cated. If we consider the behavior on MEA at 25°C as normal, the following instabilities can be stated.

Diploid homothallic cells (either homozygous or heterozygous for the h^{90} allele) normally sporulate directly (azygotic asci). On YEA at 30°C they also copulate frequently. In the latter case a further ambivalence is obvious: the nuclei either fuse (zygotic asci with four large spores, see *6*) or do not fuse but undergo separate meioses (twin meiosis, zygotic asci with eight haploid spores). Thus two conclusions can be drawn for homothallic diploid cells: although having a nucleus able to perform meiosis, the cells still have a tendency for copulation. Also, their nuclei still have an ability to fuse. In about 50 percent of the zygotes karyogamy occurs, whereas the rest of the copulations result in twin meiosis.

The diploid cells homozygous for incompatible mating-type alleles ($h+/h+$ or $h-/h-$) are not able to sporulate but can copulate with cells of compatible mating type. Normally asci with four diploid spores are formed (*2, 6*). However, on YEA at 30°C twin meioses also occur frequently (see *12*). The occurrence of twin meiosis in crosses with $h+/h+$ and $h-/h-$ cells demonstrates an interesting complementation mechanism since their nuclei individually do not have the complete genetic information for meiosis. In each of the crosses of types II to IV, one of the diploid nuclei involved in the twin meioses was able to perform meiosis. In these cases, it is therefore sufficient to assume that due to the diploid homothallic nucleus all substances necessary for meiosis are present in the common cytoplasm, and that these substances enable the $h+/h+$ or $h-/h-$ nucleus to undergo meiosis too. In the cross of type V, however, neither nucleus was capable of independent meiosis. Here one has to conclude that complementing gene products are formed by the $h+/h+$ and $h-/h-$ nuclei, and that these substances, when combined, enable the nuclei to undergo meiosis regardless of whether they are fused or not.

The latter situation is surprising: after copulation of the diploid cells ($h+/h+ \times h-/h-$), their nuclei have a tendency to fuse, but are obviously producing at the same time the (incomplete) gene products necessary for meiosis. Whether twin meiosis or karyogamy will occur seems to depend merely on whether the "meiotic" gene products complement each other to a sufficient level before the nuclei have fused.

The observed ambivalences might offer a possibility of evaluating in further experiments the regulatory mechanisms involved in copulation, karyogamy, and meiosis.

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2. ———, *Cold Spring Harbor Symp. Quant. Biol.* **23**, 161 (1958).
3. The three mating types of *S. pombe* are determined by a short region (1.1 map units) on the genetic map (*2*). Whether this region consists of one or more cistrons cannot be decided at present. In the present report the terms mating-type "alleles" and "locus" are used for simplicity.
4. "Homothallic" means that the descendants of a single cell are compatible with each other (copulation), whereas the descendants of a heterothallic cell are incompatible with each other but can copulate with cells of a compatible mating type.
5. For the selection of diplonts (*2*) strains with complementary growth requirements are mated, and young zygotes are transferred to minimal agar. A small number of the zygotes grow up to diploid colonies. In $h+/h-$ diplonts nonsporulating cells ($h+/h+$ or $h-/h-$) occur by mitotic crossing over between the centromere and the mating-type locus.
6. U. Leupold, *Compt. Rend. Lab. Carlsberg, Sér. Physiol.* **26**, 221 (1956).
7. ———, *Arch. Julius Klaus-Stift.* **30**, 506 (1955). YEA, yeast-extract agar (0.5 percent Bacto-yeast extract, 3 percent glucose, 2 percent agar); MEA, malt-extract agar (3 percent Bacto-malt extract, 2 percent agar).
8. Zygotic asci with only two or three large spores also occur rarely. A few of the six-spored asci have two large spores and four of normal size. Leupold (*6*) has already observed zygotic asci with seven to eight spores in a homothallic diploid strain. They occurred only rarely under his experimental conditions (beer wort, 25°C), and he did not carry out a genetic analysis of these asci. In agreement with Leupold's observation I found that zygotic asci (especially with six to eight spores) are much less frequently formed on MEA at 25°C than on YEA at 30°C.
9. In the postulate of "brachymeiosis" it was assumed that a tetraploid nucleus is reduced to eight haploid nuclei in three consecutive meiotic divisions. For references see E. Gäumann, *Die Pilze* (Birkhäuser, Basel and Stuttgart, ed. 2, 1964), p. 198.
10. Notation: *ura-1, lys-2, ade-6*: genes involved in the synthesis of uracil, lysine, and adenine, respectively; *M216, M210*, and *L702* are nonidentical *ade-6* mutations; *let*: lethal factor.
11. H. Gutz, *Habilitationsschrift* (Technische Universität, Berlin, 1963); U. Leupold and H. Gutz, *Proc. Intern. Congr. Genet. 11th* **2**, 31 (1964). The complementing *ade-6* alleles were used to select the diploid strains.
12. The frequencies of zygotic asci with four and six to eight spores, respectively, fluctuated from cross to cross. In the crosses with diploid homothallic strains (types I to IV) four-spored zygotic asci were approximately as frequent as six- to eight-spored. In the cross $h+/h+ \times h-/h-$ (type V) about 30 percent six- to eight-spored asci were found. When plated on MEA (25°C), the latter cross showed approximately 96 percent four-spored and only about 4 percent six- to eight-spored asci.
13. I thank Mrs. Lois Wilson for excellent technical assistance. Supported by NSF grant GB-4286 and NIH grant GM 13234.

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Boron in Plants: A Biochemical Role

Abstract. *Boron, as borate, appears to have a role in partitioning metabolism between the glycolytic and pentose-shunt pathways. This effect results from the association of borate with 6-phosphogluconic acid, forming a virtual substrate that inhibits the action of 6-phosphogluconate dehydrogenase. In the absence of borate, the inhibition of the enzyme is released, and excess phenolic acids are formed. These acids also associate strongly with borate and thus develop an autocatalytic system for production of excess phenolic acids which cause necrosis of tissue and eventual death of the plant.*

A biochemical syndrome of boron deficiency in plants (*1, 2*) is the accumulation of phenolic acids; their excessive concentration appears to be the immediate cause of necrosis and ultimate death from this nutritional deficiency (*1*). One may therefore presume that boron plays a role in the regulation of phenol synthesis, either directly or indirectly—for example, by control of substrate permeability through membranes, or by interaction with enzymes of the glycolytic or pentose-shunt pathway, or of both. The metabolism of

Table 1. Radioactivities of selected compounds from B+ and B— sunflower fed $^{14}CO_2$ for 30 minutes under light.

Compound	Time after feeding with $^{14}CO_2$ (hr)			
	0 (count/min)		30 (count/min)	
	B+	B—	B+	B—
Citric acid	614 ± 11	421 ± 10	54,010 ± 73	16,620 ± 41
Glyoxylic acid			19,540 ± 44	38,462 ± 62
Phenylalanine	633 ± 12	576 ± 10	10,950 ± 33	25,080 ± 50

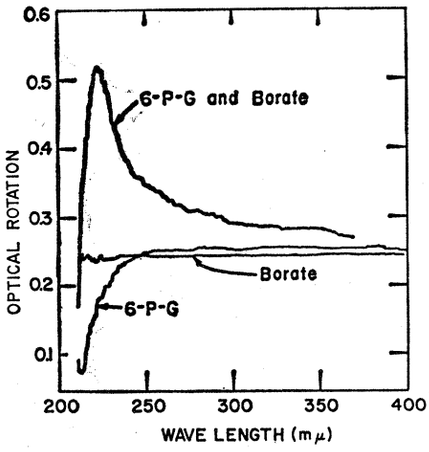

Fig. 1. Relative optical rotatory dispersion of borate ($2.0 \times 10^{-1}M$) and 6-phosphogluconate (6-P-G) ($2.5 \times 10^{-3}M$), and of their mutual complex. The apparent optical activity of borate was the result of the strain in the vessel, the inherent value being zero. As a consequence, the dispersions were those in fact observed, the proper values of 6-P-G in the region 200 to 250 nm being negative, and those of the complex from 220 nm onward being positive. The solutions were aqueous, *p*H 7.8.

the latter is inferred from the knowledge that phenolic acids arise from 3-deoxy-D-arabinoheptulonic acid-7-phosphate, which in turn is formed from the condensation of erythrose-4-phosphate (from the pentose shunt) and phosphoenol pyruvate (from glycolysis).

The complexing of borate with sugars has long been known, and has provided the basis for earlier suggestions regarding its metabolic role (*3*). However, association constants of borate with α-hydroxy acids (for example, citric acid) and phenolic acids are equal to those of the sugars or greater by orders of magnitude [as is reflected in conductivity data (*5*)] and are thus expected to be more prominent as regulators. We have tested this hypothesis on 6-phosphogluconate, an initial substrate of the pentose shunt, and found the result compatible with a model of control by borate of formation of the virtual substrate, 6-phosphogluconate/borate. Kinetics suggests that this complex combines with the enzyme 6-phosphogluconate dehydrogenase, inhibiting the oxidative decarboxylation of 6-phosphogluconate. In the absence of boron, and the corresponding absence of the complex, the enzyme operates at greater capacity, providing additional erythrose-4-phosphate for increased synthesis of phenol.

Borate thus acts as a modulator, determining the level of activity of the pentose shunt. In plants in which the product of the pentose shunt includes phenols that may also complex with borate, the borate available for complexing with 6-phosphogluconate is decreased still further; consequently this inhibition by borate of the pentose shunt is further diminished, and autocatalytic rates of phenol formation ensue.

The general outlines of this thesis may be indicated by (i) physical demonstration of the existence of the 6-phosphogluconate borate complex, (ii) inhibition of the 6-phosphogluconate dehydrogenase by the complex, and (iii) the metabolic pattern in B+ and B− sunflower leaves following incorporation of $^{14}CO_2$ by way of photosynthesis.

The formation of the 6-phosphogluconate/borate complex (i) may be demonstrated by formation of a unique optical rotatory dispersion curve (Fig. 1). The sign of the Cotton effect of 6-phosphogluconate is reversed when borate is added, and the extent of complexing may be calculated by solution of a pair of simultaneous equations involving the [α]'s of the 6-phosphogluconate and the complex.

The effect of this complex on the action of 6-phosphogluconate dehydrogenase (ii) may be demonstrated by the use of the labeled substrate ^{14}C-6-phosphogluconate, synthesized from ^{14}C-glucose-6-phosphate by reaction with nicotinamide adenine dinucleotide phosphate and glucose-6-phosphate dehydrogenase. The latter substrate, in turn, was prepared from ^{14}C-glucose by reaction with adenosine triphosphate and hexokinase.

Autoradiography of the chromatogram of the action of 6-phosphogluconate dehydrogenase on the radiomeric substrate results in only a single new spot, presumed to be ribulose-5-phosphate. Kinetics of the rate of reaction of the enzyme with substrate show 33-percent inhibition by $6 \times 10^{-4}M$ borate and 60-percent by $3 \times 10^{-3}M$ borate (Fig. 2). These concentrations of borate are not considered to be excessive, being commensurate with the level of leaf borate determined analytically.

Both B+ and B− plants (iii) were allowed to photosynthesize in $^{14}CO_2$ for 30 minutes; they were then analyzed immediately for soluble constituents, and again after 30 hours in darkness. The most conspicuous differences (Table 1) may be interpreted as follows: The difference between activities at 0 and at 30 hours reflects the dependence of the respiratory pathways upon reserve substances such as starch

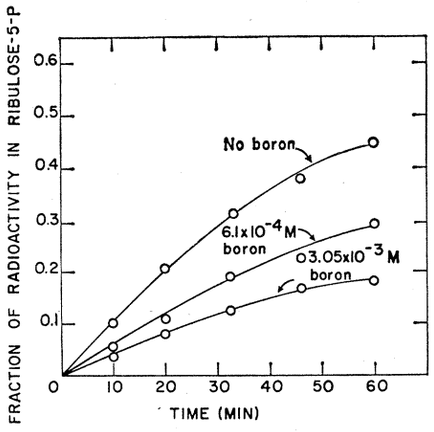

Fig. 2. The influence of borate on the activity of 6-phosphogluconate dehydrogenase with its substrate 6-phosphogluconate. The composition of the reaction mixture was: nicotinamide adenine dinucleotide phosphate, 0.5 μmole; 6-phosphogluconate, 0.25 μmole; ^{14}C-6-phosphogluconate, 1.1 μc, 0.25 μmole; Hepes buffer (Calbiochem), 25 μmole, *p*H 7.55; $Ca(NO_3)_2$, 15 μmole; and enzyme, 0.087 mg. The amounts of borate resulting in the concentrations noted for the middle and bottom curves were 1 to 5 μmole, respectively. The volume of the reaction mixture was 1.63 ml; *p*H 7.7. The product was separated by paper electrophoresis.

and sucrose; these become available only after the indigenous (nonradioactive) substrate is used. The increased amount of citric acid in B+ is then thought to arise either by inhibition of the aconitase reaction concomitant with the formation of the strong citrate-borate complex, or from inhibition of isocitrate dehydrogenase by virtue of the isocitrate-borate complex. We favor the latter alternative since it is analogous to the 6-phosphogluconate experiments described above, and because of the increase of glyoxylate in the B− plants. We reason that the isocitratase reaction becomes more prominent when the isocitrate dehydrogenase is inhibited.

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