ЕКОЛОГІЯ І ВИРОБНИЦТВО

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ACCUMULATION OF CAESIUM BY ARBUSCULAR MYCORRHIZAL SYMBIOSES ON A CELLULAR LEVEL

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The distribution of stable ^{133}Cs isotope in structures of arbuscular mycorrhizal fungi *Glomus intraradices* and *Glomus mosseae* as well as in root tissues of *Plantago lanceolata* on the microscopic level is studied. The role of arbuscular mycorrhizal fungi in caesium accumulation in plant root system and impact of high caesium concentrations in soil on mycorhhizal colonization parameters were considered. The comparative analysis of caesium and potassium uptake by mycorrhizal and nonmycorrhizal *P. lanceolata* was conducted. It was found that arbuscular mycorrhizal fungi could participate in accumulation and transport of Cs to plants. The obtained results suggest that mycorrhiza limits caesium transfer and at the same time enhances potassium transport to aboveground part of plants. ***Keywords:*** caesium, arbuscular mycorrhiza, scanning electron microscopy, arbuscular mycorrhizal fungi, mycorrhizal colonization.

Накопичення цезію арбускулярними мікоризними симбіозами на клітинному рівні. Сергій Валерійович Дубчак. Досліджено розподіл стабільного ізотопу ^{133}Cs на мікроскопічному рівні в структурах арбускулярних мікоризних грибів *Glomus intraradices* і *Glomus mosseae*, а також в кореневих тканинах рослин *Plantago lanceolata*. Розглянуто роль арбускулярних мікоризних грибів у накопичення цезію в кореневій системі рослин та вплив високих концентрацій цезію в грунті на параметри мікоризної колонізації. Проведено порівняльний аналіз накопичення цезію і калію мікоризованими і немікоризованими рослинами *P. lanceolata*. Встановлено, що арбускулярні мікоризні гриби можуть брати участь в накопиченні Cs та в його переносі до рослин. Отримані результати свідчать про те, що мікориза обмежує надходження цезію, і в той же час збільшує накопичення калію в надземній частині рослин. *Ключові слова:* цезій, арбускулярна мікориза, сканувальна електронна мікроскопія, арбускулярні мікоризні гриби, мікоризна колонізація.

Накопление цезия арбускулярными микоризными симбиозами на клеточном уровне. Сергей Валериевич Дубчак. Исследовано распределение стабильного изотопа ^{133}Cs на микроскопическом уровне в структурах арбускулярних микоризных грибов *Glomus intraradices* и *Glomus mosseae*, а также в корневых тканях растений *Plantago lanceolata*. Рассмотрена роль арбускулярных микоризных грибов в накопление цезия в корневой системе растений и влияние высоких концентраций цезия в почве на параметры микоризной колонизации. Проведен сравнительный анализ накопления цезия и калия микоризоваными и немикоризоваными растениями *P. lanceolata*. Установлено, что арбускулярные микоризные грибы могут участвовать в накоплении Cs и в его переносе к растениям. Полученные результаты свидетельствуют о том, что микориза ограничивает поступление цезия, и в то же время увеличивает накопление калия в надземной части растений. *Ключевые слова*: цезий, арбускулярная микориза, сканирующая электронная микроскопия, арбускулярные микоризные грибы, микоризная колонизация.

Statement of the problem. The naturally occurring stable ^{133}Cs, whose main natural source is pollucite (an aluminosilicate mineral) is the rarest of alkali metals with petty economic value. Up to date, no substantial biological role of caesium has been found, although its trace quantities occur in most living organisms [1]. The average concentration of ^{133}Cs in different soil types varies between 0.3 and 25.7 mg·kg^{-1} dry soil weight. This corresponds to micromolar Cs^+ concentrations in soil solutions. ^{133}Cs is the only stable caesium isotope, however it exists in various isotopic forms with atomic masses in the range from ^{112}Cs to ^{151}Cs [2]. The extended interest to caesium properties was arisen in recent several decades due to numerous releases (totally about 10^{18} Bq) of long-lived ^{137}Cs radioisotope to the environment after nuclear weapon tests and nuclear accidents resulting in contamination of large areas [3].

The significant part of radiocaesium isotopes in the soil is accumulated and retained in "immobile" condition for a long time. The mycorrhizal fungi make a considerable contribution to this process. Their mycelia could slow down the vertical migration of radiocaesium in soils and consequently maintain this radionuclide in upper soil layers replete with plant roots and mycorrhizal mycelium. In turn, the radiocaesium immobilized in fungal structures could be transferred by the mycelia to their plant hosts [4].

Analysis of recent studies and publications.It was demonstrated that wide number of soil fungal species are involved in radiocaesium immobilization including arbuscular mycorrhizal (AM) fungi [5]. These fungi are important participants in the Cs cycle in the upper layers soils. They have strong impact on mobility of radiocaesium in the soil and result to unavailability of this radionuclide to the other components in various terrestrial ecosystems [6].

The role of arbuscular mycorrhizal (AM) fungi on the acquisition of radiocaesium by plants remains poorly understood and rather controversial. Also, the concentration of potassium in the soil could have interfered with the capacity of AM fungi to accumulate or transport Cs. Furthermore, the various AM fungi and plants studied could also explain the controversial conclusions obtained, since AM fungi and plants have probably different capacity to accumulate and transport radiocaesium. As a consequence, it was successively suspected that AM fungi could accumu-

late radiocaesium in their extraradical or intraradical structures, transport Cs to their hosts and influence on its distribution among plant roots/shoots [7].

In addition, only a few pilot studies were related to analysis of caesium uptake and distribution in plant root tissues and AM fungal structures at cellular level [8,9]. Thus, the evidence of AM fungi participation in caesium accumulation and transport to plants was shown in from results of X-ray microanalysis of *Plantago lanceolata* mycorrhizal root material using the stable ^{133}Cs isotope [9]. Being added in moderate concentration (400 $mg \cdot kg^{-1}$) to the soil substrate as the complete analogue of radiocaesium, caesium was found both in extraradical and intraradical structures of AM fungi used in this research.

Objectives of research. Accordingly, the objectives of this work were therefore to identify the capacity of AM fungi to take up and transfer stable caesium isotope (^{133}Cs) to their hosts and, to study the influence of arbuscular mycorrhiza stable caesium uptake by plants and reveal the capacity of AM fungi to accumulate Cs isotopes in their extraradical and intraradical structures.

The main material of the study. The pot substrata for plant cultivation were composed of sterilized sand and clay (volumetric proportion v:v = 3:1) and fertilized with the rock phosphate (50 $g \cdot l^{-1}$ of substrate). The sand was preliminarily sterilized (heated twice in the electric oven for 1 hour at 100^{o} C with 48 hours interval in between). Two weeks after the sterilization, the clay and rock phosphate were added to the sand, and the prepared mixture was saturated with solutions containing stable ^{133}Cs isotope. The caesium source (in form of caesium carbonate powder Cs_2CO_3 obtained from Sigma-Aldrich) was dissolved in deionised water. According to the pilot experiments related to impact of different levels of stable caesium in the soil on *Plantago laceolata* plants as a model species, the distinct and strong toxic inhibition of plant growth (death of more than 70 % of plants during first two weeks, smaller size and yellowish colours of shoots) was observed at 1000 $mg \cdot kg^{-1}$ of ^{133}Cs concentration in the soil. Based on these results, the caesium concentration in substrata was adjusted as 400 $mg \cdot kg^{-1}$ that is below the inhibition level and thus minimizes Cs toxic impact on plants.

The pH of prepared $CsCO_3$ solutions was adjusted to 6.5 with 10 % KOH. The substrata in pots were watered with caesium solutions from bellow using a capillary system, according to the technique developed by Walker and Lewis [10]. It allowed reaching the homogeneous distribution of caesium isotopes though the pot volume. The mass of substratum in each pot was 1.3 kg.

Plantago lanceolata L. as a plant species capable to form efficient association with a broad range of AM fungi was selected for our study. This plant was chosen due to its prevalence in diverse habitats as well as widespread application in biological studies as model mycorrhizal species [11]. The plants were cultivated in the presence or absence of the following AM fungal species: *Glomus intraradices* (syn. *Rhizophagus intraradices*; strain BIO, obtained from BIORIZE, Dijon, France); *Glomus mosseae* (syn. *Funneliformis mosseae*; strain BEG 12, obtained from Banque Européenne des Glomeromycota).The AM fungal inoculum of *G. in-*

traradices or *G. mosseae* (ca. 5 g inoculum per pot) was added to the substrata simultaneously with seedlings of *P. lanceolata*.

The plants were cultivated for three months in pots sealed in transparent Sun bags (Sigma^{TM} Aldrich, Poznan, Poland) kept in a growth chamber at 20 °C, with a photoperiod of 12 h light and 12 h darkness and at photosynthetic photon flux density 30 ± 6 μmol·(s·m^2)$^{-1}$.

The scanning electron microscopy (SEM) and energy-dispersive X-ray microanalysis (EDX) were applied to analyse the uptake and distribution of ^{133}Cs in plant root tissues and AM fungal structures at cellular level. The biological samples were prepared according to the procedure described by Tylko [12]. The roots of mycorrhizal and nonmycorrhizal *P. lanceolata* cultivated on substrata spiked with stable Cs (400 mg·kg^{-1}) were dug out, washed in distilled water to remove soil grains and gently dried with filter paper. Young parts of plant roots were first analysed with light microscope, and root fragments with distinctly visible attached extraradical AM fungal hyphea were selected for the further treatment and analysis. These roots were dissected and cut into pieces of about 5 mm long. Then, root samples were collected into bundles (10–15 pieces of roots), immersed in a OCT compound (Sakura Finetek Europe, NL) and quickly plunge frozen in isopentan precooled to –145 °C with liquid nitrogen. Frozen standards were cut in cryomicrotome into 30 μm sections at –30 °C (Shandon OT, Astmoor, Runcorn, Cheshire, UK) and placed into precooled adhesive graphite laminas. Dried specimens were coated with 15 nm layer of carbon to prevent charging (JEE 4B, JEOL, Tokyo, Japan).

The prepared specimens were analysed using JEOL JSM 5410 scanning electron microscope equipped with Noran energy-dispersive spectrometer (Noran Instruments Inc., WI, USA). Elemental analyses were performed at plant cell walls of cortical and vascular tissues of *P. lanceolata* and structures of AM fungi *G. intraradices* and *G. mosseae* in both in point and raster mode. During the measurements the electron beam was focused on the walls of cortex and vascular tissues as well as on fungal structures (arbuscules, extraradical hyphae), where the dead-time was about 25 – 30 %. The concentration of Cs was calculated using its L_α emission line in X-ray spectrum (E_{emis} = 4.286 keV, critical ionization energy $E_{cr.ion}$ = 5.714 keV), on the basis of gelatine standard with known concentration of caesium.

The statistical analysis of data was performed with Statistica 8 (Statsoft, Krakow) software. The parametric one way ANOVA test was applied. The normality of data distribution was checked by the Levene's test. The significance between treatment means was ranked by the Tukey's test for unequal numbers at $p < 0.05$. In cases of data on mycorrhizal colonization or when the transformation was not successful to obtain the normal distribution of data, the significance between treatment values was ranked by non-parametric U Mann-Whitney test with significance level $p < 0.05$.

The measured dry weights of roots and shoots of mycorrhizal and nonmycorrhizal *P. lanceolata* plants collected from soil treated with ^{133}Cs and nonpolluted soil did not differ significantly.

No significant differences were also observed while two different AM fungal species were used for the experiment. Although, a tendency of higher biomass and shoot length in case of mycorrhizal plants grown both on ^{133}Cs spiked and non-spiked soil was visible.

Plants noninoculated with AM fungi had no structures typical for mycorrhizae. Significant differences in *P. lanceolata* plants colonization were found between two AM fungal species used. Much higher colonization parameters (frequency of mycorrhiza F, mycorrhizal intensity M,m and arbuscular richness A,a) were obtained in case of *G. intraradices* than in *G. mosseae* (Fig. 1). In case of the second fungal species, the differences between AM colonization parameters of plants grown on Cs spiked and non-spiked substrata were not found, while significant differences were obtained for *G. intraradices* which colonization was reasonably lower in plants grown on soil spiked with ^{133}Cs.

The intraradical structures of both AM fungi were morphologically typical for *Arum* type of mycorrhizae. No morphological differences were observed between intraradical fungal structures in mycorrhizal roots between plants from Cs spiked and non-spiked soil.

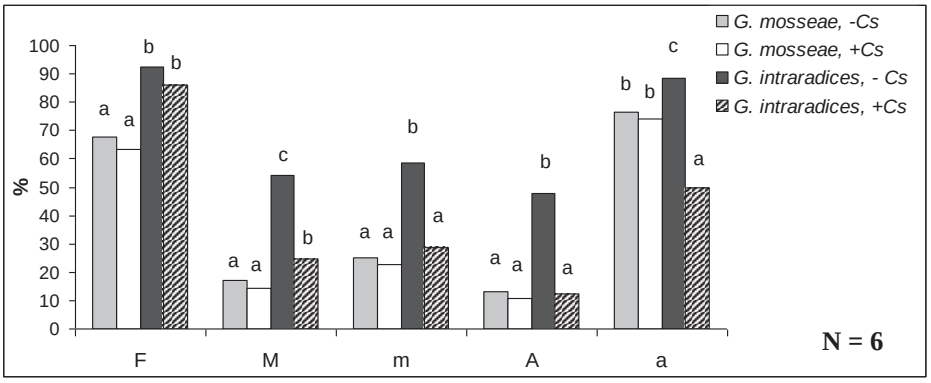

Figure 1. AM colonization parameters (%, medians) of Plantago lanceolata mycorrhizal with Glomus mosseae or Glomus intraradices: F%, frequency of mycorrhiza; M%, mycorrhizal colonization intensity for all roots; m%, mycorrhizal colonization intensity within individual mycor rhizal roots; A%, arbuscule richness for all roots; a%, arbuscule richness in root fragments where the arbuscules were present. Plants were cultivated on non-spiked soil (–Cs) and soil spiked with ^{133}Cs (+Cs, 400 mg·kg^{-1}); the different letters above bars mean statistically significant differences ($p < 0.05$).

Part 2. ^{133}Cs distribution in plant and AM fungal tissues on the microscopic scale.

The numerous arbuscules of *G. mossseae* and *G. intraradices* located within cortical layer cells were revealed in analysed root fragments of *P. lanceolata* plants (Fig. 2A-D). The extraradical fungal hyphea was found at the outer surface of only few plant root pieces. *P. lanceolata* plants colonized with *G. intraradices* had nearly 60 % higher Cs concentration in cell walls of root cortex than nonmycorrhizal ones.

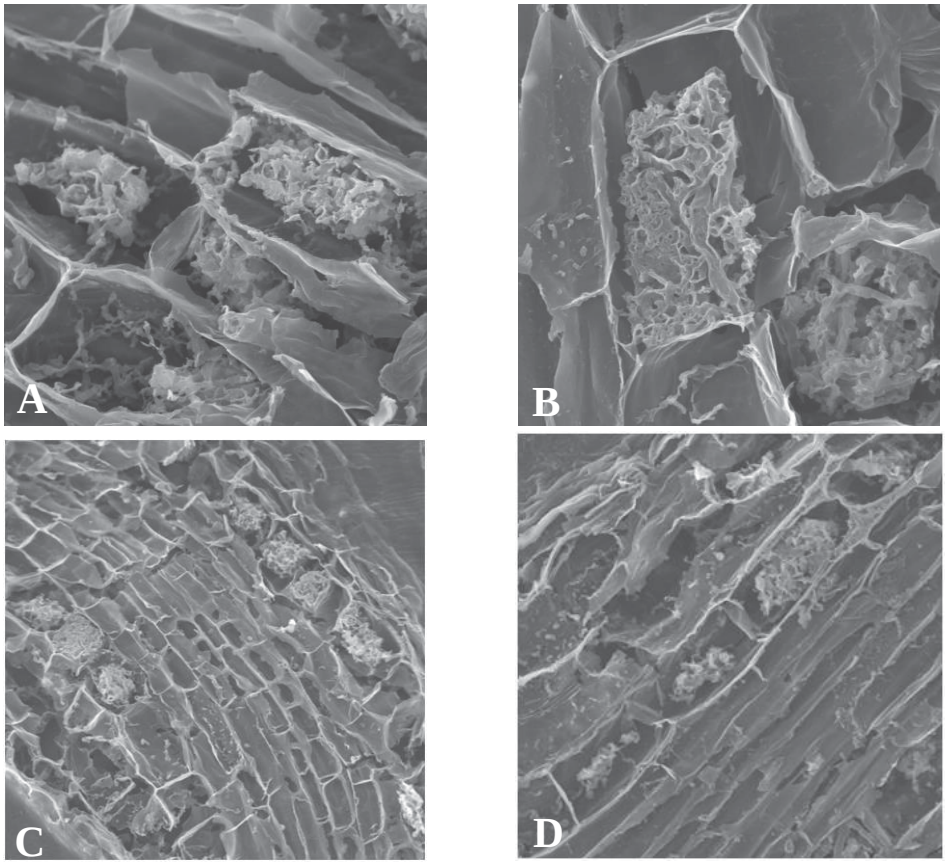

Figure 2. Arbuscules of Glomus mosseae (A, x1500; C, x500) and Glomus intraradices (B, x2000; D, x750) localized in cortical root cells of Plantago lanceolata grown on substrata spiked with ^{133}Cs (400 mg·kg^{-1})

At the same time, Cs concentration in root cortex of plants mycorrhizal with *G. mosseae* was ca. 50 % lower than that in nonmycorrhizal ones. The root vascular tissue of mycorrhizal and nonmycorrhizal plants was characterized by nearly the same levels of Cs content. No significant differences were found between Cs concentration in arbuscules and extraradical hyphea of both AM fungal species. Although, the moderate increase of Cs levels was noticed in arbuscules and extraradical hyphea of *G. intraradices* (ca. 10% and 14 % respectively) as compared to those of *G. mosseae* (Fig.3A).

The concentration of Cs in the cortex and vascular tissue of plants colonized with *G. mosseae* was approximately the same, whereas plants mycorrhizal with *G. intraradices* had about threefold higher Cs concentration in their root cortex than in the vascular tissue (Fig. 3A). This fact shows the significant reduction of Cs translocation to aboveground part of plants in case of their colonization with *G. intraradices*.

^{133}Cs is a stable isotope that reveals similar biochemical properties to K [13]. Therefore the concentration of potassium as the element having chemical affinity with caesium was measured as well. In the root cortex of nonmycorrhizal *P. lanceolata* plants it was respectively fivefold and twofold higher than those of plants colonized with *G. mosseae* and *G. intraradices* (Fig. 3B). The opposite tendency was revealed in vascular tissue of nonmycorrhizal plants, where K concentration was respectively 24 % and 115 % lower than those of plants mycorrhizal with *G. mosseae* and *G. intraradices*. Similarly to Cs, the K concentration in structures of both AM fungi did not differ significantly, although arbuscules and extraradical hyphae of *G. intraradices* showed moderate increase of K concentration (15 % and 10% respectively) as compared to those of *G. mosseae* (Fig. 3B).

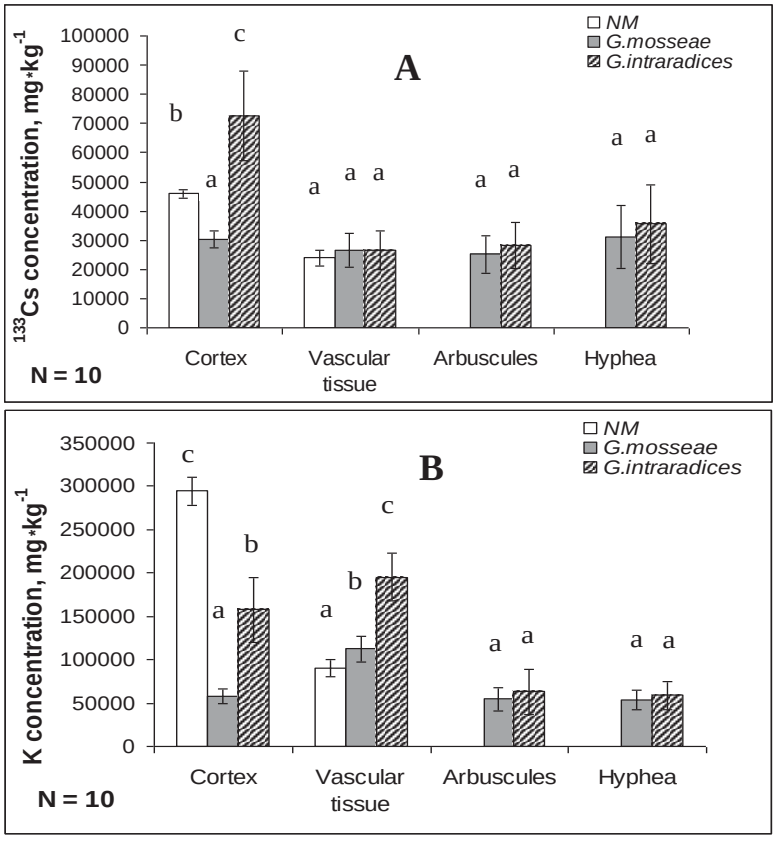

Figure 3. Concentrations of ^{133}Cs (A) and K (B) in cortex and vascular tissue of Plantago lanceolata mycorrhizal or not (NM) with Glomus mosseae or Glomus intraradices; arbuscules and extraradical hyphae of Glomus mosseae and Glomus intraradices (mg·kg^{-1}). Plants were cultivated on substrata spiked with ^{133}Cs (400 mg·kg^{-1}). The results are presented as mean ± standard deviation; the different letters above bars mean statistically significant differences ($p < 0.05$).

The obtained results are in good agreement with the data of total reflection X-ray fluorescence analysis (TXRF) presented in [9] that demonstrate significant reduction of ^{133}Cs concentration in the root system (from 2200 to 1400 mg·kg^{-1}) and aboveground organs (from 4300 to 3900 mg·kg^{-1}) of *P. lanceolata* inoculated with *G. intraradices* as compared to nonmicorrhizal plants. At the same time, the colonization of *P. lanceolata* with AM fungus *G. mosseae* led to 25% and 37% increase of ^{133}Cs concentration in their aboveground parts as compared correspondingly to control plants and those inoculated *G. intraradices*.

The translocation of Cs and K within roots (i.e. the ratio of element concentrations in vascular and cortical root tissues) of plants colonized with *G. intraradices* and *G. mosseae* was different. Thus, the concentration of K in mycorrhizal plants raised in their vascular tissues in comparison with the root cortex, however such tendency was not noticed for Cs. Accordingly, the translocation of K from cortical to vascular tissue of mycorrhizal *P. lanceolata* plants was more intensive than Cs translocation within root tissues of these plants. It demonstrates that significant amount of Cs is retained in the root cortex of mycorrhizal plants since it is not transferred to vascular tissue, while K is more efficiently transported from cortical to vascular tissue. This result suggests that mycorrhiza limits the Cs transport and at the same time enhances the K transport to aboveground part of plants.

Conclusions. ^{133}Cs is a stable isotope which average environmental concentration in different soil types is from 2 to 3 mg·kg^{-1} [1]. In the present research Cs soil concentration (400 mg·kg^{-1}) was nearly two orders higher than the natural ones. Although, in case of stable Cs there are no radiation effects, it was important to study effects of Cs presence on distribution of elements in plant tissues. Such studies are not possible with radioactive Cs isotopes due to low levels used and lack of equipment capable to distinguish distribution of elements in such case.

The significant differences in colonization degree of *P. lanceolata* plants with *G. intraradices* or *G. mosseae* revealed in the experiment confirm the results previously obtained by Calvet [14], who found a higher extent of *G. intraradices* colonization as compared with *G. mosseae*. Also *G. intraradices* was more sensitive to increased concentration of ^{133}Cs in the soil that testifies about its better availability in application for environmental monitoring. The effect of Cs on mycorrhizal colonization could be explained since this element, similarly to its chemical analogue K, is toxic for the mycorrhizal fungus at high concentrations in the soil [15].

The evidence of AM fungi participation in accumulation and transport of Cs to plants was shown from results of X-ray microanalysis for *P. lanceolata* root material. ^{133}Cs was found both in arbuscules and extraradical hyphea of *G. intraradices* and *G. mosseae* used in this study. The distribution of Cs and K in arbuscules and extraradical hyphea of both AM fungi correlated well, since ratios of K/Cs concentrations in these structures did not differ significantly. Such similarity in K and Cs localization suggests the existence of common uptake and translocation mechanisms for these elements by AM fungi. The similar conclusions were made by Dupré de

Boulois [6], who supposed that the uptake of Cs is mediated by K transporters as observed for plants.

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