The Use of Antibiotics in the Plant Protection

The Phytopathological Section of the Czechoslovak Botanical Society held on February 16, 1965 a working conference on the use of antibiotics in the plant protection. Summaries of papers are as follows (translation Dr. V. B r e j c h a).

Member of Academy

Ctibor B l a t t n ý

Antibiotics

At what phase of evolution are we actually with regard to the application of antibiotics in plant protection? At that where exaggerated hopes diminish, where discoveries become to be subjected to criticism but where in the meantime the theme itself happens to be extended due to enrichments resulting from recent findings. The proper mechanism of antibiotical effectiveness toward plants begins to be elucidated. There are copious and difficult problems to be dealt with, not even the idea of antibiotics as such seems to be clear; what is the difference between antibiotics „produced" by microorganisms and between these found in higher plants. Many economical questions remain still to be very important. Medical officers rise objections against the use of human antibiotics for phytotherapeutical purposes and claim for development of specifically effective phytotherapeutical antibiotics.

Antibiotics and their way of activity could serve for demonstrative purposes as a model of the so called deep or systemic intervention e.g. as a model for the study of systemic fungicides in action. Antibiotics can be used the same way as curative agents with plants—a treatment lying outside of our possibilities until now. Basic research upon the mechanism of antibiotical mode of action should throw some light upon many dark parts hithertoo remaining within the plant-life.

Our conference may offer both a review and a stimulus for further work indeed. On the one hand it will contribute to reveal our insufficiencies together with partial succesfulness, on the other hand it will show where to insist with our endeavour first and where to do so afterwards.

J. B a b i č k a

A world review of use of antibiotics in plant protection

Although the use of antibiotics in plant protection happens to be a question of but twoo decades of years, more than hundred treateases, many books and numerous meetings in abroad dealt with them. A more reserved point of view seems to replace today some seemingly promising results of the times at beginning, because achievements are not inasmuch convincing as they had been in the human or in the veterinary medicine, due to quite a different metabolism with animals and with plants.

Two antibiotics still remain to be suitable for practical purposes among a large number of those, introduced into experimentations: the streptomycin and the actidione. Both of them are marketed abroad under various forms of complementary formulations and under various names. As a whole, their use brought good experience achieved with them in the plant protection. A preparation on the basis of streptomycin called PLANTASOL has been admitted for treatment of seed-crops in the CSSR by the Board of the Medical Officer-in-Chief of Public Health under the signature: HE-554.1-24-464.

There exists a prospective way with the use of antibiotics in plant protection which could have a good mission indeed, provided a careful examination being undertaken. Perhaps in such instance where other preparations would fail to prove abreast. It seem necessary to set on with the antibiotical research broadly and extendly.

V. Z a l a b á k

To some questions about the mechanism of antibiotical action in the phytopathology

Antibiotics are effective against quite a many contagious diseases due to the principle of microbial antagonism. They are operating even within the plant tissues. The matter is to recognize the mechanism of the antibiotical activity as it takes place relative to the physiological processes occuring within the plants, further to understand how the metabolic products in microorganisms do chemically influence the host plant and it is necessary to trace antibiotics on the way of biological interference with the plant tissue of higher plants. The relationship between micro-

organisms and higher plants represent a basis which would enable us to attack the immunity problem. Many authors, e.g. A. STOCKES (1954), N. A. KRASILNIKOV, A. G. KUČAJEVA and others. (1958), E. GÄAUMANN (1958), B. A. RUBIN (1960) have dealt with these problems very profoundly. The pathway of the biosynthesis in green pigments was investigated besides of the inhibitory antibiotical action against the enzymes in microorganisms upon the inducing of reversal geotropism T. JAMADA, (1953), or albinism, the chlorophyll disturbation and the coagulation in the DNA (G. HAMMER, TUCKEY et al. 1951). It seems necessary to pay more attention to the study how to find more profit within the phytoncides. Many features were illustrated and described, on this field. K. V. THIMANN (1963) pointed out—along with other opportunity—the fact valuable even for other antibiotics being contemporarily used whilst telling: "The problem does not remain in the existence of those matters, but in their action, in that, how they act on the molecular level, how they exert their influence. This question lacks answering."

E. Valášková

The spectrum of effectiveness and the mechanism of action in some antifungal antibiotics

Fungicidin (syn.: Mycostatin, Nystatin) made in the CSSR was compared with foreign antibiotics in a set of in vitro and in vivo performed experiments. These antibiotics were: griseofulvin, actidione, trichothecin and others. Tests on 42 microorganisms proved a relatively broad activity spectrum of fungicidin. It was specifically different from e.g. that of actidione. Reciprocal combination of both antibiotics fortifies therefore their effectiveness. Fungal spores react upon the presence of fungicidin by a sharp drop in germination within critical concentrations. Doses ranging at 1000 μg/ml and above do inhibit germination for more than 12 days. Fungicidin remains non toxic even at doses of 2 000 μg/ml and easily enters into the plants tissues. Its content e.g. in the sap of tobacco plants reaches a maximum value within 48 hours, following the last spray, and it decreases approximately to 1/3 in the course of 4 days. The mixture of fungicidin with actidione penetrates more slowly but the antibiotical level in the plant tissue remains for 6 next days. Among practical experiments the application against *Botrytis tulipae* on tulips, done under field conditions, showed to be successful; similar results were obtained against *Botrytis cinerea* on pellargonies kept in the greenhouse and against *Penicillium* sp. on stored tulip bulbs. A mixed preparation of fungicidin with actidione worked better, but the obtained results failed to be equal to those of chemical fungicides. In experiments with mildew and during the application against downy mildew in tobacco proved the combined preparation of streptomycin with terramycin—the Plantasol.

V. Linětová, V. Brejcha and F. Starý

Changes in effectiveness of some antibiotics as influenced by light and temperature

The effectiveness of PLANTASOL, ACTIDIONE and FUNGICIDIN was investigated under various physical conditions. The same way different carriers for PLANTASOL were examined. The effectiveness of ACTIDIONE and that of FUNGICIDIN undergone but slight differences after six months elapsed, although that of PLANTASOL dropped significantly, due to environmental conditions characterised as alternating temperature and light intensity levels. SILOXYD (kieselguhr) proved best among carriers; bentonite, however, reduced significantly the effectiveness of PLANTASOL.

V. Brejcha, V. Melicharová and F. Starý

Phytotoxicity bioassay using wheat stem elongation test with antibiotics ACTIDIONE, FUNGICIDIN and PLANTASOL

Within sets of decreasing concentrations from overdosing to underdosing a pronounced phytotoxicity of ACTIDIONE took place and lasted until 0,0002% concentration in concordance with the litterature. Phytotoxic concentrations of FUNGICIDIN were 1,4% and 2,8%. No phytotoxicity took place at 0.4% concentration. Stimulative effects of PLANTASOL appeared at 0.05% concentration on the one hand whilst PLANTASOL removed the phytotoxicity of ACTIDIONE (0.05%) and FUNGICIDIN (0.2%) mixture if added at 0.02% concentration on the other hand.

E. Kováčíková, Z. Petrlík, M. Staněk, E. Staňková, Z. Štys and I. Ujević

Experiments with antibiotics used in plant protection

In the course of 1956—1961 years experiments with the use of antibiotics against bacterial and fungal plant diseases were in course. Plantasol, formerly called Fytostrept, a preparation containing streptomycin and chlorotetracycline, proved suitable to protect early growed cucumbers against *Pseudomonas lachrymans*. These phytopathogenic bacteria were removed from the seeds due to 1 hour lasting dip in a 0.4% conc. solution of this preparation and a three times repeated spraying of plants (0,05—0,2%) reduced the incidence of bacterial spot disease on cucumbers. The growth of plants showed stimulative effects and yields were increased up to 8—22% whilst a 0.075% concentration of the preparation had been applied. This preparation of streptomycin and chlorotetracycline proved even suitable to protect tomatoes against *Corynebacterium michiganense*, further if calla were treated against damping mould caused by the bacteria *Erwinia aroideae* and also against *Pseudomonas atrofaciens*, causing leaf spot disease of barley.

The antifungal antibiotic fungicidin failed to act phytotoxically within doses 250—2000 μg/ml on cucumbers, tomatoes and hops. Tomatoes infested by the fungus *Cladosporium fulvum*, also cucumbers infected by *Cladosporium cucumerinum* and lastly hops infected by the fungus *Pseudoperonospora humuli*, all these plants revealed lower degree of infestation but if higher doses of antibiotic had been applied (1000—2000 μg/ml).

Actidione caused a decrease in hops infestation by hte fungus *Pseudoperonospora humuli* under application of doses ranging between 5—25 μg/ml. Hop cones were dammaged by these doses and necrotic spots appeared on leaves following some higher doses application then 10 μg/ml. In order to protect apple trees against the mildew disease (*Podosphaera leucotricha*), also to protect hops against *Pseudoperonospora humuli* and to protect roses against *Actinonema rosae*, actidione was applied and proved fully against apple mildew, partially against both other diseases mentioned on hops and roses.

The sanitary conditions of plants turned considerably amended by dipping bean seeds infected with the fungus *Colletotrichum lindemuthianum* and with the bacteria *Pseudomonas phaseoli* into a native preparation of garlick.

M. Povolný

Some aspects in connection with antibiotics applied on plants

The influence of the antibiotic chlorotetracycline (CTC) and other prepared derivatives of chlorotetracycline ($CTCD_1$ and $CTCD_2$) was investigated upon the growth and development of plants. Experiments took place in the gardens belonging to the Chair of Horticulture, High Agronomy School at Prague. Sprays of fruit trees using aqueous solutions of CTC in concentrations 0.01 and 0.005% at blossom time and after blossoming regulate the setting on, the fall and the harvest of fruits.

It was found that the CTC and its derivatives $CTCD_2$ at concentrations 2.5 mcg/ml significantly stimulated the growth of cucumbers under natural and laboratory conditions.

The stimulation is not induced by the chlorotetracycline itself but by degradative products previously formed during the biosynthesis of the antibiotic.

E. Kováčíková, I. Ujević, Z. Petrlík and Z. Štys

Experiences with the in vitro effectiveness of some antifungal antibiotics upon the downy mildew of hops

We have examined the effectiveness of some antibiotics (actidione from USA and fungicidin) and of their combinations (technically pure preparations of actidione and fungicidin with chloroahylline admixed) using the method of dipping and that of small volume sprays against the *Pseudoperonospora humuli* Miy et Tak., on the cuttings to be explanted.

An effectiveness of 95—99% was found when cuttings had been dipped into solutions or into suspensions of the preparations but a lowered effectiveness was recorded with te small volume sprays used. In both instances actidione (USA) was the most effective one.

Using the method of glass slides treated by immersing into solutions or suspensions of preparations, their effectiveness to control the zoospore liberation has been found to be high indeed, namely with the combination of both actidione and fungicidin, the suppression degree in zoospore liberation equalled to 91,2, with glass slides treated by means of small volume sprays, the effectiveness appeared to be entirely a lower one.

Further, we have found that among all antibiotics in question, the actidione (USA) or the technically pure actidione in mixture penetrate most quickly into the cell saps of the twig leaves whilst using the highest doses.

Z. Petrlík, Z. Štys, E. Kováčíková and I. Ujević

A contribution to the influence of actidione and fungicidin upon the quality of hops

Field experiments with actidione application (0.025% and 0.05%) and their combinations (i.e. actidione with fungicidin), (0.25% and 0.5%) in the form of aerosols in amounts of 40–60 lit./ha were carried out at the Research Station for Hops (Žatec), in cooperation with the Central Research Institute for Plant Production (Ruzyně) in the course of 1961–1964, to protect hops against downy mildew disease. The experimental results revealed that the biological effectiveness of used antibiotics had not been adequate to that of classical copper fungicides applied in the form of aqueous sprays.

Chemical analyses showed that hop treatment with actidione caused a decrease of active ingredients present in the cones, a very important item for brewery purposes. The drop as compared with controls, amounted till17% p.c. whilst a simultaneous sharp decrease in alpha-acid content (32.8%) took place. To the contrary, a combination of actidione with fungicidin (0.25%) rised the total resin content up to 3.8%. The ifluence of the mentioned concentrations prevails over that, resulting from the treatment numbers, with respect to the active ingredients, very important for brewery purposes, found in the cones. No relevant differences appeared during commercial estimations of hop samples.

V. Melicharová

The use of Plantasol against bacterial diseases in calla and in hyacinths

Plantasol was applied to protect hyacinths against bacterial wilt caused by *Pseudomonas hyacinthi*. Following the isolation of the pathogen and following to ascertain the in vitro inhibitory effects of Plantasol, treatments with this antibiotic both in the form of watering the plots (aqueous solutions containing 4–8–16 g/lit/m²) and in the form of bulb dressing closely to the moment when they were lifted from the soil, in the predormancy state took place. (The found results were statistically evaluated.)

Withwatering, Plantasol proved best at concentrations 8 g/lit., lower doses being ineffective, higher being toxic ones. With bulb dressing, Plantasol proved best at concentrations ranging between 1–9 g per lit., provided a 2-hours lasting treatment had took place.

Treatments of calla against bacterial damping caused by *Erwinia aroideae* proceeded with Plantasol too. The infected subterranean parts having been detached, Plantasol was applied at various concentrations on the cutting surfaces either diluted with talc, or with lanoline and with indole acetic acid, used for stimulative purposes. Treated plants were layed down into chests filled with peat and supervised 3 weeks later with respect to the root formation rate and to the degree of infection. Among all modifications tested a mixture of Plantasol (0.4 g) with talc (50 g) and with indole acetic acid (100 mg) proved best. The disease outbreak was controlled.

V. Táborský and J. B. Novák

The effect of antibiotics upon *Pseudomonas syringae* v. Hall. and *Pseudomonas mors-prunorum* Wormald bacteria isolated from cherries, plums and durancies

Antibiotical effectiveness of streptomycin, Plantasol, penicillin, chlorotetracycline, oxytetracycline and actidione was tested against *Pseudomonas syringae* and *P. mors-prunorum* (isolates Nos. 65, 134, 156 and 168). Experimental results evaluated by means of the variance analysis showed the existence of high statistically significant differences among individual antibiotics tested and among concentrations being used. These results may serve as a basis to select suitable antibiotic and its concentration for further studies on infected plant material. With isolate No. 65 it is the concentration of streptomycin 0.5%, the conc. of Plantasol 0.8% and the conc. of penicillin 0.5%; with isolate No. 136 it is the conc. of streptomycin 0.25%, the conc. of Plantasol 0.4% and the conc. of penicillin 0.25%; with isolate No. 156 it is the conc. of streptomycin 0.25%, the conc. of penicillin 0.25% and with isolate No. 168 it is the conc. of streptomycin 0.125%, the conc. of Plantasol 0.2% and the conc. of penicillin 0.125%. As the oxytetracycline and the actidione failed to reveal any effectiveness, their evaluation did not take place.

V. Jančařík, B. Temmlová and V. Brejcha

The use of antibiotics against needle-cast of pine *Lophodermium pinastri* (Schrad.) Chev.

(Preliminary communication)

An antibiotical mixture was applied in a region of endemic needle-cast of pine outbreak at the Forest District Holá, Forest entreprise Mladá Boleslav during 1963/1964. The mixture contained:

Actidione 0.017 g/m² with the activity of 40 800 I U/mg
Fungicidin 0.094 g/m² with the activity of 2 576 I U/mg
Plantasol (Fytostrept) 0,1 g/m²

with chlorophylline addition to remove phytotoxicity of actidione.

Two years old pine seedlings *Pinus silvestris* L.) were successively three times treated in the form of either watering or dusting by the above mentioned mixtures. A further non treated area besides of another area, standardly sprayed by means of a fungicidal preparation Novozir N-50 (Zineb), all partly exposed to sun and partly protected through the shade of trees, formed a basis to compare the effectiveness of antibiotics.

In the course of the evaluation of experimental results it was found that the sunlight and/or the shading over the parcels of the experimental plots exerced a pronounced influence upon the effectiveness of the tested antibiotics. With plants growing in the shade of the neighbouring trees, the antibiotical effects equaled to those of Novozir N-50 (standard); with plants exposed to sunlight, antibiotical treatments appeared to be only of an attenuated effectiveness and, apart from this feature, even a phytotoxicity occured.

S. Navrátil

Experimental application of antibiotics against *Melampsora* sp. on poplars

Along with the proceeding vegetative period, treatments of poplars against rust diseases *Melampsora* sp. showed the need to be several times repeated. Systemic effects of fungicidal antibiotics may represent a starting point. Preliminary, in field conditions, antibiotical mixture sprays of Plantasol and fungicidin (2576 I U per mg) in doses at 150 p.p.m. + 150 p.p.m. and 1 500 p.p.m. + 1 500 p.p.m. were tested on parental heads of young american poplar trees. All sprays were two times repeated. (VII. and VIII.). A positive effect of sprays upon the degree of leaf injury caused by the disease was not observed.

PRESLIA, časopis Československé botanické společnosti
ročník 38, číslo 1, říjen 1965

Vydává: Československá botanická společnost v Academii, nakladatelství ČSAV, Vodičkova 40, Praha 1. Nové Město - dod. pú. 1 — *Redakce:* Benátská 2, Praha 2-Nové Město, dod. pú. 2, tel. 232851

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