und 3). Die Bestimmung des Sulfatschwefelgehaltes zeigte eine deutliche, konzentrationsabhängige Zunahme des Sulfatgehaltes (Figur 3, Tabelle).

Diskussion. Wachstumsuntersuchungen bei *Lemna minor L.* haben ergeben, dass bis zu Konzentrationen von 0,3 ppm SO_2 in der Begasungsluft keine Wachstumshemmung eintritt[4]. Die Toxizitätsgrenze für diese Pflanze liegt bei 0,6 ppm[4]. Bei dieser Konzentration ist das Wachstum zunächst gehemmt; die Organismen passen sich jedoch innert 4 Wochen den veränderten Bedingungen an und zeigen gegenüber der Kontrolle keine Wachstumshemmung mehr[7]. Die vorliegenden Resultate und die zugehörigen Kontrollexperimente[4,7] lassen den Schluss zu, dass die beobachtete Hemmung der Sulfataufnahme direkt auf das der Begasungsluft zugemischte SO_2 zurückzuführen ist. Diese Tatsache deckt sich mit den Ergebnissen von BRUNOLD[8], welcher denselben Effekt mit H_2S beobachtete. Bei nachgewiesener, gleichbleibender Wachstumsrate und gleichem oder höherem Sulfatgehalt muss also der Schwefelbedarf der Organismen zum Teil aus der Gasphase gedeckt werden. Dieser Anteil kann aus dem zweiten kinetischen Experiment (Figur 3) als auch aus den statistischen Experimenten berechnet werden und beträgt in beiden Fällen um 45% des gesamten aufgenommenen Schwefels. Dabei ist die zusätzlich aufgenommene Schwefelmenge, welche durch den erhöhten Sulfatgehalt erwiesen ist, nicht berücksichtigt. Diese Befunde sind insofern von ökologischer Bedeutung, als eine erhebliche entgiftende Wirkung der Vegetation auf die Luftverunreinigung mit SO_2 nicht übersehen werden darf[9]. Andererseits ist diese Tatsache auch von wirtschaftlicher Bedeutung, da in Gebieten mit Schwefeldefizit im Boden eine Ertragssteigerung durch eine gasförmige Schwefelzufuhr nicht ausgeschlossen werden kann. Allerdings dürfen dabei nur geringe SO_2-Konzentrationen erreicht werden, da einerseits die toxische Wirkung des SO_2 in Verbindung mit anderen Schadgasen multiplikativ erhöht wird[10], andererseits die absoluten Grenzwerte der SO_2-Toleranz für verschiedene Pflanzen weit unter jenen der Lemnen liegen können.

Sulfatschwefelgehalt SO_2-begaster Lemnen. Mittelwerte und Standarabweichung 6 paralleler Proben

Kontrolle	1,33	µg S/mg TG ± 0,253
0,13 ppm SO_2	1,24	µg S/mg TG ± 0,211
0,3 ppm SO_2	1,89	µg S/mg TG ± 0,312
0,6 ppm SO_2	2,28	µg S/mg TG ± 0,231

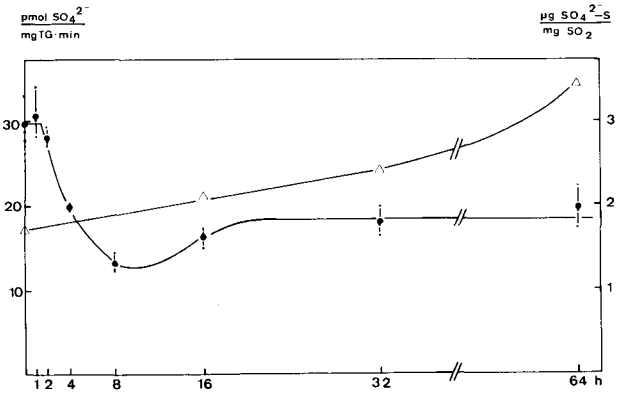

Fig. 3. Sulfataufnahmerate (●) und Sulfatschwefelgehalt (△) unter SO_2-Begasung. Begasung: 0,3 ppm SO_2 zu Versuchsbeginn. Sulfataufnahmerate, Mittelwerte dreier Proben.

Die Steigerung des Sulfatgehaltes unter SO_2-Einwirkung wurde schon andernorts beschrieben[11,12] und konnte von uns auch durch statistische Experimente gesichert werden (s. Tabelle). In guter Übereinstimmung mit unseren Resultaten steht auch der Befund von BRUNOLD[13], welcher zeigte, dass Sulfit vorerst zu Sulfat oxidiert wird und dann erst den normalen Metabolisierungsweg eingeht. Aus diesen Versuchen geht ebenfalls hervor, dass 40–50% des gesamten aufgenommenen Schwefels aus der Gasphase stammt.

Summary. The sulfate uptake of *Lemna minor* L. is very rapidly inhibited by SO_2-concentrations of 0.15, 0.32 and 0.61 ppm. The sulfate concentration in the plant material is increased. At least 40% of the total sulfur in the organisms origins from SO_2.

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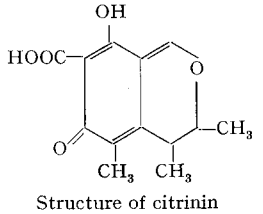
Citrinin, a Phytotoxin?

Citrinin is a benzopyran compound produced by *Penicillium citrinum*, whose occurrence in soil is known[1]. A few other soil-borne fungi also produce this yellow pigment, citrinin. Its toxicity to animals has been well studied[2] and its effect on seed germination and composition has been reported[3]. The present note is a preliminary report of a study on the possibility of citrinin being toxic to plants.



Structure of citrinin

In our studies pure citrinin crystals were isolated from cultures of *Penicillium citrinum* or *Aspergillus candidus*[2]. Solutions were prepared by dissolving the crystals in alkaline, pH 8.5, and then adjusting to pH 7.0. Control

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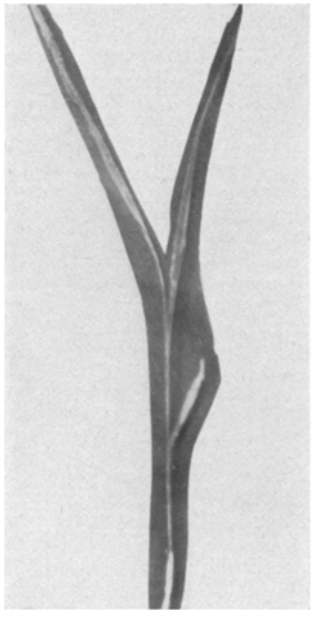
System	Concentration of citrinin			Wilting observed
	10^{-3} *M*	10^{-4} *M*	10^{-5} *M*	
Bean				
Dolichos lab lab	+++	++	+	Leaf
Phaseolus vulgaris	++	++	+	flaccidity;
Phaseolus mungo Co-1	+++	++	+	peripheral necrosis;
Cotton (*Gossypium arboreum*)				vein clearance;
K_6	++	++	+	finally
0320-1	++	++	+	total wilting
Sorghum				
Sorghum vulgare Co-20	+++	++	+	yellowing effect at 10^{-5} *M* concentration[a]

+++, Early wilt (after 2 h); ++, moderate (after 6 h); +, slow (after 24 h). [a]In all 3 bean species; but not in others.

was distilled water with pH adjusted to 7.0. 2 ml of solution (experimental/control) was taken in a sample tube providing enough depth for each plant kept in one such tube.

Three types of bean plants (*Dolichos lab lab*, *Phaseolus vulgaris* and *Phaseolus mungo* Co-1), 2 types of cotton (*Gossypium arboreum* K_6 and 0320-1) and 1 sorghum (*Sorghum vulgare* Co-20) were tested. For the in vitro experiments involving whole plants, they were grown in sterile soil and whole plants (with intact root system) were carefully removed after 7–8 days, washed cautiously in water to remove the adhering soil particles and immediately placed in their respective solution. Symptoms of wilting were observed.



Autoradiographic picture of a sorghum plant provided in vitro with ^{14}C-citrinin solution. After development, the X-ray film was covered, leaving a small margin around the image of the plant, and then exposed for print. ^{14}C-citrinin was prepared (specific activity 1.9×10^6 dpm/mmole) as described by RODIG et al.[6]. Preparation of citrinin solution (10^{-5} *M*) and other details were as given for wilting experiment (see text); after 3 h the plant was taken for autoradiography.

Three different concentrations of citrinin were tried, and the Table shows the data obtained. It is interesting to note that citrinin, which is an animal toxin, possesses a wilting effect on plants, as can be seen in the Table. Another experiment with cotton grown in *Penicillium citrinium*-infected soil and citrinin-supplemented soil reveals inhibited seed germination and stunted growth. There had been attempts to relate fungal toxicity, both in animals and plants[4]. However, this being the first report, before going ahead one has to answer fully: Is citrinin really a phytotoxin?

Here it is worthwhile mentioning some observations in this field. The autoradiographic picture (Figure) shows ^{14}C-citrinin distributed much in the stem near the root and in the leaves, more so in the primary ones. In the case of cotton and bean, accumulation of radioactivity in the petioles was significant. This might prompt one to think of both physiological and biochemical mechanisms involved in the production of citrinin-induced wilt. Citrinin may bind with the cellular components present along the vascular setup, and possibly in the leaves also, and thereby affect the osmotic balance and translocation. This seems a possibility since citrinin is known to bind with pure bovine serum albumin and human serum albumin[2].

Initial experiments with citrinin-treated leaves of sorghum and bean plants did not show any change in the ATPase activity, when compared with the control. Similarly, incubation of bean leaf cells with citrinin did not produce altered respiratory/photosynthetic activity. There is no clue from these preliminary experiments as to

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any biochemical action attributable to citrinin. However, it is necessary to note that citrinin causes a definite inhibition of both respiration and photosynthesis in the blue green alga, *Anacystis nidulans*[5].

To sum up, there seems to be a positive indication that citrinin is a phytotoxin; the details are yet to be worked out.

Summary. Observations from preliminary experiments to discover the phytotoxicity, if any, of the fungal metabolite, citrinin, are presented. There seems to be a positive indication, warranting further investigation.

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Inhibitory Effect of β-Hydroxyglutamic Acid on a Molluscan Giant Neurone

Hayashi[1] reported in 1959 that β-hydroxy glutamic acid caused convulsions when injected into the cerebrospinal fluid or when applied to the motor cortex of mammals. At the cellular level, Curtis and Watkins[2] demonstrated the excitatory effect of this substance on spinal neurones, but its effect was weaker than that of glutamic acid.

We observed in the present study that erythro-β-hydroxy-L-glutamic acid (erythro-L-BHGA) had an inhibitory effect on the electrical activity of a spontaneously firing giant neurone, periodically oscillating neurone (PON) identified in the subesophageal ganglia of an African giant snail (*Achatina fulica* Férussac). We observed also some decrease of the neuromembrane electrical resistance under this substance. This neurone was excited by 5-hydroxytryptamine and dopamine, inhibited by L-homocysteic acid and L-homocysteine sulfinic acid, and not affected by acetylcholine, glutamic acid or glycine[3–6].

One or two glass micropipettes filled with 2 *M* potassium acetate were implanted in the neurone. The biopotential was recorded by a pen-writing galvanometer, and the spike discharges were recorded by a spike counter. As an indicator of the neuromembrane resistance, the current-voltage relationships (I–V curve) of the neuromembrane were determined by applying a triangular current (hyperpolarizing, depolarizing and hyperpolarizing) into the soma via the implanted micropipette. In this circuit, a resistor of 70 MΩ was connected in series. Substances to be examined were applied to the ganglia in 2 ways: the bath application and the microdrop application. In the former, we applied to the ganglia the substances dissolved in the physiological solution of the snail[7]. In the latter, a micropipette was filled with the substance to be examined; a microdrop of the solution (100 μm in diameter) was made in the open air at the tip of the micropipette by oil pressure; and this microdrop was placed on the surface of the examined neurone. We avoided the iontophoretic application because of its current effect[8].

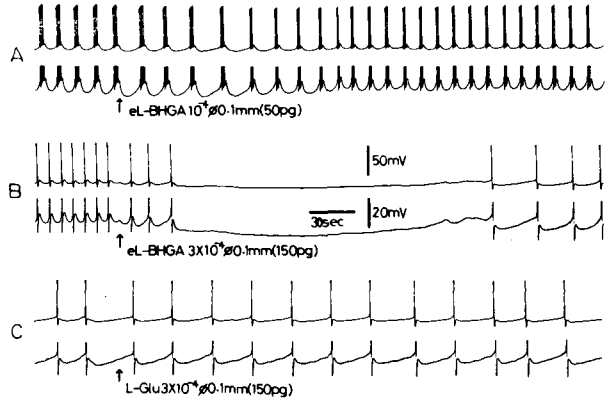

Fig. 1. Inhibitory effect of erythro-β-hydroxy-L-glutamic acid (eL-BHGA, microdrop application) on the electrical activity of the periodically oscillating neurone (PON). The upper traces of A, B, C, are the full-spike recordings of the pen-writing galvanometer. The lower traces of A, B, C, are the recordings in high amplification of the same biopotentials as the upper traces. (The spike peaks have been cut off by an electronic voltage clipper.) In A), a microdrop of eL-BHGA solution (100 μm in diameter) at 10^{-4} g/ml (50 pg) was applied on the surface of the examined neurone (arrow). In B), a microdrop of the same substance (the same diameter) at 3×10^{-4} (150 pg) was applied (arrow). In C), a microdrop of L-glutamic acid (150 pg) was applied (arrow). Note that a microdrop of even 50 pg of eL-BHGA produced some hyperpolarization of the PON membrane, inspite of the ineffectiveness of L-glutamic acid (150 pg) on the same neurone.

The bath application of erythro-L-BHGA (donated by Ono Pharmaceutical Co. Ltd., Osaka; Anal. C 36.56%, H 5.65%, N 8.57%, Calc. for $C_5H_9O_5N$ C 38.81%, H 5.56%, N 8.59%, $[\alpha]_D^{20}$ + 31.91°(C = 1.99 in 20% HCl), confirmed by NMR and IR) in a concentration of $10^{-6} \sim 3\times10^{-6}$ g/ml hyperpolarized the PON membrane and reduced the number of spontaneous spike discharges. Both erythro-D-BHGA and threo-D,L-BHGA also showed some inhibitory effect on the PON. But the effect of the latter two substances was much weaker than erythro-L-BHGA. The PON was completely insensitive to L- and D-glutamic acid, L-aspartic acid, glycine and β-alanine.

Figure 1 shows the biopotential change of the PON caused by the microdrop application of erythro-L-BHGA. A slight hyperpolarization of the PON membrane was produced by the application of 50 pg of this substance dissolved in the microdrop (Figure 1, A). The application of 150 pg erythro-L-BHGA produced a strong hyperpolarization, and no spike discharges of the PON were

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