bausch and Lomb colorimeter.

Results and Discussion. Optimal conditions for reaction: Polyphenoloxidase activity of healthy plant extracts was assayed in the pH range of 3.6–7.8. Acetate buffer was used for pH 3.6–5.6, and phosphate buffer for 5.7–8.0. The activity curve obtained for this pH range showed that the whole span between 4.8 and 7.8 was optimal for this purpose. Similar results regarding this enzyme, though in apple tissue, were reported by PONTING and JOSLYN[8].

The effect of temperature on this activity was tested in the range of 10–65°C following 4 min reaction. An optimum plateau was recorded between 30 and 50°C. Even at 60°C activity was still very high, while at a temperature higher than 65°C the substrate was decomposed.

Increase in the molar concentration of the substrate from 0.0625 to 2.0 *M* resulted in an increase of activity, though the rate of increase beyond the 1 *M* point was fairly low. Activity rose linearly with the increase of plant-extract volume up to the point of 0.4 ml.

The initial velocity curve of this reaction showed that an activity period of 4 min, under the above-mentioned conditions, fits well within the linear part of the diphasic enzymatic activity curves.

Based upon these preparative results, the conditions for the reaction comparing healthy and diseased plants were set at pH 4.8, 37°C, 0.2 ml plant extract, 1 *M* substrate concentration, and a 4-min incubation period, after which the reaction was terminated by the addition of 0.5 ml of 20% H_2SO_4.

Polyphenoloxidase activity in healthy and diseased plants: Each one of the 6 maize stalk samples was assayed in 5 replicates with appropriate blanks. The average net values of this enzymatic activity, for the 6 sample plants at each date, are presented in the Table. The Table shows a remarkable increase in activity following the systemic spread of the virus in the plant. At 28 days after inoculation, the value for infected plants was nearly 3 times as high as that of healthy plants. From that age onwards there is a constant decrease of this enzyme activity in infected tissue until the difference between healthy and infected plants becomes insignificant at 56 days after inoculation, when non-infected plants of this variety begin to flower.

The respective dry matter percentages, included in the Table, clearly demonstrate that, contrary to expectation, the virus tends rather to delay the senescence of the infected tissue by keeping its dry matter content at a considerably lower level than that of non-infected plants of the same age. Hence, the difference in activity between healthy and infected plants is actually even greater than that indicated in the Table. At any rate, no prevention of spread or localization of infection of this particular virus seems to take place, even when the stimulation of polyphenoloxidase activity reaches an extent comparable to that of the instances of other viruses where localization does occur[3]. It should, however, be admitted that these two situations are not wholly comparable, as local lesions are formed within 1–2 days after mechanical inoculation of an entire leaf surface, whilst in the case of MRDV, in-

Polyphenoloxidase activity[a] in healthy and MRDV-infected maize plants at different ages

Days after inoculation	28 days		42 days		56 days	
	Optical density	% dry matter in plant	Optical density	% dry matter in plant	Optical density	% dry matter in plant
Non-infected	0.016	8.13	0.022	9.16	0.024	15.11
MRDV-infected	0.046	8.56	0.036	7.65	0.028	9.68

[a] Expressed as optical density, at 430 mμ, per 100 mg dry matter after 4 min reaction.

[1] This work was supported in part by a grant from the Agricultural Research Service of the United States Department of Agriculture, under Public Law 480.

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fection spreads from a very small number of insect feeding sites, and the amount of inoculum introduced into the plant is so minute that after 2 days any increase in enzymatic activity is still immeasurably low.

The hitherto accepted view, though with some reservations, that cereal plants do not contain polyphenoloxidases, and that the activity which has nevertheless been recorded in such plants is attributable to fungus spores or mycelia[2], appears to be an overstatement in the light of the present results.

Zusammenfassung. Der Wirkungsgrad der Polyphenoloxydase gesunder Maispflanzen blieb während der Gesamtdauer ihres Wachstums praktisch unverändert. Er verdreifachte sich hingegen in jungen Pflanzen, die mit dem Virus der Rauhverzwergung des Maises systematisch infiziert wurden.

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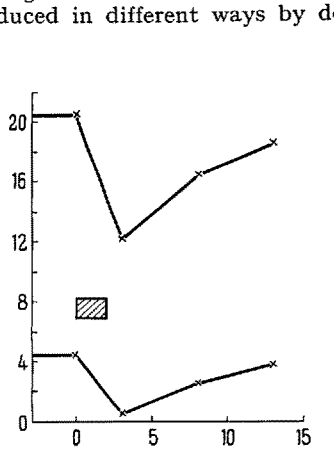
Action of Acetylcholine on the Responsiveness of Effector Cells

The responsiveness of submaxillary gland cells to chemical stimuli increases gradually when the gland has been disconnected from the central nervous system by section of the chorda tympani[1] or treatment with ganglion blocking agents[2]. Lack of secretory impulses seems to be responsible for this effect, for some supersensitivity can be brought about simply by reducing the inflow of secretory impulses to the salivary nuclei; this is attained by cutting afferent fibres of the secretory reflex[3]. This regulatory function of the secretory impulse on the responsiveness of the gland cell is exerted by acetylcholine, as shown by the facts that supersensitivity develops when the transmitter is prevented from being released by the nerve impulse, after administration of botulinum toxin[4], or from acting on the glandular cells because of prolonged treatment of the experimental animal with atropine or atropine-like drugs[5]. The supersensitivity created by these procedures, or by postganglionic parasympathetic denervation[6], is more pronounced than that following section of the preganglionic chorda fibres, suggesting that the responsiveness of the gland cells is dependent not only on transmitter released by the secretory impulse but also on acetylcholine leaking from the endings of the postganglionic parasympathetic fibres[7].

In the investigations described so far, supersensitivity has been produced in different ways by depriving the gland for a long time of some action of acetylcholine. In the present experiments the gland has, instead, been subjected to the action of acetylcholine in excess, and the consequential effect on the responsiveness studied. The sensitivity of the submaxillary gland of cats to adrenaline was examined using a method which permits repeated observations at intervals of some days[8]. By injecting eserine sulphate subcutaneously for two days (0.5 mg/kg in the morning and 1.0 mg/kg in the evening), the gland was exposed to increased concentrations of endogenous acetylcholine. 24 h after the last injection the responsiveness of the gland was estimated, and again repeatedly during the following days. Eserine treatment was found to lower the sensitivity of the gland, as shown in the Figure. This was the case in a normally innervated gland, and also in a gland previously decentralized by section of the chorda. When the treatment with eserine was discontinued, the sensitivity gradually rose again.



Responses (drops of saliva as ordinates) of the submaxillary glands of a cat to 5 μg/kg adrenaline, given intracardially on four different occasions (abscissa: time in days). Lower line: normal gland. Upper line: contralateral gland, decentralized three weeks earlier by section of the chorda-lingual nerve. The rectangle demonstrates the time of treatment with eserine, as described in the text.

The acetylcholine preserved by the eserine probably originated from the gland, leaking from the postganglionic endings and, in the case of the normal gland, also liberated by the secretory impulse. It cannot be excluded, however, that in the presence of eserine some acetylcholine reached the gland from extraglandular sources by way of the blood stream.

The experiments support the view that the concentration of acetylcholine in contact with the effector cells determines the responsiveness of these cells; even the so-called 'normal' level of sensitivity is variable and dependent on the amounts of acetylcholine acting on the cells.

Zusammenfassung. Bei der Katze wird die Empfindlichkeit der Submaxillarisdrüse gegenüber chemischen Reizen verringert, wenn während zwei Tagen subkutane Eserininjektionen verabreicht werden. Damit ist bewiesen, dass das Empfindlichkeitsniveau der Drüse von der Acetylcholinkonzentration abhängig ist.

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