BIOLOGIA PLANTARUM (PRAHA)

25 (5) : 366—372, 1983

Characterization of Phosphate Absorption in Maize Root Cortex Segments

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Abstract. Uptake of phosphate ions by 1 mm segments of isolated maize root cortex layers was studied. Cortex segments (from roots of 8 days old maize plants) absorb phosphate ions from 1 mM KH_2PO_4 in 0.2 mM $CaSO_4$ at the average rate of 34.3 ± 3.2 μg P_i g^{-1} (fr. m.) h^{-1}, *i.e.* 0.35 ± 0.02 μmol P_i g^{-1} (fr. m.) h^{-1}. Phosphate uptake considerably increases after a certain period of "augmentation", *i.e.* washing in aerated 0.2 mM $CaSO_4$. This increase is completely blocked by the presence of 10 μg ml^{-1} cycloheximide.

The relation of uptake rate to phosphate concentration in the medium was shown to have 3 phases in the concentration range of 0.02—40 mM. Transition points were found between 0.8—1 mM and 10—20 mM. Following K_m and V_{max} values were found:

K_m[mM] : 0.37 — 3.82 — 27.67

V_{max}[μg P_i g^{-1} (fr. m.) h^{-1}] : 3.33 — 39.40 — 66.67

We have found no sharp pH optimum for phosphate uptake. It proceeds at almost constant rate till pH 6.0 and then the uptake rate drops with increasing pH. At low phosphate concentrations (1 mM) the lowest uptake rate was found at 5 and 13 °C, while the uptake is higher at 5 °C than at 13 °C at phosphate concentrations higher than 1 mM. At these concentrations uptake rate at 35 °C is lower than at 25 °C.

Phosphate uptake considerably decreased in anaerobic conditions. DNP and iodoacetate (0.1 mM) completely blocked phosphate uptake from 1 mM KH_2PO_4, while uptake from 5 and 10 mM KH_2PO_4 was left unaffected by these substances. The inhibitors of active —SH groups NEM and PCMB inhibited phosphate uptake: 10^{-3} M NEM by 81.6%, 10^{-4} M NEM by 42%, and 10^{-4} M PCMB by 42%.

Phosphorus (in the form of inorganic phosphate) is one of the most important plant nutrients and its uptake and translocation in plants have been studied for a long time. Plant roots absorb primarily $H_2PO_4^-$ ions, but it was shown in some algae that also HPO_4^{2-} ions could be absorbed (ULLRICH-EBERIUS 1973, RAVEN 1974). Phosphate absorption was proved to be active be means of various inhibitors of energetic metabolism (METLER and LEONARD 1979, LIN 1980) and by means of changes in membrane potential (BOWLING *et al.* 1978, ULLRICH-EBERIUS *et al.* 1981). ATP is considered the main energy source (*e.g.* SMITH and RAVEN 1974). BORST-PAUWELS and DOBBLEMAN (1972) described phosphate uptake in yeast as a P_i—OH^- antiport. This was recently confirmed in *Lemna gibba* — uptake of monovalent

Received July 16, 1982; accepted March 17, 1983

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phosphate proceeded as a H^+ cotransport (ULLRICH-EBERIUS *et al.* 1981), which is experimentally indistinguishable from OH^- antiport.

Most of the mentioned papers dealt with algae, fungi, plant protoplasts or intact roots. Very seldom work comparing absorption into root cortex with that into xylem is found (LATIES and BUDD 1964, VAN IREN *et al.* 1981). We anticipate that such detailed comparison could help us to answer some questions about the mechanism and eventual barriers in phosphate ion absorption. This paper reports on properties of phosphate uptake system in maize root cortex tissue.

MATERIAL AND METHODS

Maize (*Zea mays* L., hybrid CE-240 from Breeding Station in Čejč) seeds were surface sterilized, thoroughly washed with bidistilled water and left to imbibe for 20 h at room temperature. Imbibed seeds were transferred to Petri dishes with filter paper discs soaked in 0.2 mM $CaSO_4$ and germinated in the dark at 25 ± 1 °C for 2 days. Well germinated seeds were transferred to glass dishes (3 l volume) covered with black PVC foil, in the top of which small holes for plants were perforated. Plants were raised for 3 days on 10 times diluted Knop's nutrient solution (LAŠTŮVKA and MINÁŘ 1967) in a controlled-environment glasshouse, where temperature of 20 ± 1 °C and relative humidity of 50 % were kept. Plants were illuminated 14 h a day with sodium discharge lamps (100 Wm^{-2}). The nutrient solution was kept at 18 ± 0.5 °C and was continuously aerated. On the fourth day the nutrient solution was replaced with 0.2 mM $CaSO_4$ and plants were kept for further two days under the same conditions.

Cortex layers of roots (from the region 5—100 mm behind the tip) were stripped and placed into aerated 0.2 mM $CaSO_4$. These cortex layers were cut into 1 mm segments by means of a device made from stainless blades. Cortex segments were then incubated for 3 h in aerated 0.2 mM $CaSO_4$ at room temperature (so called "augmentation" — fresh segments have very low uptake rate and this is increased by washing in $CaSO_4$ solution (LEONARD and HANSON 1972, VAN STEVENINCK 1975)). We call augmented segments such segments, the absorption rate of which does not substantially increase on further incubation in $CaSO_4$ solution. Aliquots of washed segments were replaced into the incubation solution: various concentrations KH_2PO_4 in 0.2 mM $CaSO_4$ (pH 4.7—4.9) with or without addition of salts, inhibitors *etc.* Incubation proceeded on a temperature-controlled water bath at 25 ± 0.5 °C (unless indicated otherwise) for 5 h under continuous shaking. At the end of the incubation period an aliquot of incubation solution was withdrawn and kept on ice till the phosphorus content determination. The segments were filtered off and replaced into 10 ml cold 0.2 mM $CaSO_4$ for 10 min to remove free space and surface-sorbed phosphate (CRAM 1973). Aliquots of washing media were also analyzed for phosphorus content. These values were subtracted from values of absorption to obtain net absorption into cortex tissue.

Phosphorus content was determined on a Technicon analyzer (colorimetrically) using the photometric method of MURPHY and RILEY (1962).

Abbreviations used: P_i = inorganic phosphate, DNP = 2,4-dinitrophenol, NEM = N-ethyl maleiimide, PCMB(S) = *p*-chloromercuribenzoate (benzene-sulfonic acid).

All experiments were performed in triplicate. All absorption values are expressed on a fresh matter basis. Control for real absorption into cells was performed with boiled and frozen-thawed segments.

RESULTS

Cortex segments absorb phosphate ions from bathing solution. The mean value of uptake rate (for "augmented" segments) from 1 mM KH_2PO_4 in 0.2 mM $CaSO_4$ is 34.3 $\pm$ 3.2 $\mu gP_i\ g^{-1}\ h^{-1}$, *i.e.* 0.35 $\pm$ 0.02 $\mu mol\ P_i\ g^{-1}\ h^{-1}$.

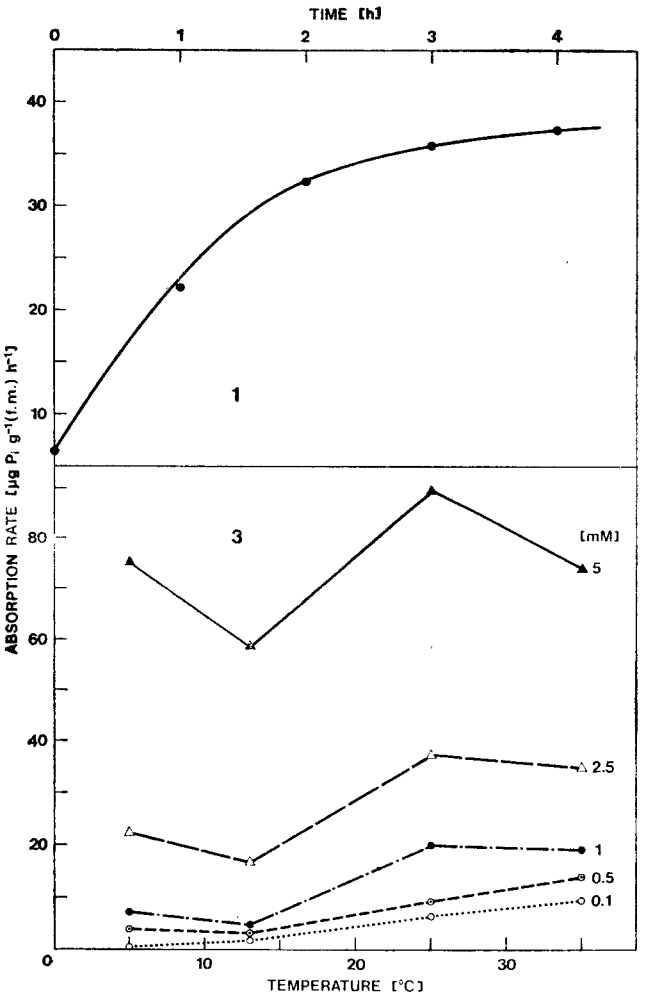

Fig. 1. The dependence of phosphate uptake rate on the duration of washing ("augmentation") period in aerated 0.2 mM $CaSO_4$. 1 mm cortex layer segments were washed 0—4 h in 0.2 mM $CaSO_4$ at room temperature. Then the segments were incubated in 1 mM KH_2PO_4 in 0.2 mM $CaSO_4$ for 5 h at 25 $\pm$ 0.5 °C. The mean SE of given values is 8.5%.

Fig. 3. Temperature dependence of phosphate uptake in maize root cortex segments. 1 mm segments were incubated in various concentrations of KH_2PO_4 in 0.2 mM $CaSO_4$ for 5 h at various temperatures. The mean SE of given values is 8.5%.

The uptake rate depends on the duration of preincubation in aerated 0.2 mM $CaSO_4$. Freshly cut segments absorb only slightly (5.67 $\pm$ 0.31 $\mu g\ P_i\ g^{-1}\ h^{-1}$); the uptake rate increases with the time of preincubation till 4 h (see Fig. 1). This increase in uptake rate is fully inhibited by the presence of 10 $\mu g\ ml^{-1}$ cycloheximide. DNP(10^{-4} M) in the preincubation solution inhibited the

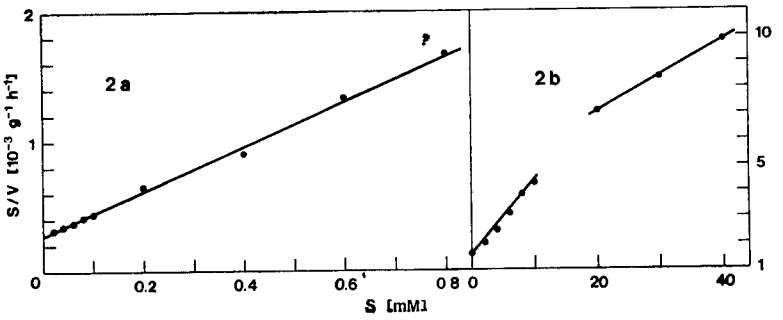

Fig. 2. Hanes's transformation of reaction isotherm for phosphate uptake. 1 g of 1 mm cortex layer segments were incubated in 10 ml of various concentrations of KH_2PO_4 in 0.2 mM $CaSO_4$ for 5 h at 25 $\pm$ 0.5 °C. The mean SE of given values is 9.3%.

subsequent P_i uptake (in the absence of DNP in incubation solution) by 34 %. Addition of 0.5 or 1 mM KH_2PO_4 to the pretreatment solution had no effect on the increase of uptake rate.

Boiled (5 min in 0.2 mM $CaSO_4$) and frozen-thawed segments did not absorb any phosphate ions.

The absorption isotherm (in Hanes's transformation) is shown in Fig. 2. It is obvious that there are 3 distinct phases in the concentration range of 0.02—40 mM P_i (correlation coefficients for the straights are 0.998, 0.991 and 0.998, respectively). The transitions occurred at the following concentrations: 0.8—1 mM and 10—20 mM. Following K_m and V_{max} values were read from the plot:

K_m[mM] :	0.37 $\pm$ 0.06	3.82 $\pm$ 0.37	27.67 $\pm$ 0.65
V_{max}[$\mu g\ P_i\ g^{-1}\ h^{-1}$] :	3.33 $\pm$ 0.29	39.40 $\pm$ 3.91	66.67 $\pm$ 6.70

The temperature dependence of phosphate uptake rate is quite complex (Fig. 3). In the range of low phosphate concentrations (0.1—1 mM) the lowest uptake was recorded at low temperatures (5—13 °C). At higher phosphate concentrations uptake is higher at 5 °C than at 13 °C and uptake from 5 mM KH_2PO_4 is at 5 °C even equal to that at 35 °C. Absorption minimum at higher phosphate concentrations was found at 13 °C.

Phosphate uptake by cortex tissue has no sharp pH optimum. The highest uptake was recorded at low pH values (pH 3.5—5.5), then a slight decrease of uptake rate till pH 7.0 and a sharper decrease in the alkaline region were recorded. But during the relatively long incubation period the pH of bathing solution had changed: in the acid region it was slightly alkalinized (about 0.5 pH unit) and in the alkaline region more pronouncedly acidified (1—2 pH units).

DNP (10^{-4} M) completely inhibited the uptake of phosphate from 0.1 and 1 mM KH_2PO_4, but failed to inhibit phosphate uptake from 5 mM KH_2PO_4. Similarly, 10^{-3} and 10^{-4} M iodoacetate inhibited phosphate uptake from 1 mM KH_2PO_4 completely and 10^{-5} M iodoacetate by 55.7%, but the uptake from 5 mM phosphate solution was left unaffected.

The sulfhydryl reagents NEM and PCMB(S) actively suppressed phosphate uptake. 10^{-4} M NEM inhibited phosphate uptake from both 1 mM and 5 mM KH_2PO_4 by 42.4%, 5.10^{-4} NEM by 72% and 10^{-3} M NEM by 81.6%. 10^{-4} M PCMB inhibited phosphate uptake from both 1 and 5 mM P_i by 42%.

Cycloheximide (10 μg ml^{-1}) and actinomycin D(10 μg ml^{-1}) suppressed phosphate uptake only by 14.5 and 10%, respectively (these values are not statistically significant-*t*-test, $n = 3$).

DISCUSSION

Maize root cortex tissue actively absorbs phosphate ions from bathing medium. Higher uptake rates were determined only after a certain washing (or so called "augmentation") period (1—4 h). This is a well known phenomenon (*e.g.* LEONARD and HANSON 1972, VAN STEVENINCK 1975) which is mostly explained as a period necessary for damage repair. Cycloheximide when present in washing solution completely blocked this process, which suggests the need of *de novo* proteosynthesis in the augmentation process.

Our results show that isolated cortex tissue is able to absorb phosphate, so that it does not lack absorption capacity. But VAN IREN and BOERS-VAN DER SLUIJS (1980) proved that mature cortex cells lack this capacity *in vivo*. We cannot exclude that absorption capacity found in our experiments is the result of some changes of metabolism and membrane properties due to cut injury.

In the concentration range 0.02—40 mM P_i the dependence of uptake rate gave in Hanes's plot (Fig. 2) 3 straight lines. Although there were 4 phases in Lineweaver-Burk's transformation, the analysis of correlation coefficients of data in Hanes's transformation showed higher probability of only one phase in the region of low concentrations (0.02—0.8 mM). Moreover, HARRISON *et al.* (1981) found Hanes's transformation to be most reliable. The K_m and V_{max} values found correspond well with those published recently by MICHALÍK (1982) for roots of intact maize plants. Our results support Nissen's multiphasic theory (NISSEN 1973, 1977). But we are aware of the relatively high experimental error of our measurements (8.8%) and we cannot exclude the possibility of multiphasic interpretation of continual reaction isotherm as suggested by BORSTLAP (1981).

We have found no sharp pH optimum for phosphate uptake. Very similar pH dependence was found by ULLRICH-EBERIUS *et al.* (1981) in *Lemna gibba* and by LIN (1980) in maize root protoplasts. As the pH value of the medium changed during our measurements (these changes could be due to K^+ uptake, which is known to proceed as $K^+—H^+$ antiport), we can with certainty say only that the phosphate uptake is reduced in the alkaline region, which fact complies with the proposed mechanism of $P_i—OH^-$ antiport.

The complex temperature dependence of phosphate uptake is difficult to explain. Recently PEDRO-BRAVO and URIBE (1981) studied the temperature

dependence of phosphate uptake in intact maize roots. They found 13 °C to be the transition temperature and suggest that some changes in cell membrane structure are induced with temperature changes. Relatively high uptake rates found at 5 °C at higher phosphate concentrations show that uptake in this concentration range is only slightly dependent on metabolic energy (POLLEY and HOPKINS 1979, PEDRO-BRAVO and URIBE 1981). This view is further supported by the inability of DNP and iodoacetate to suppress phosphate uptake from solutions of higher phosphate concentrations. Thus it is evident that at higher phosphate concentrations (>1 mM) mostly diffusion is responsible for phosphate entry into maize root cortex tissue.

The inhibiting effect of sulfhydryl agents on transport processes is well known (LIN 1979, 1981). But recently LICHTNER *et al.* (1981) proved that NEM and PCMBS caused membrane potential depolarization, which could be the cause of changed transport properties of the membranes. Therefore the inhibitory effect of sulfhydryl agents need not necessarily be proof of active —SH groups in the carrier molecules.

Acknowledgement

The technical assistance of Miss Ivana Vurmová is gratefully acknowledged.

REFERENCES

BORSTLAP, A. C.: Invalidity of multiphasic concept of ion absorption in plants. — Plant Cell Environ. **4** : 189—195, 1981.

BORST-PAUWELS, G. W. F. H., DOBBLEMAN, J.: The mechanism of inhibition of anaerobic phosphate uptake by fatty acids in yeast. — Biochim. biophys. Acta **290** : 348—354, 1972.

BOWLING, D. J. F., GRAHAM, R. D., DUNLOP, J.: The relationship between the cell electrical potential difference and salt uptake in the roots of *Helianthus annuus*. — J. exp. Bot. **108** : 135—140, 1978.

CRAM, W. J.: Chloride fluxes in cells of the isolated root cortex of *Zea mays*. — Aust. J. biol. Sci. **26** : 757—779, 1973.

HARRISON, S. J., NICHOLAS, W. L., PHIPPS, D. A.: Calculation of kinetic constants for ion uptake studies. A comparison of methods in relation to their effects on data interpretation. — J. Plant Nutr. **3** : 181—192, 1981.

LAŠTŮVKA, Z., MINÁŘ, J.: [Water culture of higher plants.] In Czech. — Fol. Fac. Sci. nat. Univ. Purk. brunensis **8** [Biol. 16(2)] : 1—83, 1967.

LATIES, G. G., BUDD, K.: The development of differential permeability in isolated steles of corn roots. — Proc. nat. Acad. Sci. USA **52** : 462—469, 1964.

LEONARD, R. T., HANSON, J. B.: Induction and development of increased ion absorption in corn root tissue. — Plant Physiol. **49** : 430—435, 1972.

LICHTNER, F. T., LUCAS, W. J., SPANSWICK, R. M.: Effect of sulfhydryl reagents on the biophysical properties of the plasmalemma of *Chara corallina*. — Plant Physiol. **68** : 899—904, 1981.

LIN, W.: Potassium and phosphate uptake in corn roots. Further evidence for an electrogenic H^+/K^+ exchanger and an OH^-/P_i antiporter. — Plant Physiol. **63** : 952—955, 1979.

LIN, W.: Corn root protoplasts. Isolation and general characterization of ion transport. — Plant Physiol. **66** : 550—554, 1980.

LIN, W.: Inhibition of anion transport in corn root protoplasts. — Plant Physiol. **68** : 435—438, 1981.

METLER, I. J., LEONARD, R. T.: Ion transport in isolated protoplasts from tobacco suspension cells. I. General characteristics. — Plant Physiol. **63** : 183—190, 1979.

MICHALÍK, I.: The influence of phosphate concentration on the kinetics of uptake by maize roots. — Biol. Plant. **24** : 161—169, 1982.

MURPHY, J., RILEY, J. P.: A modified single solution method for the determination of phosphate in natural waters. — Anal. chim. Acta **27** : 31—36, 1962.

NISSEN, P.: Multiphasic uptake in plants. I. Phosphate and sulphate. — Physiol. Plant. **28** : 304—316, 1973.

NISSEN, P.: Ion uptake in higher plants and KCl stimulation of plasmalemma adenosine triphosphatase: comparison of models. — Physiol. Plant. **40** : 205—214, 1977.

PEDRO-BRAVO, F., URIBE, E. G.: Temperature dependence of the concentration kinetics of absorption of phosphate and potassium in corn roots. — Plant Physiol. **67** : 815—819, 1981.

POLLEY, L. D., HOPKINS, J. W.: Rubidium (potassium) uptake by *Arabidopsis*. A comparison of uptake in suspension culture and by roots of intact seedlings. — Plant Physiol. **64** : 374 to 378, 1979.

RAVEN, J. A.: Phosphate transport in *Hydrodictyon africanum*. — New Phytol. **73** : 421—432, 1974.

SMITH, F. A., RAVEN, J. A.: Energy-dependent processes in *Chara corallina*: absence of light stimulation when only photosystem I is operative. — New Phytol. **73** : 1—12, 1974.

ULLRICH-EBERIUS, C. I.: Die pH-Abhängigkeit der Aufnahme von $H_2PO_4^-$, SO_4^{2-}, Na^+ and K^+ und ihre gegenseitige Beeinflussung bei *Ankistrodesmus braunii*. — Planta **109** : 161—176, 1973.

ULLRICH-EBERIUS, C. I., NOVACKY, A., FISCHER, E., LÜTTGE, U.: Relationship between energy-dependent phosphate uptake and the electrical membrane potential in *Lemma gibba* G 1. — Plant Physiol. **67** : 791—801, 1981.

VAN IREN, F., BOERS-VAN DER SLUIJS, P.: Symplasmic and apoplasmic radial ion transport in plant roots. Cortical plasmalemmas lose absorption capacity during differentiation. — Planta **148** : 130—137, 1980.

VAN IREN, F., LOULOU JOOLEN, M., GERRITSEN, A. F. C., NOORDERVLIET, M. A. W., BOERS-VAN DER SLUIJS, F. P.: The lag-phase of potassium translocation from the root to the shoot of low-salt barley plants. — Kinetic and localization studies. — Physiol. Plant. **52** : 15—22, 1981.

VAN STEVENINCK, R. F. M.: The "washing" or "ageing" phenomenon in plant tissues. — Annu. Rev. Plant Physiol. **26** : 237—258, 1975.

BOOK REVIEW

DATENSAMMLUNG ZUR TOXIKOLOGIE DER HERBIZIDE. — Verlag Chemie, Weinheim 1981. 308 S. Lfg. 1/DM 58,—; 2/DM 98,—; 3/DM 196,—.

Mit der Einführung zahlreicher neuer, organischer Herbizide, Insektizide und Fungizide in die landwirtschaftliche Praxis konnten manche bis dahin ungelöste Probleme optimal gelöst werden. Freilich gab es nicht nur hervorragende Problemlösungen, sondern es entstanden auch neue Probleme. Der Pflanzenschutz bedient sich kulturtechnischer, biologischer, physikalischer und chemischer Methoden, deren Anwendung Eingriffe in das Umweltgefüge darstellen. Das Buch, herausgegeben von der "Arbeitsgruppe Toxikologie" der Kommission für Pflanzenschutz-, Pflanzenbehandlungs- und Vorratsschutzmittel der Deutschen Forschungsgemeinschaft (BRD), informiert verständlich und umfassend über die Eigenschaften der Herbizide, die heute vollauf verwendet werden. Zur Charakterisierung der einzelnen Herbizide werden folgende Angaben gemacht: 1. Die wichtigsten chemisch-physikalischen Daten, 2. Anwendungsbereich, 3. Allgemeiner Wirkungscharakter, 4. Tierexperimentelle Grundlagen, 5. *In-vitro*-Versuche bzw. Versuche zur Aufklärung des Wirkungsmechanismus, 6. Schicksal im tierischen Organismus, 7. Schicksal in Pflanzen, Boden und Wasser, 8. Wirkungen auf den Menschen, 9. Wirkungen auf Haus-, Nutz- und Wildtiere, 10. Wechselwirkungen mit anderen Stoffen, 11. Therapie bei Vergiftungsfällen, 12. Zusammenfassende Beurteilung und 13. Literatur. Die Ergänzungslieferungen der Dokumentationen sollen die Datensammlungen zur Toxikologie der Herbizide auf den neuesten Stand bringen. Der Möglichkeit der Ergänzung dieser Datensammlung wurde dadurch Rechnung getragen, dass zur Veröffentlichung eine ergänzungsfähige Ringbuchausgabe gewählt wurde. Der Anhang ist in zwei Teile gegliedert: 1. Wirkstoffe, Handelsbezeichnungen und Hersteller herbizider Pflanzenschutzmittel, und 2. Anschriften der Hersteller. Das Buch ist für Biochemiker, Agronomen und Umweltschutzbeauftragte bestimmt.

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