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BOTANICAL BRIEFING

Twenty Years of Brassinosteroids: Steroidal Plant Hormones Warrant Better Crops for the XXI Century

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The discovery of brassinosteroids (BS) just over 20 years ago opened a new era in studies of bio-regulation in living organisms. Previously, the only known role of steroids as hormones was in animals and fungi; now a steroidal hormone in plants had been added. Progress in brassinosteroid research has been very rapid. Only 20 years passed between the discovery of brassinolide, the first member of the series, and the application of brassinosteroids in agriculture. Although the other plant hormones have been studied for a much longer period, there has not been similar development. Within the last couple of years two books on brassinosteroids (Khripach VA, Zhabinskii VN, de Groot A. 1999. *Brassinosteroids—a new class of plant hormones*. San Diego: Academic Press; Sakurai A, Yokota T, Clouse SD, eds. 1999. *Brassinosteroids: steroidal plant hormones*. Tokyo: Springer Verlag) have been published, but many new data have appeared since that time. Many of the more recent data is devoted to molecular biological aspects of BS and has helped to create a vision of their role in plants and their mechanisms of action. New discoveries of the physiological properties of BS allow us to consider them as highly promising, environmentally-friendly, natural substances suitable for wide application in plant protection and yield promotion in agriculture. This aspect of BS is the main subject of this Botanical Briefing.

Key words: Review, brassinosteroids (BS), practical application, plant protection, crop increase, toxicology.

INTRODUCTION

Plants possess the ability to biosynthesize a large variety of steroids whose function as hormones has frequently been postulated. However, it was not until 1979 that the presence of steroidal hormones was confirmed in plants. In that year, American scientists published data on a new steroidal lactone called brassinolide, which was isolated from pollen of *Brassica napus* L. that had been collected by bees (Grove *et al*., 1979). Three years later, castasterone, a biosynthetic precursor of brassinolide, was isolated from insect galls of *Castanea crenata* (Yokota *et al*., 1982). Today, more than 40 structurally and functionally related steroids have been identified from natural sources. These compounds are the brassinosteroids (BS) and are a new class of plant hormone. The structure of brassinolide is shown in Fig. 1. The structures of other BS differ from it within the boxed areas I and II. For example, castasterone has a keto group at the C6 position of the steroidal skeleton instead of a lactone group, and two other important BS, 28-homobrassinolide and 24-epibrassinolide, differ from brassinolide by the substituent in the side chain at C24 or by its configuration at C24, respectively.

Since the discovery of BS, more than 1000 articles have been published on various aspects of their research, mainly by scientists from Japan (45 %), USA (15 %), Germany (10–15 %), China (10–15 %) and the former Soviet Union (10–15 %). At first, investigations focused on the occurrence of BS in plants and their chemical synthesis. Later, attention concentrated on the physiological properties of BS and their mode of action. From the beginning of investigations in the USA (Maugh, 1981), BS were considered promising compounds for application in agriculture, because they showed various kinds of regulatory activity on growth and development of plants (Table 1), and their economic value as yield-promoting agents was predicted by the early 1990s (Cutler, 1991).

The practical value of BS in promoting yield was confirmed by studies in various countries, especially Japan, China and the USSR. The results obtained in this branch of

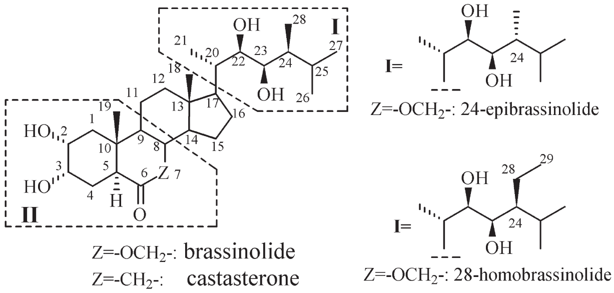

FIG. 1. Structures of some brassinosteroids.

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TABLE 1. *Some physiological effects of brassinosteroids in plants*

Cell level	Whole plant level
Stimulation of elongation and fission	Growth promotion
Effect on hormonal balance	Increase in the success of fertilization
Effect on enzyme activity; H^+-pump activation	Shortening the period of vegetative growth
Activation of protein and nucleic acid synthesis	Size and quantity of fruits increase
Effect on the protein spectrum and on the amino acid composition of proteins	Effect on the content of nutritive components and fruit quality improvement
Effect on the fatty acid composition and on the properties of membranes	Increased resistance to unfavourable environmental factors, stress and diseases
Enhancement of the photosynthetic capacity and of translocation of products	Crop yield increase

BS research have been summarized in a number of reviews (Maugh, 1981; Fujita, 1985; Abe, 1989; Mandava, 1991; Takeuchi, 1992; Prusakova and Chizhova, 1996; Khripach *et al*., 1997) and in two recent books (Khripach *et al*., 1999; Sakurai *et al*., 1999). In this Botanical Briefing we will concentrate on the most recent results of BS research; results that have appeared following the publication of these books. New data on physiological properties of BS and their application in agriculture, including those that are difficult for Western readers to access, will be presented.

BS ARE WIDE-SPREAD IN THE PLANT KINGDOM AND CONTROL THE PROCESSES OF PLANT GROWTH AND DEVELOPMENT

Brassinosteroids are found in gymnosperms, monocotyledonous and dicotyledonous plants, and in algae. New studies (Fujioka *et al*., 1998; Sasse *et al*., 1998; Schmidt *et al*., 1998; Shim *et al*., 1998; Yokota *et al*., 1998) confirm that BS are obligatory plant constituents, with the highest concentrations being found in the reproductive organs and in growing tissues (pollen, immature seeds, shoots). The range of concentrations and structures of BS that can be found in plants is wide and characteristic for a given species at specific periods of development. The highest measured concentration is about 10^{-1} nmol g^{-1} fresh weight (brassinolide in the pollen of *Brassica napus* and *Vicia faba*) and the lowest is about 10^{-7} nmol g^{-1} (homocastasterone in immature seeds and sheaths of Chinese cabbage, *Brassica campestris* var. *pekinensis*). The average situation is illustrated by recent data of Fujioka *et al*. (1998) on the concentrations of BS (brassinolide and its biosynthetic precursors) in mature seeds of *Arabidopsis thaliana*: brassinolide, $3{\cdot}9 \times 10^{-3}$ nmol g^{-1}; castasterone, $9{\cdot}5 \times 10^{-4}$ nmol g^{-1}; typhasterol, 3×10^{-3} nmol g^{-1}; 6-deoxocastasterone, $3{\cdot}5 \times 10^{-3}$ nmol g^{-1}; 6-deoxotyphasterol, $2{\cdot}1 \times 10^{-3}$ nmol g^{-1}; 6-deoxoteasterone, $1{\cdot}2 \times 10^{-3}$ nmol g^{-1}.

Brassinosteroids are integral components of the plant hormonal spectrum, influencing the total hormonal balance and the concentrations of other phytohormones. For example, BS restored the normal responses to other phytohormones in an *Arabidopsis* mutant with altered hormonal sensitivity (Ephritikhine *et al*., 1999). This can be interpreted as confirmation of an earlier proposal by Takematsu (pers. comm.) for a leading role of BS among phytohormones. The search for new interactions continues (Korableva *et al*., 1998; Shakirova and Bezrukova, 1998).

Brassinosteroids are involved in the process of cell enlargement through their effects on gene expression and on enzyme activity (Mussig and Altmann, 1999). A better understanding of the absolute requirement for BS in cell elongation has become possible from studies on mutants and from molecular genetic approaches (Nicol *et al*., 1998; Azpiroz *et al*., 1998; Salchert *et al*., 1998; Munoz *et al*., 1998). Thus the importance of BS in cell division was recently confirmed by the finding that brassinolide can accelerate or inhibit cell division in isolated leaf protoplasts of *Petunia hybrida*, depending on the phase of cell development and auxin and cytokinin conditions of the culture (Oh and Clouse, 1998).

Many results suggest that BS are required for optimal productivity and resistance to unfavourable influences of the environment (stresses, diseases: see below). A lack of BS in mutants, caused by breaks in BS biosynthesis, leads to dramatic deviations from normal development, which can often be rescued by application of exogenous BS (Altmann, 1998, 1999; Choe *et al*., 1998, 1999*a*,*b*; Klahre *et al*., 1998; Bishop *et al*., 1999; Ephritikhine *et al*., 1999; Nomura *et al*., 1999).

PLANTS RESPOND TO VERY SMALL DOSES OF BS, COMPARABLE TO THOSE FOUND NATURALLY

BS-induced changes in growth and development are the result of a cascade of biochemical reactions, which can be initiated via direct action of BS on the genome or by an extra-genetic route. Both routes assume the participation of a system of secondary messengers and can act together. An important feature is the ability of BS to act in extremely low concentrations. An indirect confirmation of this phenomenon is the very low concentration of BS in plants. A typical quantity of BS in agricultural applications is between 5 and 50 mg per hectare for growing plants. If it is assumed that the highest dose is fully absorbed by plants, it means that the average BS concentration is 10^{-7} % or $2{\cdot}1 \times 10^{-3}$ nmol g^{-1} if the total weight of plants were 50 tonnes. If only a part of the BS were to be absorbed by the plants, the concentration would be diminished. Although this concentration looks extremely low, the final result is close to the natural BS concentration in plants, and still much more than the 10^{10} molecules of BS per plant that are likely to initiate growth responses.

BS HAVE A BROAD SPECTRUM OF STIMULATIVE AND PROTECTIVE ACTIVITIES THAT HAVE A POSITIVE EFFECT ON THE QUANTITY AND QUALITY OF CROPS

Experiments to investigate the potential of BS for use in agriculture began in the 1970s in the USA and showed beneficial effects (Maugh, 1981; Mandava, 1991). In the early eighties, studies on BS in Japan and the USSR confirmed their usefulness as agricultural chemicals (Fujita, 1985; Abe, 1989; Takeuchi, 1992; Khripach *et al*., 1993). Since then, numerous reports from all over the world have appeared and many potential practical uses have been patented. Along with growth alteration, BS can influence plant development, in particular reproduction, maturation, senescence, and seed yield.

Brassinolide, 24-epibrassinolide, and 28-homobrassinolide and some other BS have been tested in field trials to determine their influence on plant growth and development and on crop yield in natural conditions. The results obtained in field trials do not always coincide with those predicted from bioassays. Under field conditions, better results can be obtained with 24-epibrassinolide and 28-homobrassinolide, even though the activity of brassinolide is usually higher in bioassays. One reason for this effect could be the lower stability of brassinolide compared to 24-epibrassinolide and 28-homobrassinolide under field conditions. This, together with the fact that 24-epibrassinolide and 28-homobrassinolide can be synthesized more cheaply than brassinolide, has promoted use of these two natural hormones in agricultural applications. Nowadays, 24-epibrassinolide is used as the active ingredient in preparations that are officially registered and have large-scale application. Nevertheless rapid development of the application of 28-homobrassinolide in agriculture might be expected because of its specific action in plants.

An important feature of BS is their ability to increase not only the yield, but also the quality of crops (Prusakova *et al*., 1999*a*). Thus, the application of brassinolide, 28-homobrassinolide, and 24-epibrassinolide to potato plants in a dose of 10–20 mg ha^{-1} gives, along with a 20 % rise in productivity, a better quality of crop with regard to a diminished nitrate content and enhanced starch and vitamin C content (Khripach *et al*., 1996*b*). Treated plants are damaged less by phytophthora and have better consumer properties. A similar influence of BS has been reported for tomato, cucumber, sugar beet and some other plants.

Another new aspect of the influence of BS is their ability to regulate the uptake of ions into the plant cell. BS can be used to reduce the accumulation of heavy metals and radioactive elements when plants are grown in areas that are polluted by these contaminants (Khripach *et al*., 1995, 1996*a*).

New promising findings in food and non-food applications are growth stimulation in mushrooms (Alexeeva *et al*., 1999), production of ornamental plants (Runkova *et al*., 1999), enhanced resistance of lawns in contaminated environments (Kalashnikov and Melnikov, 1999), improvement of the introduction and adaptation of new crops in new areas (Belova, 1999) and production of grapes (Chirilov *et al*., 1997). The capability of BS to stimulate root growth (Davidtchuck, 1999) that is important for plant adaptation is, however, still the subject of discussion in the literature (Khripach *et al*., 1999).

In Russia and Belarus, 24-epibrassinolide is the active ingredient of the plant growth promoter Epin™ (Moiseev, 1998), which has been officially registered since 1992 and is recommended for treatment of agricultural plants such as tomato, potato, cucumber, pepper and barley (State Chemical Commission of Belarus, 1998; State Chemical Commission of Russian Federation, 1999). A preparation with the same active ingredient was extensively investigated in Japan in the middle of the 1980s (Nippon Kayaku Co., 1988). It was found to be efficient for increasing the yield of many crops. Another chemical, Tianfengsu, has been developed in China as the result of a joint research programme between Japanese and Chinese scientists (Ikekawa and Zhao, 1991). This preparation is mainly a mixture of natural 24-epibrassinolide and its unnatural 22S, 23S isomer; Tianfengsu is widely used in China to increase yield of crops such as rice, maize, wheat, cotton, tobacco, vegetables and fruit (Ikekawa, pers. comm.).

It is worth mentioning here the potential role of BS in countries with highly developed agriculture—countries such as the USA, Japan and in Europe. Information on any official registration of epibrassinolide in Japan is lacking, probably because of the variable responses obtained in field trials (Cutler, 1991). American researchers have also reported erratic responses to brassinolide in field evaluations—the main reason for 'little interest in developing brassinosteroids for agricultural usage in the US' (Steffens, 1991). The relative effects of BS may be low when the conditions under which plants are growing are generally favourable. This could explain the consistently good results obtained in China, compared to those in Japan (Ikekawa and Zhao, 1991). However, it has become clear that not only crop yields, but also the quality of crops and their resistance to diseases, could be positively influenced by application of BS. This allows a more environmentally-friendly type of agriculture, which may become an issue in the near future.

BS INCREASE PLANT RESISTANCE TO STRESS AND PHYTOPATHOGENS, AND CAN BE USED AS A SUBSTITUTE FOR SOME TRADITIONAL PESTICIDES

The potential applications of BS in agriculture and horticulture are based not only on their ability to increase crop yield, but also to stimulate other physiological processes. As a result, it may become feasible to grow crops under unfavourable (stressful) conditions, such as high salinity, drought or insufficient nutrients (Prusakova *et al*., 1999*a*,*b*). Although these stress-protective properties of BS have been known for some time, systematic investigations into the potential of BS to enhance plant resistance to diseases have only recently been undertaken. Among the results obtained to date, most data are related to the influence of BS on fungal phytopathogenesis. The treatment of potato plants with BS decreased the level of phytophthora infection: it was shown, in field experiments, that spraying plants with BS (brassinolide, 24-epibrassinolide, and 28-homobrassinolide) solutions at the beginning of the bud formation period was most effective (Khripach *et al.*, 1996*c*). In some cases, the protective effect of BS against fungi was even higher than for plants treated with standard fungicides. A single dose of 20 mg ha^{-1} of 28-homobrassinolide on potato was comparable to the effect produced by a double treatment with the standard fungicide, Arcerid (composition of ridomil and polycarbacine) at 2 kg ha^{-1}. Crop yield was higher for the plants treated with 28-homobrassinolide than for those treated with Arcerid, and about 20 % greater than the untreated control. Recent data (Savel'eva *et al.*, 1999) confirmed the efficiency of BS application in the reduction of phytophthora infection (up to a maximal 40 %). Usually, the higher the level of pest development, the higher the expression of the protective properties of BS observed. Treatment with 24-epibrassinolide prolongs dormancy of potato tubers and increases their resistance to sprouting and diseases, changes that are associated with enhancement of ABA and ethylene levels, and also the presence of phenolic and terpenoid protective substances (Korableva *et al.*, 1999).

Field application of 24-epibrassinolide to barley plants in doses of about 5–15 mg ha^{-1} significantly decreased the extent of leaf diseases induced by mixed fungal infection, along with an increase in crop yield. A comparison of the results with those obtained after application of Bayleton (0·5 kg ha^{-1}), the traditional fungicide (Pshenichnaya *et al.*, 1997) used under these conditions, showed the higher capacity of 24-epibrassinoide as a protective factor against fungi. A protective effect of 24-epibrassinolide against fungi was established in field trials with cucumber (Churikova *et al.*, 1999). The data obtained illustrate significant suppression of mildew in cucumber plants treated twice with 24-epibrassinolide. First, seeds were soaked in a 0·1 mg l^{-1} solution of 24-epibrassinolide and then the plants were sprayed with a dose of 25 mg ha^{-1} at the flowering stage. An increase in the activity of some enzymes (peroxidase, polyphenoloxidase) in the leaves of cucumber plants has also been found. Since these enzymes are involved in the metabolism of polyphenols, a change in their activity may be considered as one of the factors connected with increased plant resistance to infection.

A newly discovered aspect of the protective action of BS on plants is related to their ability to stimulate resistance to viral infection (Bobrik *et al.*, 1998). Potato cuttings cultured in a medium containing brassinolide, 24-epibrassinolide, or 28-homobrassinolide showed a significant increase in all the parameters that characterize growth and development, in comparison with a control, and this led to a higher yield of plants. One of the most important effects was a reduction of viral infection in the material, and this effect was found in all stages of plant development. Along with an essential lowering of viral infection, the plants obtained from BS-treated cuttings gave a higher crop yield when compared to plants from untreated cuttings. The maximum increase in crop yield, 56 %, occurred in plants whose previous generation was produced from material that was grown in a culture medium with 24-epibrassinolide at a concentration of 0·25 mg l^{-1}. The highest increase in yield corresponded with the lowest level of disease.

These data indicate that exogenous BS can act efficiently in plants as immuno-modulators when applied at the appropriate dose and at the correct stage of plant development. As in other cases of BS-regulated stress response, the pathogen protective action of BS is the result of a complex sequence of biochemical shifts such as activation or suppression of key enzymatic reactions, induction of protein synthesis, and the production of various chemical defence compounds. These relatively little investigated properties of BS are promising from a practical point of view. They open up new approaches for plant protection, based on the employment of very small amounts of environmentally-friendly natural substances instead of traditional pesticides, which are often environmentally-unfriendly.

BS HAVE BEEN COMPONENTS OF ANIMAL FOOD CHAINS THROUGHOUT THEIR EVOLUTION

The metabolism of BS in mammals has not yet been investigated. It may be speculated, however, that a 'normal' catabolism of the steroidal skeleton will take place. Being normal constituents of practically all plants, BS have been, and are, consumed by mammals, and so additional harmful effects are not likely from their use in agriculture. This assumption is an important prerequisite for considering BS as ecologically safe, non-toxic chemicals for agriculture. However, confirmation of their safety can be obtained from toxicological studies.

The acute toxicity data obtained at the Sanitary-Hygienic Institute of Belarus for 24-epibrassinolide are: LD_{50} (orally) in mice (female) is more than 1000 mg kg^{-1}; LD_{50} (orally and dermally) in rats (male/female) is more than 2000 mg kg^{-1}. Dermal toxicity in rats (male/female) is more than 2000 mg kg^{-1}. The formulation, Epin (0·025 % solution of 24-epibrassinolide), in mice and rats (orally and dermally) has an LD_{50} of more than 5000 mg kg^{-1}. Repeated experiments confirmed the value of LD_{50} for 24-epibrassinolide orally in mice and showed a value for Epin which was higher than 15 000 mg kg^{-1} (white rats, orally or intranasally). In concentrations of 0·2 %, 24-epibrassinolide did not irritate mucous membranes of rabbits' eyes; this compound, or a solution of Epin, did not irritate the skin. The Ames test for mutagenic activity carried out at the Scientific Research Center of Toxicologic and Hygienic Regulation of Biopreparations of Russia, with or without metabolic activation, was negative (*Salmonella typhimurium* TA 1534, TA 1537, TA 1950, TA 98, TA 100). In micronuclear or chromosome abberation tests (mice CBAB1/6) neither 24-epibrassinolide nor Epin caused spontaneous mutations.

Complex biological testing on *Tetrahymena pyriformis* carried out at the Sanitary-Hygienic Institute of Belarus has confirmed the genetic safety of 24-epibrassinolide and the absence of mutagenic activity over seven generations. In acute, subacute, and chronic experiments, 24-epibrassinolide showed low toxicity and very little cumulative effect. In prolonged experiments, 24-epibrassinolide showed no toxicity but a pronounced adaptogenic effect (increasing adaptive ability of the population). Studies on fish toxicity showed no negative effects, but pronounced stimulative and toxico-protective properties (Vitvitskaya *et al*., 1997*a*,*b*).

AGRICULTURALLY APPLIED BS, ALTHOUGH THEY ARE PREPARED SYNTHETICALLY, ARE IDENTICAL TO THE NATURAL PHYTOHORMONES

The extremely low BS content in plants means that chemical synthesis is vital in this field of research. Synthesis is the only source of BS for physiological studies and for practical application in agriculture. The studies on chemical synthesis of BS and their analogues (compounds, structurally related to BS, which do not occur in nature) began immediately after the elucidation of the structure of brassinolide and are ongoing. Chemical synthesis of BS can be considered as reasonably efficient, and for some BS economically feasible, large-scale syntheses are available for the production of sufficient quantities for agricultural application.

The most convenient starting materials for BS syntheses are sterols that are easily available from natural sources and contain the same carbon skeleton as the target hormone. In such cases, only the introduction of the chemical functionalities that are typical for BS is necessary. Unfortunately, 22-dehydrocampesterol, which has the correct carbon skeleton and suitable starting functionalities for the synthesis of brassinolide, is not available in sufficient quantities from nature and for that reason most brassinolide syntheses are still rather long and relatively complicated. For 24-epibrassinolide and 28-homobrassinolide, both promising for agricultural use, relatively simple and straightforward large-scale syntheses are available, starting from ergosterol and stigmasterol, respectively.

The constantly increasing demands for the ecological safety of agricultural chemicals introduces a significant cost for toxicological studies on *unnatural* pesticides. For the immediate future, a much wider employment of *natural* products may be profitable, perhaps together with the application of traditional chemicals when necessary. The long-term toxicological effects of natural products (such as BS) are either non-existent or dramatically lower than those of synthetic, unnatural compounds. It is important in this respect that the synthetically prepared BS, which are intended for agricultural application (State Chemical Commission of Russian Federation, 1999), are pure compounds and fully identical to the corresponding natural phytohormones. This is also necessary in studies of the physiological effects of BS, especially those concerning the mechanisms of BS action, in order to prevent mistakes that could be the result of effects of unnatural compounds that are present as 'impurities'.

Although the term 'BS' includes only naturally occurring compounds, the story of their agricultural application is not complete without mentioning BS analogues. Many analogues have been synthesized and patented as new plant growth promoting substances, especially those that were found to be more easily synthesized than natural BS. Following an intensive search for new BS in plants, some analogues were recognized as naturally-occurring compounds. This was the case for 24-epibrassinolide, which was described by Thompson *et al*. (1979) purely as a synthetic compound, but was found by Ikekawa *et al*. in pollen of broad bean (*Vicia faba* L.) in 1988. Among the well-investigated BS analogues are isomers with unnatural configurations of the hydroxy groups at C22 and C23 (22S, 23S- or 22β, 23β-isomers). These compounds, for example 22S,23S-homobrassinolide, can be synthesized much more easily than the natural compounds and they have a relatively high biological activity. Another group of analogues possesses all the characteristic structural elements of BS in a modified chemical form, which allows their transformation in living cells into the active phytohormone. One representative of this group is (22R,23R)-epoxy-homobrassinolide, 2,3-diacetate, also known as TS303. In many field trials this BS analogue showed good results with respect to germination, root formation, growth, fruit-setting, crop yield and stress resistance for a number of agricultural plants (Takatsuto *et al*., 1996; Kamuro *et al*., 1997; Kamuro and Takatsuto, 1999).

BS CAN BE APPLIED FOR THE TREATMENT OF PLANTS OR SEEDS USING EXISTING EQUIPMENT AND TECHNOLOGIES

The growth-promoting activity of BS usually takes place only after treatment of plants in the appropriate phase of development and within a certain concentration range, which is different for each plant species and type of BS. For large-scale field application, two modes of BS application are possible: seed soaking and foliar spray (State Chemical Commission of Belarus, 1998; Vakulenko and Shapoval, 1998; State Chemical Commission of Russian Federation, 1999). Both methods have been investigated extensively, but results with the latter method were found to be highly dependent on the phase of plant development. Generally, better results can be obtained when young rather than old plants are treated. The formulation of the spraying solution is very important, and additives are necessary to facilitate the spreading of active substance, to prevent early drying and to ensure penetration of BS via cell walls. The working solution should be prepared by dilution with water shortly before application, which can be done with normal agricultural sprayers. The ability to combine BS treatment of plants with other pesticides, for example with fungicides for potato treatment, allows BS to be used with existing technologies for plant protection, with no significant additional expense.

A new variant of BS application has been developed recently (Pirogovskaya *et al*., 1996), based on the use of combinations of BS with mineral fertilizers. Field trials showed an increase in yield and an improvement in the quality of crops of barley, oat, potato, winter rye and wheat. In this approach, 24-epibrassinolide was recommended as an addition (10^{-4}–10^{-6} %) to nitrogen–phosphorus–potassium fertilizers.

CONCLUSIONS

Data obtained by American investigators on the application of BS resulted in the conclusion in 1981 that: 'New chemicals promise larger crops' (Maugh, 1981). Now, almost 20 years later, all the collected data lead us to the conclusion that the use of BS can produce larger and better quality crops. Research suggests that BS can be developed to be a new generation of agricultural chemicals, which do not interfere with the environment, act in natural doses and in a natural way. For these reasons BS have good prospects for enhancing plant production and protection in the near future. Being natural compounds, which stimulate the plant itself via activation of gene expression in a certain period of development, BS do not initiate co-evolution of pests. Such an approach, based on a wider use of existing genetic resources looks promising, may be better accepted by the consumer and can enrich our arsenal of plant protection strategies in the twenty-first century.

LITERATURE CITED

Abe H. 1989. Advances in brassinosteroid research and prospects for its agricultural application. *Japan Pesticide Information* 10–14.

Alexeeva KL, Malevannaya NN, Khripach VA, Zhabinskii VN. 1999. A method of growth stimulation of meadow mushrooms. *Pat. Appl.* **RU 99,106,737**.

Altmann T. 1998. A tale of dwarfs and drugs: brassinosteroids to the rescue. *Trends in Genetics* **14**: 490–495.

Altmann T. 1999. Molecular physiology of brassinosteroids revealed by the analysis of mutants. *Planta* **208**: 1–11.

Azpiroz R, Wu Y, LoCascio JC, Feldmann KA. 1998. An *Arabidopsis* brassinosteroid-dependent mutant is blocked in cell elongation. *Plant Cell* **10**: 219–230.

Belova TA. 1999. Efficacy of growth regulator epin for soy-bean introduction In: Shevelucha VS, Karlov GI, Karsunkina NP, Salnikova EI, Skorobogatova IV, Siusheva AG, eds. *Regulators of plant growth and development* **5**. Moscow: Agricultural Academy, 156.

Bishop GJ, Nomura T, Yokota T, Harrison K, Noguchi T, Fujioka S, Takatsuto S, Jones JD, Kamiya Y. 1999. The tomato DWARF enzyme catalyses C-6 oxidation in brassinosteroid biosynthesis. *Proceedings of the National Academy of Sciences, USA* **96**: 1761–1766.

Bobrick AO, Khripach VA, Zhabinskii VN, Zavadskaya MI, Litvinovskaya RP. 1998. A method of production of sanitated seed potato. *Pat. Appl.* **BY 19,981,189**.

Chirilov A, Khripach V, Toma S, Scurtul A, Zhabinskii V, Cozmic R, Zavadskaya M, Erlihman J. 1997. Method of grape production. *Pat.* **MD 701F1**.

Choe S, Dilkes BP, Fujioka S, Takatsuto S, Sakurai A, Feldmann KA. 1998. The DWF4 gene of *Arabidopsis* encodes a cytochrome P450 that mediates multiple 22α-hydroxylation steps in brassinosteroid biosynthesis. *Plant Cell* **10**: 231–243.

Choe S, Noguchi T, Fujioka S, Takatsuto S, Tissier CP, Gregory BD, Ross AS, Tanaka A, Yoshida S, Tax FE, Feldmann KA. 1999*a*. The *Arabidopsis* dwf7/ste1 mutant is defective in the Δ^7 sterol C-5 desaturation step leading to brassinosteroid biosynthesis. *Plant Cell* **11**: 207–221.

Choe S, Dilkes BP, Gregory BD, Ross AS, Yuan H, Noguchi T, Fujioka S, Takatsuto S, Tanaka A, Yoshida S, Tax FE, Feldmann KA. 1999*b*. The *Arabidopsis* dwarf1 mutant is defective in the conversion of 24-methylenecholesterol to campesterol in brassinosteroid biosynthesis. *Plant Physiology* **119**: 897–907.

Churikova VV, Chozhainova GN, Eprintsev AT. 1999. The action of epin on the activity of malatdehydrogenase in seedlings of cucumber plants In: Shevelucha VS, Karlov GI, Karsunkina NP, Salnikova EI, Skorobogatova IV, Siusheva AG, eds. *Regulators of plant growth and development* **5**. Moscow: Agricultural Academy, 141.

Cutler HG. 1991. Brassinosteroids through the looking glass. In: Cutler HG, Yokota T, Adam G, eds. *Brassinosteroids. Chemistry, bioactivity, and application. ACS Symposium Series, 474*. Washington: American Chemical Society, 334–345.

Davidtchuck NV. 1999. Effect of various epin treatment methods on growth of seedlings of sugar-beet and maize In: Shevelucha VS, Karlov GI, Karsunkina NP, Salnikova EI, Skorobogatova IV, Siusheva AG, eds. *Regulators of plant growth and development* **5**. Moscow: Agricultural Academy, 89.

Ephritikhine G, Fellner M, Vannini C, Lapous D, Barbier-Brygoo H. 1999. The sax1 *dwarf* mutant of *Arabidopsis thaliana* shows altered sensitivity of growth responses to abscisic acid, auxin, gibberellins and ethylene and is partially rescued by exogenous brassinosteroid. *Plant Journal* **18**: 303–314.

Fujioka S, Noguchi T, Yokota T, Takatsuto S, Yoshida S. 1998. Brassinosteroids in *Arabidopsis thaliana*. *Phytochemistry* **48**: 595–599.

Fujita F. 1985. Prospects for brassinolide utilization in agriculture. *Chemical Biology* **23**: 717–725.

Grove MD, Spencer GF, Rohwedder WK, Mandava N, Worley JF, Warthen JD, Steffens GL, Flippen-Anderson JL, Cook JC. 1979. Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. *Nature* **281**: 216–217.

Ikekawa N, Zhao YJ. 1991. Application of 24-epibrassinolide in agriculture. In: Cutler HG, Yokota T, Adam G, eds. *Brassinosteroids. Chemistry, bioactivity, and applications. ACS Symposium Series, 474*. Washington: American Chemical Society, 280–291.

Ikekawa N, Nishiyama F, Fujimoto Y. 1988. Identification of 24-epibrassinolide in bee pollen of the broad bean, *Vicia faba* L. *Chemical and Pharmaceutical Bulletin* **36**: 405–407.

Kalashnikov DV, Melnikov GA. 1999. Application of growth regulators for increasing of lawn resistance in cities and highways In: Shevelucha VS, Karlov GI, Karsunkina NP, Salnikova EI, Skorobogatova IV, Siusheva AG, eds. *Regulators of plant growth and development* **5**. Moscow: Agricultural Academy, 188.

Kamuro Y, Takatsuto S. 1999. Practical application of brassinosteroids in agricultural fields. In: Sakurai A, Yokota T, Clouse SD, eds. *Brassinosteriods: steroidal plant hormones*. Tokyo: Springer Verlag, 223–241.

Kamuro Y, Takatsuto S, Watanabe T, Noguchi T, Kuriyama H, Suganuma H. 1997. Practical aspects of brassinosteroid compound [TS303]. *Proceedings of the Plant Growth Regulation Society of America* **24**: 111–116.

Khripach VA, Lakhvich FA, Zhabinskii VN. 1993. *Brassinosteroids*. Minsk: Science and Technique.

Khripach VA, Voronina LV, Malevannaya NN. 1996*a*. A preparation for decreasing of heavy metals absorbtion by agricultural plants. *Pat.* **RU 2,119,285**.

Khripach VA, Zhabinskii VN, de Groot A. 1999. *Brassinosteroids—a new class of plant hormones*. San Diego: Academic Press.

Khripach VA, Zhabinskii VN, Malevannaya NN. 1997. Recent advances in brassinosteroids study and application. *Proceedings of the Plant Growth Regulation Society of America* **24**: 101–106.

Khripach VA, Zhabinskii VN, Litvinovskaya RP, Zavadskaya MI, Deeva VP, Vedenéev AN. 1995. Preparation for the diminishing of radionuclides accumulation by plants and method of its application. *Pat. Appl.* **BY 950,941**.

Khripach VA, Zhabinskii VN, Litvinovskaya RP, Zavadskaya MI, Savel'eva EA, Karas II, Vakulenko VV. 1996*b*. A method of increasing of potato food value. *Pat. Appl.* **BY 960,345**.

Khripach VA, Zhabinskii VN, Litvinovskaya RP, Zavadskaya MI, Savel'eva EA, Karas II, Kilcchevskii AV, Titova SN. 1996*c*. A method for protection of potato from phytophthorosis. *Pat. Appl.* **BY 960,346**.

Klahre U, Noguchi T, Fujioka S, Takatsuto S, Yokota T, Nomura T, Yoshida S, Chua NH. 1998. The *Arabidopsis* DIMINUTO/

DWARF1 gene encodes a protein involved in steroid synthesis. *Plant Cell* **10**: 1677–1690.

Korableva NP, Platonova TA, Dogonadze ME. 1998. Effect of brassinolide on the ethylene biosynthesis in potato tuber meristems *Solanum tuberosum* L. *Doklady Akademii Nauk (Russia)* **361**: 113–115.

Korableva NP, Platonova TA, Dogonadze MZ, Bibick ND. 1999. A stability change of potato to premature germination and diseases under the brassinosteroid action In: Shevelucha VS, Karlov GI, Karsunkina NP, Salnikova EI, Skorobogatova IV, Siusheva AG, eds. *Regulators of plant growth and development* **5**. Moscow: Agricultural Academy, 102–103.

Mandava NB. 1991. Brassinosteroids. U.S. Department of Agriculture contributions and Environmental Protection Agency registration requirements. In: Cutler HG, Yokota T, Adam G, eds. *Brassinosteroids. Chemistry, bioactivity, and application. ACS Symposium Series, 474*. Washington: American Chemical Society, 320–323.

Maugh TH. 1981. New chemicals promise larger crops. *Science* **212**: 33–34.

Moiseev A. 1998. Preparation epin: water of life. *Eureka*: 68–69.

Munoz FJ, Labrador E, Dopico B. 1998. Brassinolides promote the expression of a new *Cicer arietinum* β-tubulin gene involved in the epicotyl elongation. *Plant Molecular Biology* **37**: 807–817.

Mussig C, Altmann T. 1999. Physiology and molecular mode of action of brassinosteroids. *Plant Physiology and Biochemistry* **37**: 363–372.

Nicol F, His L, Jauneau A, Vernhettes S, Canut H, Hoefte H. 1998. A plasma membrane-bound putative endo-1,4-β-D-glucanase is required for normal wall assembly and cell elongation in *Arabidopsis*. *EMBO Journal* **17**: 5562–5576.

Nippon Kayaku Co., Ltd. 1988. JRDC-694 (epi-brassinolide). Technical information.

Nomura T, Kitasaka Y, Takatsuto S, Reid JB, Fukami M, Yokota T. 1999. Brassinosteroid/Sterol synthesis and plant growth as affected by *lka* and *lkb* mutations of *Pea*. *Plant Physiology* **119**: 1517–1526.

Oh MH, Clouse SD. 1998. Brassinolide affects the rate of cell division in isolated leaf protoplasts of *Petunia hybrida*. *Plant Cell Reports* **17**: 921–924.

Pirogovskaya GV, Bogdevitch IM, Naumova GV, Khripach VA, Azizbekyan SG, Krul LP. 1996. New forms of mineral fertilizers with additives of plant growth regulators. *Proceedings of the Plant Growth Regulation Society of America* **23**: 146–151.

Prusakova LD, Chizhova SI. 1996. The role of brassinosteroids in growth, tolerance and productivity of plants. *Agrokhimiya*: 137–150.

Prusakova LD, Ezhov MN, Salnikov AI. 1999*a*. The use of emistim, epibrassinolide and uniconazole to overcome quality difference of buckwheat grains. *Agrarian Russia*: 41–44.

Prusakova LD, Chizhova SI, Tretyakov NN, Ageeva LF, Golantseva EN, Yakovlev AF. 1999*b*. Ecost and epibrassinolide antistress functions on spring wheat under the conditions of the Central Non-Chernozem zone. *Agrarian Russia*: 39–41.

Pshenichnaya LA, Khripach VA, Volynetz AP, Prokkhorchik RA, Manzhelesova NE, Morozik GV. 1997. Brassinosteroids and resistance of barley plants to leaf diseases In: Parfenov VI, ed. *Problems of experimental botany*. Minsk: Byelorussian Science, 210–217.

Runkova LV, Alexandrova VS, Belyaeva GE. 1999. The action of epin on taking roots of perspective varieties of roses In: Shevelucha VS, Karlov GI, Karsunkina NP, Salnikova EI, Skorobogatova IV, Siusheva AG, eds. *Regulators of plant growth and development* **5**. Moscow: Agricultural Academy, 245–246.

Sakurai A, Yokota T, Clouse SD, eds. 1999. *Brassinosteroids. Steroidal plant hormones*. Tokyo: Springer.

Salchert K, Bhalerao R, Koncz-Kalman Z, Koncz C. 1998. Control of cell elongation and stress responses by steroid hormones and carbon catabolic repression in plants. *Philosophical Transactions of the Royal Society of London. B. Biological Sciences* **353**: 1517–1520.

Sasse JM, Griffiths PG, Gaff DF, Yokota T, Cameron DW. 1998. Brassinosteroids of a resurrection grass. *16th International Conference on Plant Growth Substances*. Chiba, (Japan), 131.

Savel'eva EA, Karas II, Kilchevskii AV, Titova SN, Khripach VA, Zhabinskii VN, Litvinovskaya RP, Zavadskaya MI. 1999. New properties of natural brassinosteroids. Method of potato protection from phytophthorosis In: Shevelucha VS, Karlov GI, Karsunkina NP, Salnikova EI, Skorobogatova IV, Siusheva AG, eds. *Regulators of plant growth and development* **5**. Moscow: Agricultural Academy, 247.

Schmidt J, Porzel A, Adam G. 1998. Brassinosteroids and a pregnane glucoside from *Daucus carota*. *Phytochemical Analysis* **9**: 14–20.

Shakirova FM, Bezrukova MV. 1998. Effect of 24-epibrassinolide and salinization on changes in abscisic acid and lectin concentration in wheat seedling roots. *Fiziologiya Rastenii (Moscow)* **45**: 451–455.

Shim JH, Kim IS, Lee KB, Suh YT, Morgan ED. 1998. Determination of brassinolide in rice (*Oriza sativa* L.) by HPLC equipped with a fluorescence detector. *Agricultural Chemistry and Biotechnology* **39**: 84–88.

State Chemical Commission of Belarus. 1998. *Chemicals for plant protection and plant growth regulation* (Suppl.). Minsk: Republic state station of plant protection, 59.

State Chemical Commission of Russian Federation. 1999. *Handbook on pesticides and agrochemicals allowed for application in Russian Federation*. Moscow: Agrorus, 195–199.

Steffens GL. 1991. U.S. Department of agriculture brassins project: 1970–1980. In: Cutler HG, Yokota T, Adam G, eds. *Brassinosteroids. Chemistry, bioactivity and application. ACS Symposium Series, 474*. Washington: American Chemical Society, 2–17.

Takatsuto S, Kamuro Y, Watanabe T, Noguchi T, Kuriyama H. 1996. Synthesis and plant growth promoting effects of brassinosteroid compound TS303. *Proceedings of the Plant Growth Regulation Society of America* **23**: 15–20.

Takeuchi Y. 1992. Studies on the physiology and applications of brassinosteroids. *Chemical Regulation of Plants* **27**: 1–10.

Thompson MJ, Mandava N, Flippen-Anderson JL, Worley JF, Dutky SR, Robbins WE, Lusby W. 1979. Synthesis of brassinosteroids: new plant-growth-promoting steroids. *Journal of Organic Chemistry* **44**: 5002–5004.

Vakulenko VV, Shapoval OA. 1998. Plant growth regulators for seed treatment. *Protection and Quarantine of Plants*: 44.

Vitvitskaya LV, Tikhomirov AM, Nikonorov SI, Egorov MA, Malevannaya NN, Khripach VA, Zhabinskii VN. 1997*a*. The method of survival increase of larvae and fingerlings of sturgeons in fish breeding. *Pat. Appl.* **RU 97,117,958**.

Vitvitskaya LV, Tikhomirov AM, Nikonorov SI, Egorov MA, Malevannaya NN, Khripach VA, Zhabinskii VN. 1997*b*. The method for estimation and improvement of activity and viability of spermatozoons in animal breeding. *Pat. Appl.* **RU 97,117,959**.

Yokota T, Arima M, Takahashi N. 1982. Castasterone, a new phytosterol with plant-hormone potency from chestnut insect gall. *Tetrahedron Letters* **23**: 1275–1278.

Yokota T, Higuchi K, Takahashi N, Kamuro Y, Watanabe T, Takatsuto S. 1998. Identification of brassinosteroids with epimerized substituents and/or the 23-oxo group in pollen and anthers of Japanese cedar. *Bioscience, Biotechnology and Biochemistry* **62**: 526–531.