The effect of plant growth regulators on strains of *Fusarium moniliforme* Cheld. [*Gibberella fujikuroi* (Saw.) Wr.].

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In studies on the biological activity and mutual interactions of plant growth regulators a useful subject material is provided by the fungus *Fusarium moniliforme* Sheld., which is the conidial state of *Gibberella fujikuroi* (Saw.) Wr., the fungus producing gibberellins. This is particularly well demonstrated in studies of Lang and collaborators on the mechanism of action of the growth retardants such as (2-chloroethyl)trimethylammonium chloride (CCC), 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methyl chloride (AMO 1618) and others of the growth of higher plants (Kende et al. 1963; Ninnemann et al. 1964; Baldew et al. 1965; Harada and Lang 1965; Paleg et al. 1965). From these studies and from those on the biogenesis of gibberellin molecules which have been conducted by numerous authors (Birch et al. 1959; Sander mann and Burns 1962; Cross et al. 1963; Hanson 1963; Cross, Galt and Hanson 1964; Dennis et al. 1964; Graebe et al. 1965) it appears that the biosynthesis of gibberellin in strains of *Fusarium moniliforme* Sheld. is probably analogous or very close to that in higher plants. Thus it appears promising to investigate the influence of several other plant growth regulators on the biosynthesis of gibberellin in the fungus *Fusarium moniliforme* Sheld. and to explain to what extent these regulators affect other intracellular biochemical processes taking place in the microorganism. In the studies the following plant growth regulators were employed: indole-3-acetic acid (IAA), kinetin, gibberellin A-3 (GA-3) as compounds stimulating plant growth and CCC, 2,3,5-triiodobenzoic acid (TIBA) and maleic hydrazide (MH) as compounds having a growth inhibiting effect on plants. GA-3 was included in the study since far there is a lack of information on the physiological role of this compound in the cells of *Fusarium moniliforme* Sheld. Apart from the influence of these compounds on the biosynthesis of gibberellin their effect was also studied on the respiratory processes of the cells, on the dry weight of the mycelium, on the pH of the culture medium, on the colour of the mycelium, on its utilization of sugars and the intracellular levels of the sugar fractions, on the utilization of phosphorous and on the dynamics of the intracellular phosphate metabolism. The studies were conducted on the two cultured strains, one of which produced gibberellins while the other was a mutant lacking the ability to synthesize them. The use of both these strains was necessary to establish the extent to which the biochemical effects of the applied growth regulators were related to processes of gibberellin biosynthesis and to the general intracellular metabolism.

MATERIALS AND METHODS

The experiments were conducted on two strains of the species *Fusarium moniliforme* Sheld. from the strain collection of the Institute of Antibiotics. They were strain *Fusarium moniliforme* A and strain *Fusarium moniliforme* var. *minus*. Gibberellins were synthesized by the strain *Fusarium moniliforme* A. One of the convenient characteristics of this strain is that when grown on the Raulin-Thom medium it produces mainly GA-3 except for small quantities of GA-4 and GA-7. The second strain did not produce any gibberellins. This was established with the help of chemically and biologically developed chromatograms of fermentation broths of this strain (Adamiec et al. 1962; Adamiec et al. 1964). The mycelium of the strain *Fusarium moniliforme* A when grown on a synthetic medium in a submerged condition had a raspberryred colour and the mycelium of the strain *Fusarium moniliforme* var. *minus* had a pale grey colour.

The strains were cultured in flasks on a shaker. The experiments were conducted in round, flat-bottomed flasks, 750 ml in capacity with 100 ml of medium per flask. The flasks were vibrated on a reciprocal shaker at a rate of 120 shakes per min. Several experiments were conducted in Erlenmeyer flasks, 500 ml in capacity with 50 ml of medium per flasks. These flasks were vibrated on a rotary shaker at a rate of 250 r.p.m. The shakers were localized in rooms with temperature thermostatically controlled to a mean temperature of 28°C. The type of shaker used in a particular experiment is presented in a short description together with results of individual experiments.

The strains were stored on slants with potato agar. Their culture consisted of two phases, the first one involving the growing of a vegetative mycelium from spores washed off the agar slants and the other comprising an inoculation of the experimental flasks with the vegetative mycelium. Both the introductory and the experimental cultures were grown on a synthetic medium. The agar medium was prepared according to Köhler (1956) and the Raulin-Thom medium according to Kuczajewa et al. (1961).

The plant growth regulators and other substances used in the investigation were: IAA — a BDH Ltd. preparation, 96% of pure constituent; kinetin — a preparation from Dr Th. Schuchardt's firm, chromatographically pure; GA-3 the Gibbercol preparation of the Kutnowskie Zakłady Farmaceutyczne, Kutno, iso-gibberellin A-3 (iso-GA-3) obtained according to the method given by Cross, Grove and Morrison (1961); CCC — a preparation synthesized in the General Chemistry Laboratory of the Warsaw Medical Academy, chromatographically pure; TIBA — a BDH Ltd. preparation; MH- a preparation synthesized in the Institute of Organic Synthesis in Warsaw; choline chloride — an E. Merck preparation.

The substances were applied to the experimental flasks in the form of sterile solutions, sterilized by filtration through an inert Seitz bacterial filter. The solutions were applied in volumes of 1–2 ml. This volume contained the studied substance in such a quantity as to give after dilution with the necessary volume of the fermentation medium a concentration required for the experiment. GA-3, iso-GA-3, IAA and

TIBA were diluted in a 0.05 M phosphate buffer with a pH of 6.8. CCC and choline chloride were dissolved in distilled water. Kinetin and MH were dissolved in a small quantity of 0.1N HCl and then neutralised before filtration with NaOH to a pH of 3.5.

For the chromatographic separation of gibberellins the butanol, ammonia, water (4:1:5) solvent was used (according to Brian and Grove 1958) as well as the solvent developed by Adamiec et al. (1962) consisting of toluene, acetic acid, water (9:1:10). The amount of GA-3 in the fermentation medium was measured colorimetrically (Wierzchowska and Wierzchowski 1962), and fluorimetrically (Theriault et al. 1961). CCC and choline chloride were studied chromatographically (Mayr and Paxton 1962). IAA in the fermentation medium was measured with the help of thin-layer chromatography on silica gel G manufactured by E. Merck (Stahl 1962). The solvent was chloroform, amylacetate, acetic acid (5:5:0.5) and the developing was done with Ehrlich's reagent (Diamantstein and Erhart 1961).

The respiratory functions of the mycelium were studied with the help of a Wartburg apparatus. The experimental procedure was based on the method described by Umbreit et al. (1964). The experiments were performed on a washed mycelium. A homogenous mycelial suspension was washed on a Schott funnel 3G3 with a 0.05M phosphate buffer with the pH adjusted to the needs of the particular experiment. The washed medium was suspended in the phosphate buffer in a volume equivalent to that of the original mycelial suspension.

The rate of oxygen diffusion was measured by the sulphite method with Schultz and Gaden (1956) modification. Measurements were made in Erlenmeyer flasks of 500 ml capacity filled with varying amounts of fluid. The flasks were vibrated on the rotary shaker with 250 rpm and 50 mm span. The experiments were conducted in conditions equivalent to those employed in the fermentation process.

The pH values in the filtrates of the fermentation medium were measured with a pH-meter. Dry weight of the mycelium was measured for 5 ml volumes of the fermentation medium. The fermentation medium was filtrated on a Schott funnel 3G3, washed twice with 5 ml of cooled distilled water and transferred with the minimal quantity of distilled water to a weighed weighing dish. The dish was placed immediately in a drying oven at a temperature of 90°, and when the excess water had evaporated the mycelium was further dried at 95° until the weight of the material decreased no more.

Phosphates were measured in the filtrate of the fermentation medium and in the mycelium itself. In the filtrate total phosphorous was measured since as a result of the action of potassium phosphate with ammonium tartrate of the medium there is an apparent, not always uniform, lowering of the inorganic phosphorous compounds in the medium. To differentiate the phosphorus compounds in the mycelium three successive extractions of the mycelium were performed with: 10% trichloroacetic acid (TCA), a mixture of ethanol and ether (3:1) and 1N sulphuric acid. The pattern of separation of the intracellular phosphorous compounds into various fractions was based on the experimental procedures of Kaltwasser (1962).

The ashing of the samples for the measurement of total phosphorous and the determination of inorganic phosphorous was made according to the methods of Gerlach and Deuticke (1963).

Sugars in the filtrates of the fermentation medium and in the mycelium were measured by the anthron method according to Stahl and Kaltenbach (1961). Sugars were extracted from the mycelium by the method of Chester (1963). To avoid a separate extraction of sugars from the mycelium they were determined in the same fractions obtained during fractionation for phosphorous compounds. Sugars in the extract with TCA were described as fraction I, and those in the extract with 1N hot sulphuric acid as fraction II.

RESULTS OF THE EXPERIMENTS

The biosynthesis of gibberellin

The concentrations of growth regulators were adjusted depending on the physiological effects observed in the growth of the mycelium. Only such concentrations were used which do not cause a noticeable inhibition of the growth of the mycelium. Kinetin and MH were applied at lower concentrations in view of their weak solubility at pH values close to neutral.

Changes in the level of gibberellin in the fermentation medium as a result of the growth regulators applied were estimated taking aseptically at 24 hour intervals small quantities of the fermentation medium in which the level of GA-3 was then measured colorimetrically and fluorimetrically. Apart from the quantitative estimation of GA-3, in each sample of the fermentation medium a check was made chromatographically on the qualitative composition of the gibberellins. From a large number of samples separated on the chromatograms it appeared that GA-4 and GA-7 occurred in the fermentation medium only in small quantities, and that the relative proportions of the gibberellins did not undergo substantial changes as a result of the growth regulators used. In fig. 1 changes in the levels of GA-3 produced by *Fusarium moniliforme* A under the influence of growth regulators is shown. It can be seen that the biosynthesis of gibberellin is slightly stimulated by IAA and kinetin and substantially inhibited by CCC and TIBA. The application of MH and GA-3 did not produce noticeable changes.

In the experiment with GA-3 the medium was supplied with the studied metabolic product of the strain under investigation. Fig. 1 demonstrates that the level of GA-3 in the 24th hour of fermentation is the same as at the onset. The determination of the level of GA-3 was made at few hour intervals in the period from 0-24th hour of fermentation and the GA-3 level found does not represent newly synthesized gibberellin but is the same compound which was supplied to the medium at the beginning of the fermentation. This is supported by the results obtained in experiments with the strain *Fusarium moniliforme* var. *minus* (fig. 1) and in the experiments with the strain *Fusarium moniliforme* A, in which the biosyntheses of gibberellin was

inhibited with the help of CCC (fig. 2). From these experiments it appears that the level of GA-3 artificially supplied to the fermentation medium remains practically unchanged until the end of the fermentation. Thus it is possible to evaluate the true level of the newly synthesized GA-3 in the experiment where this compound was added to the culture of the gibberellin producing strain. Subtracting from

Fig. 1. The influence of plant growth regulators on the biosynthesis of GA-3 in *Fusarium moniliforme* A and *Fusarium moniliforme* var. minus

The strains were cultured in flat-bottom flasks of 750 ml capacity. The flasks contained 100 ml of medium. Reciprocal shaker (120 shakes per min., span 80 mm) Temp. 28°C. Each variable was replicated in 4 flasks. The conc. of plant growth regulators in the medium were: GA-3 — 100, IAA — 100, kinetin — 50, CCC — 100, TIBA — 30, MH — 50 mcg/ml.

the level of GA-3 at different hours of fermentation the amount of GA-3 supplied originally to the medium the amount of newly synthesized GA-3 can be obtained. At 48 hours of fermentation this will be 72 mcg/ml, at 72 hrs — 138 mcg/ml, at 96 hrs — 196 mcg/ml and at 120 hrs — 206 mcg/ml. Comparing these amounts with those for the control (fig. 1), it can be seen that they are somewhat lower, particularly in the early phase of gibberellin biosynthesis.

In the chromatographic studies it was found that both in the culture of strain *Fusarium moniliforme* A and strain *Fusarium moniliforme* var. minus, as well as in the culture of strain *Fusarium moniliforme* A in the presence of CCC there is no observable decomposition of GA-3 to gibberellenic acid or other degradation products. In one series of trials GA-3 was added to the culture of other microorganisms belonging to the streptomycetes and bacteria, namely *Streptomyces aureofaciens*, *Streptomyces erythreus*, *Streptomyces rimosus*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. In the culture of these strains it was also not possible to demonstrate any noticeable reduction in the level of GA-3 applied to the medium,

and on the chromatograms from the fermentation media of these cultures no degradation products of GA-3 were found.

In the studies with *Fusarium moniliforme* A and *Fusarium moniliforme* var. *minus* it was found that iso-GA-3 behaves in the medium and in relation to the biosynthesis of gibberellin in an analogous way to GA-3.

IAA has only a slight effect on the process of gibberellin biosynthesis by *Fusarium moniliforme* A. Towards the end of the fermentation period there is a slightly higher level of GA-3 produced in the presence of IAA than in the controls.

The effect of kinetin is more pronounced. Under the influence of this growth regulator already at the 24th hour of fermentation a noticeable amount of GA-3

Fig 2. The influence of the simultaneous action of GA-3 and CCC on the synthesis of gibberellins by the strain *Fusarium moniliforme* A.

Sterile solutions of both the growth regulators were added to the medium at the beginning of fermentation. Rotary shaker (250 rpm). Temp. 28°C. On the 120th hour of fermentation the level of gibberellins in the fermentation medium was estimated by fluorometry. The pH of the medium and the dry weight of the mycelium were also estimated. The qualitative composition of the gibberellins in the medium was controlled chromatographically. The figures on the graphs represent the concentration of GA-3 in mcg per ml of medium.

is produced. This is probably caused by a stimulation of the growth of the mycelium (compare the increase in dry weight of the mycelium in fig. 6).

The most pronounced effect on the biosynthesis of gibberellin is produced by CCC. The degree of inhibition of the biosynthesis of gibberellin by this compound is dependent on its concentration. Already a concentration of 0,1 mcg/ml inhibits the biosynthesis by a half in comparison with the control. Gibberellin biosynthesis can be inhibited by CCC at any time in the fermentation process. When CCC was applied to the medium at the 48th, 72nd or 96th hour of fermentation, at a time when the medium already contained a certain amount of gibberellin, then this level of gibberellin was maintained until the end of the fermentation process without undergoing any further changes. The inhibitive effect of CCC on the biosynthesis of gibberellin cannot be reduced by the application to the medium of GA-3. The absence of de-inhibition is independent of the relative concentrations of GA-3 and CCC, the time of application or the sequence of application of these two substances

to the medium. The results of an experiment demonstrating this are presented in fig. 2. CCC distinctly accumulates in the cells of the mycelium. When applying this compound to the medium at a concentration of 100 mcg/ml, on completion of the fermentation period chromatography of the fermentation medium revealed only small amounts of it on the chromatograms using concentrations of the order of 300 mcg/ml the chromatograms after development showed intensive spots corresponding to CCC throughout the fermentation period. During the chromatographic studies with CCC it was found that this compound reacts with sucrose and potassium phosphate of the medium giving as a result very diffuse spots on the chromatograms.

During the studies on the effect of CCC on the biosynthesis of GA-3 microscopic observations were made of the mycelium stained with Sudan Red. B. The mycelium

Fig. 3. The influence of aeration intensity on the the growth of *Fusarium moniliforme* strain A cultures and on the biosynthesis of GA-3

The strain was cultured in conical flasks of 500 ml capacity with the necks extended by glass tube 10 cm long and with a glassblown bubble with 4.5 cm diameter (which prevented the contamination of the corks by the fermentation medium when in large quantities). The culture was kept on a rotary shaker. GA-3 analysis was conducted colorimetrically in the 120th hour of fermentation. The rate of oxygen diffusion into the medium is presented in ml of O₂ per 100 ml of medium per hour; dry weight.

of *Fusarium moniliforme* strains contains up to the 96th hour of fermentation a large amount of granules staining readily with this reagent. These are lipid granules disappearing towards the end of fermentation. No changes have been found in the microscopic appearance of the mycelium as a result of treatment with CCC. Also no effect of CCC has been observed on the morphological appearance of the mycelium, its shape or texture.

In view of the structural similarity of CCC and choline the influence of the latter compound on the de-inhibition of gibberellin synthesis after inhibition with CCC was studied. To the medium CCC and choline chloride were applied simultaneously in varying relative concentrations, in different sequential order and at different times of the fermentation period. However not the slightest influence of choline chloride on the de-inhibition has been demonstrated.

Studies were also conducted on the influence of choline on the biosynthesis of gibberellin. Water solutions of choline chloride were applied in aseptic conditions,

in various concentrations and at various times, to the medium on which *Fusarium moniliforme* strain A was cultured. These studies have shown that with the help of choline it is possible to obtain marked stimulatory effects which are dependent on the intensity of aeration of the culture, that is on the rate of diffusion of oxygen to the medium. The rate of diffusion of oxygen to the medium was regulated by the volume of the medium in the fermentation flasks. The dependence of oxygen diffusion to the medium on the volume of liquid in the flasks was determined by the sulphite method. With the increase of volume in the flasks the rate of diffusion of oxygen

Fig. 4. The influence of choline on the biosynthesis of GA-3 by the strain *Fusarium moniliforme* A.

The strain was cultured in conical flasks of 500 ml capacity and with extended necks. The volume of the medium in the cultures was 150 ml. The culture was maintained on a rotary shaker. A sterile water solution of choline chloride was applied at 24 hour intervals. The level of GA-3 in the medium was estimated in the 144th hour of fermentation by the colorimetric method. The figures presented on the graphs in the figure indicate the time at which the choline chloride was applied to the fermentation medium

to the medium declines. With 250 ml of liquid in the flask the diffusion is so slow, that practically speaking it is not possible to determine by the sulphite method. In fig. 3 the dry weight of mycelium and the concentrations of gibberellins in the fermentation medium are presented in relation to the intensity of aeration, determined for the 120th hour of fermentation.

Parallel with the reduction in the rate of oxygen diffusion to the medium there is a decline in the biosynthesis of GA-3 and other gibberellins. When the flasks are filled with 250 ml of the medium the oxygen diffusion to the medium is practically nil and the biosynthesis of gibberellin also falls to zero.

When conducting experiments with choline in flasks with varying degree of aeration, that is filling the flasks with from 50 to 100 ml of medium as well as in conditions with poor aeration, that is when the flasks are filled with 250 ml of medium, it was found that choline has no marked influence on the biosynthesis of gibberellin. With moderate aeration, of the order of 23 ml O₂ per 100 ml of liquid, (filling the flasks with 150 ml of medium, corresponds to a rate of oxygen diffusion equal to 23 ml O₂/100 ml of liquid per hour) choline was found to stimulate markedly the biosynthesis of gibberellin. Results of this experiment are presented in figure 4. Choline was applied at different times during the fermentation period since it was thought likely that it might be used for other biosynthetic processes not related to

the process of gibberellin production if applied only in the early hours of the fermentation period. Choline was applied to the medium at times 0, 24, 48, and 72 hours from the onset of the fermentation. Concentrations of 10 and 50 mcg/ml were used. The amount of GA-3 was estimated in the 144th hour of the fermentation period. The control culture was common for all the trials. From the graphs in fig. 4 it can be seen that the stimulatory effect of choline increases with the passage of time of fermentation and reaches its maximum in the ultimate phase of the intensive growth of the microorganism. The influence of choline chloride on the bio-

Fig. 5

Fig. 6

Fig. 5. The influence of TIBA and GA-3 on the biosynthesis of gibberellins by the strain *Fusarium moniliforme* A.

The strain was cultured in conical flasks with 500 ml capacity, containing 50 ml. of the medium, and were kept on the rotary shaker. Sterile solutions of TIBA and GA-3 were applied at the onset of the fermentation period. The analysis of GA-3 was made in the 120th hour of the fermentation period by the colorimetric method. The figures on the graphs represent the amounts of GA-3 (mcg/ml) applied to the medium at the onset of fermentation.

Fig. 6. The increment in mycelial mass in the strains of *Fusarium moniliforme* A and *Fusarium moniliforme* var. *minus*. The amounts of plant growth regulators used in the experiment were the same as in fig. 1.

synthesis of gibberellin depends on its concentration. From the graphs it can also be seen that by application of a proper amount of choline chloride to a poorly aerated culture it is possible to obtain the same level of GA-3 production as in well aerated culture without choline. This effect is relatively well reproducible.

In view of the specific action of CCC on the biosynthesis of gibberellin several experiments were conducted applying CCC to the culture medium of streptomycetes producing antibiotics, the biosynthesis of which, according to the current biogenetic concepts, takes place similarly as gibberellin, from two carbon fragments. The influence of CCC on the biosynthesis of chlorotetracycline by *Streptomyces aureofaciens*, the biosynthesis of oxytetracycline by *Streptomyces rimosus*, and the biosynthesis of erythromycin by *Streptomyces erythreus* was investigated. In none of the experiments was any inhibitive effect of CCC on the biosynthesis of the aforementioned antibiotics observed and this, regardless of the compound's concentra-

tion used. It was also found that CCC has no inhibitive effect on the growth of streptomycetes.

The next plant growth regulator known to interfere with the process of gibberellin biosynthesis by strain A of *Fusarium moniliforme* is TIBA. The method of action of this compound is however different from that of CCC, since at higher concentrations of TIBA, of the order of 50 to 100 mcg/ml, there is a strong inhibition of the mycelium growth. At a concentration of 30 mcg/ml the mycelium develops similarly as in the control culture, while the inhibitive effect of the biosynthesis of GA-3 is still quite distinct. From the graph in fig. 1 it can be seen that under the influence of TIBA the level of GA-3 in the medium is almost three times as low as in the control culture. By increasing the level of TIBA in the medium it is possible to obtain a complete inhibition of the biosynthesis of gibberellin. The inhibitive effect of TIBA on the biosynthesis of gibberellin and at higher concentrations also on the growth of the mycelium cannot be reduced by the simultaneous addition of GA-3 to the medium. Results of this experiment are presented in fig. 5. Both compounds were added to the medium at the beginning of fermentation in various proportions and in different sequence. The final effects were always the same. The effect of inhibition on the process of gibberellin biosynthesis was always the same. The inhibitive effect on the biosynthesis of gibberellin took place regardless of the degree of aeration and composition of the medium.

From the graphs in fig. 5 it can also be seen that a low concentration of TIBA distinctly stimulates the biosynthesis of gibberellin. The amount of GA-3 in a culture containing TIBA at a concentration of 0,1 mcg/ml is after 120 hours of fermentation about 25 % higher than in the control culture. The range of concentrations at which TIBA stimulates gibberellin synthesis is very narrow — at a concentration of the order of 1 mcg/ml this effect no longer exists. The presence in the medium of GA-3 from the onset of fermentation reduces or completely abolishes the stimulatory effect of low TIBA concentrations. The stimulatory effect takes place only in cultures on a synthetic medium, regardless of the degree of their aeration. On a rich medium containing an extract from soaked maize this effect did not take place.

Maleic hydrazide has practically no effect on the biosynthesis of gibberellin. In experiments with this compound certain difficulties are created by its low solubility. It was supplied to the medium in weak solutions of brine or as a water suspension however in all situations the same result was obtained.

The respiratory function

Respiratory processes in the cells of *Fusarium moniliforme* strains play a particularity significant role in the processes of gibberellin biosynthesis. On the other hand growth phenomena are less dependent on oxygen reactions. This can be seen from fig. 3. Both the strains studied develop equally well in conditions of plentiful oxygen supply to the medium and when there is a very poor access of oxygen to the medium. The optimal aeration conditions for growth do not coincide with the optimal aeration for the biosynthesis of gibberellin. Having established the

respiratory functions of the mycelium of both strains with respect to various substrates, and the influence of the environment on the mycelial respiratory functions, a study was made of the influence of these compounds on the endogenous respiration, and on respiration in the presence of a carbon substrate. Sodium acetate was selected as the carbon substrate since it is well utilized by both strains. The experiments were conducted on a washed mycelium during the period from the 24th to the 144th hour of fermentation, adding the growth factor to Warburg cuvettes. Also the respiratory function was studied on a mycelium which developed on a medium with growth regulators from the onset of the fermentation period. Respiration of such a mycelium was studied directly and following the addition of a growth regulator to the Warburg cuvettes. The results were compared with those for the control culture. Results of these experiments are not presented numerically since in spite of numerous replications no influence whatsoever of the studied growth regulators on respiration has been observed, nor was there any effect on the oxidation of the carbon substrates.

The mycelial mass.

From the data on the control culture it was observed that the two strains differ distinctly in the dynamics of accumulation of the cellular mass and in the amount of dry weight at the time of maximal growth of the mycelium.

The influence of GA-3 on the increment of dry weight in the culture of *Fusarium moniliforme* A is to be noticed in the reduction of the rate of dry weight increment of the mycelium in the 24th hour of fermentation. In later hours of fermentation the dry weight acquires a level equal to that in the control culture and towards the end of the fermentation period it becomes greater than in the controls. The action of GA-3 on the dry weight of the mycelium of strain *minus* is analogous.

Kinetin causes a great increase in the increment of the mycelial mass at the beginning of fermentation. The maximal amount of dry weight of the mycelium for a specific volume of the fermentation broth occurs in the 72nd hour of fermentation as compared with the 96th hour for the control culture.

CCC act as similarly as kinetin in that it causes a large increase in the dry weight of the mycelium at the beginning of the fermentation period for both strains studied. In later hours of the fermentation period changes in dry weight of the mycelium have a different course for both strains.

In experiments with TIBA the strain A behaves similarly as in the control culture, however in the culture of the strain *F. moniliforme* var. *minus* the increment of dry weight in the initial phase of fermentation is somewhat inhibited after which there is an enhancement of mycelial growth which towards the end of the fermentation period gives a greater total weight than the control culture. Last of the studied growth regulators, MH, acts on the increment of dry weight in *Fusarium moniliforme* strain A in a similar manner to kinetin.

It appears therefore from the experimental results obtained that cells of *Fusarium moniliforme* strains react to the action of plant growth regulators also through a stimulation or inhibition of mycelial growth.

pH of the fermentation broth

Submerged cultures of *Fusarium moniliforme* strains are characterized by changing pH level of the medium during the culture's development. The course of pH fluctuations in the media for both strains studied is presented in fig. 7 both for the control cultures and for those with growth regulators. The two strains had different patterns of pH changes as seen in the control cultures, which indicates that they have spe-

Fig. 7. The influence of plant growth regulators on the pH of the fermentation medium in the strains *Fusarium moniliforme*.

cific differences in metabolic characters. However, it is sufficient to add GA-3 to the medium of the culture of strain *Fusarium moniliforme* var. *minus* for the change in pH level to run similarly as in the strain *Fusarium moniliforme* A.

Of the growth regulators studied a specific reaction on the pH changes has been obtained during fermentation as a result of application of TIBA and MH. Under the influence of these compounds pH declined and remained on a very low level until the end of fermentation.

The other growth regulators did not have any specific effects on the changes of pH in the fermentation broth.

Mycelial pigments

The strain *Fusarium moniliforme* A cultured on a synthetic medium of Raulin-Thom produces large quantities of a redish-brown pigment, which is partially exuded into the substratum. It has been found that the biosynthesis of this pigment can be almost completely inhibited by TIBA. The remaining growth regulators do not affect its biosynthesis in any way whatsoever. The degree of inhibition of the pigment's biosynthesis is dependent on the ammount of TIBA in the substratum. A distinct inhibitive effect is observable already at 40 mcg/ml. With an increase in TIBA level in the medium the mycelium acquires a pale-pink colour, and at a concentration of the order of 75–100 mcg/ml it is completely deprived of pigment and is pale-grey in colour. At these concentrations a marked inhibition of mycelial growth is also observable.

A similar change in mycelial pigmentation occurs also in the experiments with a varied rate of oxygen diffusion to the medium, when regulated by the amount of medium in the flask (fig. 3). When the flasks are filled with 200 ml of medium and the rate of oxygen diffusion into the medium is only about 3 ml/100 ml of medium per hour, the mycelium of strain *Fusarium moniliforme* A strains it pale-pink, and when the flasks are filled with 250 ml of medium, the rate of oxygen diffusion is no longer measurable, and the mycelium is completely lacking pigmentation. In this state the mycelium does not synthesize gibberellins, and therefore apart from the fact that its growth is not inhibited, it demonstrates a certain analogy to the situation where it is treated with higher concentrations of TIBA.

Sugar consumption and the intracellular sugar fractions

Sugar compounds were estimated in the mycelial extracts prepared with the help of cold trichloroacetic acid (I-st fraction of sugar compounds) and with the help of hot 1N sulphuric acid (II-nd fraction of sugar compounds). It was the purpose

Fig. 8. The levels of the intracellular sugar fractions in the strains *Fusarium moniliforme* A and *Fusarium moniliforme* var. minus.

of the analysis to obtain a general picture of quantitative shifts of sugar compounds in the mycelial cells as a response to treatment by plant growth regulators, and therefore a more detailed fractionation for individual sugars was not attempted. Results of some of the experiments are presented in fig. 8. The initial level of sugar in the medium is 4%. Both in control cultures and in the experimental ones with growth regulators there is a rapid utilization of the sugar from the medium. When the medium contains also GA-3, IAA or TIBA the rate of sugar consumption in the early part of the fermentation period is lowered. None of the substances studied has been able to increase the rate of sugar consumption.

A definite regularity has been observed in the changes of the level of both, sugar fractions during the mycelial development. In the 24th hour of fermentation the control culture of the strain *Fusarium moniliforme* A has about equal levels of sugars in the two fractions. In the 48th hour the level of sugars in the I fraction declines and in the II fraction increases. It can be assumed that the increase in fraction II is at the expense of fraction I. In further hours of fermentation the relative levels of sugars in the two fractions do not undergo any changes.

In the control culture of the strain *Fusarium moniliforme* var. *minus* in the 24th hour of fermentation the level of sugars in fraction II is several times greater than in fraction I. The action of GA-3 on the culture of this strain causes the difference in the proportion of sugars in the two fractions to disappear.

Of all the growth regulators studied the most marked effect on the sugar turnover has been demonstrated for CCC which substantially lowers the level in fraction II for the whole fermentation period. The remaining growth regulators do not have effect on the level of sugars in the two fractions.

No relation was found to exist between the increase in the level of GA-3 in the medium and the changes in the levels of sugars in both fractions, for cultures which biosynthesize gibberellin. The intracellular changes in the levels of sugars in both fractions are presented in fig 8 for both the strains studied.

Phosphate turnover

It was the purpose of these studies to follow the changes in the levels of phosphorous compounds within fractions which are easily differentiated on the basis of classical methods for the fractionation of these compounds. Results obtained

Fig. 9. The influence of plant growth regulators on the level of total phosphorous in the mycelium of the strains *Fusarium moniliforme* A and *Fusarium moniliforme* var. *minus* at various times during fermentation.

in this series of experiments can be considered as true reflection of the biochemical effects of applied growth regulators caused within cells of the studied strains of *Fusarium moniliforme* in the fermentation conditions provided. All the trials were made at the same time, on the same medium and using the same inoculate, thus it is safe to assume that deviations from values obtained in control cultures are the result of the action of growth regulators applied on the phosphate metabolism in the studied strains. The experiments were conducted in flat-bottom flasks of 750 ml capacity containing 100 ml of medium. The flasks were agitated on the reci-

procal shaker (120 strokes per min.) at a temperature of 28°C. Each test was run simultaneously in 4 flasks, from which at 24 hour intervals 10 ml samples of the fermentation broth were aseptically taken. The samples taken from the 4 flasks were pooled together and treated as one experimental sample. Results for individual experiments are presented in figs 9–14. Only data for the experiments with GA-3, CCC and TIBA are presented for both strains, since in these marked deviations, from the results in the control cultures were observed. The results for kinetin, IAA and MH, which have been obtained in another series of experiments with the strain

Fig. 10

Fig. 11

Fig. 10. The influence of GA-3, CCC and TIBA on the level of phosphorous measured as the inorganic phosphorous fraction in the strains *Fusarium moniliforme* A and *Fusarium moniliforme* var. *minus* at various times during fermentation.

Fig. 11. The influence of GA-3, CCC and TIBA on the level of labile phosphorous in the fraction soluble in trichloroacetic acid in the cells of the strains *Fusarium moniliforme* A and *Fusarium moniliforme* var. *minus* at various times during fermentation.

Fusarium moniliforme A did not differ much from the values obtained in control cultures and therefore are not presented here. Results of experiments are presented in the form of graphs for some of the more significant phosphate fractions. The levels of phosphate fractions in figs 10–13 are presented as percentages of the particular fraction in the whole phosphorous of the mycelium. Changes in the levels of total phosphorous in the mycelial cells of both strains are presented in fig. 9. Comparing the changes in the levels of intracellular phosphorous compounds taking place under the influence of treatment with growth regulators it was found that some of the growth regulators affect markedly phosphate metabolism in the strains under investigation. The most pronounced effect is given by the compounds that inhibit gibberellin biosynthesis, namely CCC and TIBA. Also GA-3 demonstrates a very specific influence on the phosphate metabolism in the fungus *Fusarium moniliforme*. The medium used contains on the average 4,6 mg% of phosphorous, and in spite of the fact that this phosphorous was applied to the medium in an inorganic form at no time was it possible to establish the true nature of the phosphorous turnover. The phosphorous in the fermentation medium as well as in the broth filtrate was determined as inorganic phosphorous. The results obtained always were lower than theoretical. This was a result of the interference of phosphate

salts with ammonium tartrate. It was sufficient to add a few drops of ammonium tartrate to a phosphate solution of known concentration in order to obtain much lower readings for inorganic phosphorous. For this reason only total phosphorous was estimated in the medium during fermentation. Comparing the levels of intracellular phosphate fractions for the control cultures of the strains, it was found that they differ markedly. Changes in these levels were found to vary in the opposite

Fig. 12

Fig. 13

Fig. 14

Fig. 12. The influence of GA-3, CCC and TIBA on the level of stable phosphorous, in the fraction soluble in trichloroacetic acid in the strains *Fusarium moniliforme* A and *Fusarium moniliforme* var. *minus* at various times during fermentation.

Fig. 13. The influence of GA-3, CCC and TIBA on the level of labile phosphorous in the fraction insoluble in trichloroacetic acid in the strains *Fusarium moniliforme* A and *Fusarium moniliforme* var. *minus* at various times during fermentation.

Fig. 14. The influence of plant growth regulators on the levels of phospholipids in the mycelial cells of the strain *Fusarium moniliforme* A and *Fusarium moniliforme* var. *minus* at various times during fermentation.

direction in the two strains. When the level of a certain fraction in the strain *Fusarium moniliforme* A is higher or lower, than in the mycelium of the other strain the same fraction would show an opposite relation. In particular it was noticed that there exists a relation between the ability of strains of *Fusarium moniliforme* to produce gibberellin and the level of phospholipids in their cells (fig. 14). From fig. 14 it can be seen that in the mycelium of strain *Fusarium moniliforme* A cultured on a control medium and on a medium with GA-3 the level of phospholipid phosphorous was more than 10% of the total phosphorous in the cells. In culture where the biosynthesis was completely inhibited by CCC or partially also by TIBA the level of phospholipids was lowered to 5%. In the mycelium of the strain *Fusarium moniliforme* var. *minus*, that is in the strain which does not possess the capacity to biosynthesize gibberellins, the level of phospholipids after 48 hours of fermentation is very low, fluctuating to no more than 5%. This low level of phospholipids is maintained in all experiments conducted with this strain except for the culture treated with GA-3. Under the influence of a gibberellin compound in the medium the level of phospholipids throughout the fermentation period is distinctly higher than in the other experiments. The results presented in fig. 14 indicate that there exists a definable relation between the phospholipids and the gibberellins in the cells. It is difficult to establish at the moment whether the higher phospholipid level is the cause of gibberellin synthesis or whether it is result of a certain biochemical function of gibberellins in the cells of *Fusarium moniliforme* strains.

DISCUSSION

From the experimental results presented it may be concluded that certain plant growth regulators act very distinctly on the cells of *Fusarium moniliforme* grown in submerged cultures. This action manifests itself in the process of gibberellin synthesis and also in several other biochemical functions of the mycelia. The growth regulators applied to microorganisms induce several biochemical effects analogous to those observable in higher plants under the influence of the same growth regulators.

In the studies with GA-3 it was found that the cells of both strains are neutral to the compound regardless as to whether it was synthesized by the mycelium itself or was added to the medium at any stage of development. Other microorganisms behave in a similar fashion as has been shown by Lazer, Dalziel and others (1961).

A more distinct GA-3 influence can be noticed in the sugar metabolism of both the studied strains. The composition and chemical structure of intracellular sugar compounds in strains of *Fusarium moniliforme* Sheld. are generally known, however less is known about the physiological role of these compounds (Borrow et al. 1961; Martin and Adams 1956; Siddiqui and Adams 1961). The studies of Borrow et al. (1961) have shown that intracellular sugar compounds in the strains of *Fusarium moniliforme* do not act as storage materials. They cannot be used by the mycellial cells for growth or biosynthetic functions when the sources of carbon

in the medium have been exhausted. On the other hand cellular lipids are utilizable. The data presented in fig. 8 seem to confirm the conclusions of Borrow et al. (1961), since the total sugar content of both fractions is a more or less constant value amounting to about 50 mcg/mg of dry weight of the mycelium. However depending on the age of the mycelium certain relative displacements in the levels of the two fractions can take place. It is not unlikely that the passive role of the sugar compounds in cells of *Fusarium moniliforme* strains and the complex chemical nature of these polysugars are connected with the specific action of gibberellin on the hydrolase enzymes (Ault 1961; Bawden et al. 1959; Dahlström and Sfat 1961; Sandegren and Beling 1958; Bartfay and Ostrowsky 1960; Krnigstad et al. 1960; Fleming and Johnson 1961; Fleming et al. 1962; Drews et al. 1963), which results in the hydrolization of storage sugars and the accumulation of fermenting sugars. The specific chemical structure of the intracellular polysugars in *Fusarium moniliforme* can constitute a natural protection against the action of GA-3 in the same way as in the cells of higher plants.

One of the characteristic effects of GA-3 action on the strains of *Fusarium moniliforme* is the interference with phosphorous metabolism. Among other things its action is observable in the increase of phosphorous level in the cells. It needs to be pointed out that the increase in phosphorous level in plants as a result of GA-3 treatment has been observed in the studies conducted by several authors (Denisowa and Łuninowicz 1961; Lo Shi and Lin Kyi 1962; Hashimoto and Yamaki 1962). However this is not an effect observable in all plants. The opposite reaction has also been reported (Sell et al. 1961). Sytnik (1961) on the basis of his radioisotopic studies has pointed out that an increase or decrease in phosphorous uptake by plants treated with gibberellins depends to a large extent on the composition of the medium and on the physiological characteristics of the different species and varieties of plants.

In the study on the levels of different phosphate fractions it was in particular noticeable that the inorganic phosphorous had a high concentration in the cells. On the basis of the studies of Kułajew (1964), also Kułajew et al. (1964), it is permissible to suggest that in *Fusarium moniliforme* this phosphorous includes also the extremely labile, macromolecular compounds of phosphorous. It is very unlikely that in mycelial cells as much as 30% of total phosphorous should accumulate in the inorganic form. The studies of Altmann and Kiermayer (1961) indicate that GA-3 lowers the level of macromolecular substances in the cells of plants. In the two strains studied in the present investigation the level of polyphosphates was also very low. This however does not imply that the intracellular substances exist in the form of small molecules.

An increase in the level of the phospholipid fraction in the cells of both the studied strains of *Fusarium moniliforme* as a result of treatment with GA-3 also has an analogy in the studies on higher plants. Norcia et al. (1964) have observed an increase in the phospholipid fraction from tissues of three varieties of peas, one of which was the dwarf variety. The authors have found an increase in the lipid fraction in the roots of all the three varieties treated with GA-3. In the aerial parts of

the plants an opposite effect was observed. The results obtained by these authors are particularly interesting since an ever increasing amount of experimental data seems to point to the conclusion that the site of gibberellin biosynthesis in higher plants is primarily in the roots (Butcher 1963; Radley 1958) from which they are transported to aerial parts in quantities necessary for growth in the particular phase of development (Carr et al. 1964).

The results obtained in experiments on the effect of CCC on the process of gibberellin biosynthesis are totally in agreement with those obtained from experiments conducted by the group of scientists working in Lang's laboratory (Kende et al. 1963; Ninnemann 1964; Harada and Lang 1965). From the studies conducted and on the basis of data from the literature on the influence of CCC and other growth retardants on the biosynthesis of gibberellin in the fungus *Fusarium moniliforme* (Kende et al. 1963; Ninnemann et al. 1964; Harada and Lang 1965) and in higher plants (Baldew et al. 1965; Michniewicz 1965) it can be concluded that the action of this growth retardant both in the mycelial cells and in the cells of higher plants is directed towards the process of gibberellin biosynthesis. Presently it is necessary to establish which step in the process of gibberellin synthesis is the sensitive one to the action of CCC. Harada and Lang (1965) on the basis of studies conducted by Cross et al. (1964) on the labelled (—) kaurene and on the studies on biological activity of steviol and kaurene compounds (Ruddat 1963; Phinney et al. 1964) have tried the effect of adding these compounds to the medium for the growth of a strain of *Fusarium moniliforme*, in the presence of CCC and did not find any increase in the level of GA-3 in the medium. The authors conclude that the site of CCC action in the biosynthesis of gibberellin is to be found in the process causing a change of terpene compounds with the C₂₀ structure, as (—) kaurene and steviol to terpenoid compounds with the structure C₁₉ characteristic for gibberellin. From the studies of Zeevaart (1965) it appears that employing a somewhat different technique it is possible to convert (—) kaurene to gibberellin even in the presence of growth retardants. Thus the site of CCC inhibition in the process of gibberellin biosynthesis has to be sought in the steps leading from simple carbon substrates to diterpene compounds of the type of (—) kaurene and steviol. These compounds are formed during incubation of plant tissue homogenates of *Echinocystis macrocarpa* in the presence of 2-¹⁴-C- mevalonic acid (Dennis et al. 1964). The absence of inhibition by CCC of the biosynthesis of chlorotetracycline, oxytetracycline and erythromycine, that is antibiotics produced by streptomycetes directly or indirectly from acetic fragments (Snell et al. 1960; Grisebach et al. 1960, 1961; Corcoran et al. 1960) can be considered as proof that CCC cannot inhibit the processes at the beginning of the chain of reactions leading from the pool of active acetates nor in the reactions leading from acetate to mevalonic acid through the malonyl-CoA (Brodie et al. 1963) which is an irreversible stage in the biosynthesis of terpenes (Birch et al. 1959). It has been shown by Ruddat and Hoffman (1965) that the fungus *Fusarium moniliforme* produces from mevalonic acid and in the presence of CCC considerable amounts of a substance which does not appear in the control cultures or exists there only in quantities difficult

to determine analitically. Thus the site of inhibition lies probably in that part of the chain of reactions which leads from mevalonic acid to diterpene compounds like (—)kaurene.

It can be presumed that the stage of biosynthesis inhibited by CCC depends on the presence of choline or its derivative. The following observations appear to support this: 1. Choline is a closely related substance to CCC. 2. The biosynthesis of gibberellin by a strain of *Fusarium moniliforme* cultured in suboptimal aeration conditions is stimulated by choline. 3. A higher level of the phospholipid fraction is obtained in the cells of *Fusarium moniliforme* and in the roots of higher plants in the presence of gibberellin.

The stimulating effect of choline chloride on the biosynthesis of gibberellin was obtained only in the cultures on the synthetic Raulin-Thoma medium. On a rich medium containing an extract of soaked maize this effect was not observed, presumably because the extract already contained considerable ammounts of choline in concentrations ranging from 90—100 mg% (Aurich 1960; Conrady 1962). It must be pointed out that in plants throughout the vegetation cycle considerable ammounts of choline exist, ranging from 0,001 to 0,2% of fresh weight (Guggenheim 1958).

From a microscopic study of the mycelium stained with Sudan Red it can be concluded that the culturing of the fungus in the presence of CCC does not result in an interference with the lipid metabolism in the strains of *Fusarium moniliforme*.

From literature it is known that the accumulation of lipids in the mycelial cells of *Fusarium moniliforme*, while dependent on the phase of the microorganism's development and on the level of carbohydrates in the medium (Borrow et al. 1961) it can reach as much as 35% towards the end of fermentation (Chornock and Pansack 1959). These stored lipids can be utilized in the biosynthesis of gibberellin as has been demonstrated by the studies of Muromcew and Dubov (1964).

The effect of triiodobenzoic acid TIBA on the process of gibberellin biosynthesis by the fungus *Fusarium moniliforme* depends on the concentration of this growth regulator. Low levels of TIBA, of the order of 0,1 mcg/ml stimulate the biosynthesis of gibberellin. The action of TIBA is not directed specifically on the process of gibberellin biosynthesis, since at higher concentrations TIBA inhibits also the growth of the mycelial mass. TIBA also has a very definite influence on the turnover of carbohydrates and phosphates in the mycelial cells. The presence of TIBA causes a complete absence or a very strong decrease in the level of the intracellular phosphate fraction determined as inorganic phosphorous and a lowering of the phosphorous in the phospholipid fraction. On the other hand the level of the polyphosphate fraction, both soluble and insoluble in trichloroacetic acid is markedly increased as a result of treatment with TIBA.

It is possible to find an analogy in the action of TIBA on the mycelial cells of *Fusarium moniliforme* biosynthesizing gibberellin and its action on higher plants. There are many works reporting a stimulation of growth reactions in plants by low concentrations of TIBA (Thimann and Bonner 1948; Bond 1950; Aberg 1953;

Sebanek 1953; Street 1955). These responses have been explained as a synergism with respect to auxins (Thimann and Bonner 1948; Aberg 1953). The results obtained in the present study on the action of low levels of TIBA on the biosynthesis of GA-3 by the fungus *Fusarium moniliforme* permit the hypothesis that the positive growth effects obtained on plants as reported in the above mentioned studies could have been caused by an increase in the biosynthesis of gibberellins in these plants. It remains to establish whether the promotive action of low TIBA concentrations on the process of gibberellin biosynthesis in *Fusarium moniliforme* depends on a direct action of this compound on the gibberellin producing system or whether it is a secondary effect resulting from the action of TIBA on the metabolism of compounds indirectly connected with the process of gibberellin biosynthesis.

There are several papers reporting an inhibitive effect of TIBA on the growth of plants (Galston 1947; Thimann and Bonner 1948; Pohl 1962; Linser 1954). The subject of TIBA activity and its mechanism has been dealt with in many papers and several hypotheses have been put forward. An interesting one has been proposed by Liebert (1959), according to which TIBA inhibits all intracellular transport requiring energy. This view has found a confirmation in the paper by Kentzer and Liebert (1961), who have found an inhibition in the transport of GA-3 over a segment of sunflower stem as a result of treatment with TIBA.

Considering the fact that the formation of compounds with high energy and the utilization of this energy for anabolic functions of the cell are processes strongly connected with phosphate metabolism it can be assumed that TIBA by acting on the phosphate turnover leads to violent changes in many metabolic processes in the cells. These conclusions are consistent with the results obtained from the study of the intracellular level of the phosphorous fractions during development of *Fusarium moniliforme* strains.

The specific character of TIBA action on the intracellular biochemical processes in strains of *Fusarium moniliforme* is also related to the particular biosynthetic properties. In the present study a clear relation was found between the biosynthesis of a pigment and the biosynthesis of gibberellin. All factors causing an inhibition of the biosynthesis of a pigment, as for example various levels of TIBA or a reduction in the medium aeration, simultaneously cause an inhibition of gibberellin biosynthesis. Also the strain *Fusarium moniliforme* var. *minus* which produces very limited amounts of pigment is deprived of the ability to synthesize gibberellin. On the other hand it has been shown in experiments with CCC that an inhibition of gibberellin biosynthesis is not necessarily followed by a loss of ability by the mycelium to produce the pigment.

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*Działanie regulatorów wzrostu na szczepy Fusarium moniliforme Sheld.
[Gibberella fujikuroi (Saw.) Wr.]*

Streszczenie

Badano wpływ regulatorów wzrostu roślin, gibereliny A-3 (GA-3), kwasu beta-indoliloctowego (IAA), kinetyny, chlorku (2-chloroetyl) trójmetyloamoniowego (CCC), kwasu 2.3.5-trójjodobenzoesowego (TIBA) oraz hydrazidu maleinowego (HM), na biosyntezę giberelin, czynność oddechową, masy grzybniowej, zmiany pH podłoża, biosyntezę pigmentów, oraz na przemianę fosforanową i cukrową u dwu szczepów *Fusarium moniliforme* Sheld. (*Gibberella fujikuroi* [Saw.] Wr), różniących się między sobą zdolnością do wytwarzania giberelin. Proces biosyntezy giberelin na syntetycznej pożywce Raulin-Thoma jest nieznacznie stymulowany przez kinetynę, IAA, małe stężenia TIBA, oraz chlorku choline. Proces ten jest hamowany przez CCC, oraz wyższe stężenia TIBA. TIBA hamuje ponadto biosyntezę pigmentu. HM nie wpływa na proces biosyntezy giberelin. Stosowane regulatory wzrostu nie wpływają na czynność oddechową badanych szczepów *Fusarium moniliforme*. GA-3, CCC i TIBA silnie wpływają na metabolizm fosforowy, oraz na poziom wewnątrzkomórkowych frakcji cukrowych. Stwierdzono wyraźną zależność między poziomem frakcji fosfolipidowej komórki i obecnością w komórce giberelin.