

CARBON SOURCES IN FRUIT CARBONATE OF *BUGLOSSOIDES ARVENSIS* AND CONSEQUENCES FOR ^{14}C DATING

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ABSTRACT. Fruit carbonate of *Buglossoides arvensis* (syn. *Lithospermum arvense*) is a valuable dating and paleoenvironmental proxy for late Quaternary deposits and cultural layers because CaCO_3 in fruit is assumed to be accumulated from photosynthetic carbon (C). However, considering the uptake of HCO_3^- by roots from soil solution, the estimated age could be too old depending on the source of HCO_3^- allocated in fruit carbonate. Until now, no studies have assessed the contributions of photosynthetic and soil C to the fruit carbonate. To evaluate this, the allocation of photo-assimilated carbon and root uptake of HCO_3^- was examined by radiocarbon (^{14}C) labeling and tracing. *B. arvensis* was grown in carbonate-free and carbonate-containing soils (sand and loess, respectively), where ^{14}C was provided as (1) $^{14}\text{CO}_2$ in the atmosphere (5 times shoot pulse labeling), or (2) $\text{Na}_2^{14}\text{CO}_3$ in soil solution (root-labeling; 5 times by injecting labeled solution into the soil) during one month of fruit development. Distinctly different patterns of ^{14}C distribution in plant organs after root- and shoot labeling showed the ability of *B. arvensis* to take up HCO_3^- from soil solution. The highest ^{14}C activity from root labeling was recovered in roots, followed by shoots, fruit organics, and fruit carbonate. In contrast, ^{14}C activity after shoot labeling was the highest in shoots, followed by fruit organics, roots and fruit carbonate. Total photo-assimilated C incorporated via shoot labeling in loess-grown plants was 1.51 mg lower than in sand, reflecting the presence of dissolved carbonate (i.e. CaCO_3) in loess. Loess carbonate dissolution and root-respired CO_2 in soil solution are both sources of HCO_3^- for root uptake. Considering this dilution effect by carbonates, the total incorporated HCO_3^- comprised 0.15% of C in fruit carbonate after 10 hr of shoot labeling. However, if the incorporated HCO_3^- during 10 hr of shoot labeling is extrapolated for the whole month of fruit development (i.e. 420-hr photoperiod), fruit carbonate in loess-grown plants incorporated approximately 6.3% more HCO_3^- than in sand. Therefore, fruit carbonates from plants grown on calcareous soils may yield overestimated ^{14}C ages around 500 yr because of a few percentage uptake of HCO_3^- by roots. However, the age overestimation because of HCO_3^- uptake becomes insignificant in fruits older than approximately 11,000 yr due to increasing uncertainties in age determination.

KEYWORDS: *Buglossoides arvensis*, *Lithospermum arvense*, biogenic carbonate, reservoir effect, ^{14}C labeling, radiocarbon dating, paleoenvironmental proxy.

INTRODUCTION

Buglossoides arvensis (L) I.M.Johnst., syn. *Lithospermum arvense* L., (tribe Lithospermeae, family Boraginaceae) is an annual plant 10–50 cm in height with a flowering time between April and July. *B. arvensis* is commonly found in Eurasian arable lands, grasslands, and forest margins. The fruits, which are often incorrectly considered as seeds of *B. arvensis*, are small (approximately 2 mm in diameter), ovoid, and contain CaCO_3 in their epidermal cells and parts of sclerenchyma (for more information about *B. arvensis* see Pustovoytov and Riehl [2006] and references therein) (Figure 1).

Fossil fruits of *B. arvensis* and other members of Lithospermeae are often found in late Pleistocene and Holocene deposits as well as in cultural layers of archaeological sites (Pustovoytov and Riehl 2006). This calls for testing the applicability of carbon (C) isotopes in these fruits for dating purposes and paleoenvironmental reconstructions. Previously, it has been demonstrated that fruit carbonate of another taxon, the genus *Celtis*, can be successfully radiocarbon (^{14}C) dated (Wang et al. 1997; Quade et al. 2014) and serve as a paleoclimate

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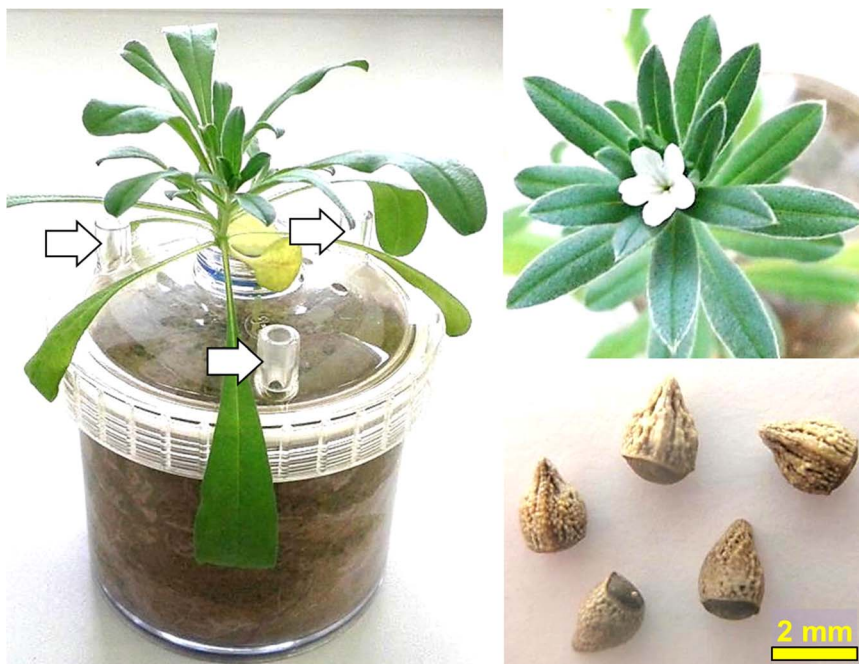


Figure 1 (Left) An approximately 1-month-old *B. arvensis* grown in a 250-mL plastic pot; (top right) *B. arvensis* flower; (bottom right) *B. arvensis* fruits. The arrows show the openings in the pot lid, which were used for irrigation and root labeling (see Labeling Procedure section).

proxy (Jahren et al. 2001). Similar results have been obtained for the tribe Lithospermeae (Pustovoytov et al. 2004; Pustovoytov and Riehl 2006; Pustovoytov et al. 2010). Aside from a few under- or overestimates the achieved ages showed good consistency with independently estimated ages for the archeological layers. The underestimated ages can be explained by post-sedimentary incorporation of fruits into the deposits (i.e. via bioturbation) (Wang et al. 1997; Pustovoytov et al. 2004; Pustovoytov et al. 2010) or slight diagenetic ^{14}C -contamination effects (Quade et al. 2014). An approximately 400-yr overestimate for a herbarium exemplar from the early 19th century has been attributed to occasional depletion in atmospheric ^{14}C concentration because of fossil fuel combustion (Pustovoytov and Riehl 2006).

However, since 1940 it has been known that plants can take up HCO_3^- from soil solution via their roots (Overstreet et al. 1940; Cramer and Richards 1999; Cramer et al. 1999; Viktor and Cramer 2005). It has been shown that the amount of HCO_3^- taken up can be 0.8–2% of the C assimilated through photosynthesis (Pelkonen et al. 1985; Brix 1990; Viktor and Cramer 2003; Ford et al. 2007). However, the HCO_3^- uptake depends on its concentration in soil solution (Cramer and Lips 1995) and the plant species (Stolwijk and Thimann 1957). Some species, for example oats, are tolerant of high HCO_3^- concentrations in the rhizosphere (up to 6.5% CO_2 concentration), but some like tomato may show toxicity symptoms at comparatively low concentrations (approximately 1% CO_2) (Stolwijk and Thimann 1957). HCO_3^- uptake via roots is mostly passive and depends on transpiration rates (Stolwijk and Thimann 1957; Brix 1990; Amiro and Ewing 1992). This may explain why soil-derived HCO_3^- is found at highest concentrations in roots, and decreases with distance from the roots (Brix 1990). However, these concentrations can increase locally in specific plant organs such as newly formed stems or fine roots, through unknown active mechanisms (Vuorinen et al. 1989; Ford et al. 2007).

The HCO_3^- concentration in soil solution is determined by the dissolution of root- and microbe-respired CO_2 , exchange of CO_2 between the soil and atmosphere and dissolution of carbonate containing minerals such as CaCO_3 . The isotopic composition of C in these HCO_3^- sources differs: while HCO_3^- from carbonate minerals is often totally ^{14}C depleted, the ^{14}C content of respired CO_2 is almost identical with the ^{14}C concentration in modern atmospheric CO_2 . Therefore, even a few percent of old C from carbonate minerals can modify ^{14}C ages of a sample. We hypothesize that ^{14}C ages based on fruit carbonate could overestimate the true age of a sample if part of the C comes from soil HCO_3^- . Therefore, the main aims of this experiment were (1) to identify the origin of C in CaCO_3 of fruits, (2) to quantify the contribution of absorbed HCO_3^- from soil, and (3) to calculate the potential effect of root HCO_3^- uptake on ^{14}C dates based on fruit carbonates of *B. arvensis*.

MATERIAL AND METHODS

Experimental Layout

We used 250-mL plastic pots with lids (Sartorius AG, Germany) for plant growth (Figure 1, left). The lids had one main hole in the middle, for the growing plant stem, and three smaller openings, which were used for soil labeling and irrigation. To make a carbonate-containing and a carbonate-free medium for plant growth, a carbonate-free loamy soil (Haplic Luvisol, originated from loess) was mixed with loess and sand particles, respectively, at a 1:1 ratio (200 g of loamy soil to 200 g of loess or sand). The loamy soil, loess, and sand particles were air-dried and passed through a 2-mm screen before mixing. Loess samples containing 30% CaCO_3 were taken from an open mine at Nussloch, southwest Germany, from 10 m below the soil surface (see Kuzyakov et al. [2006] for details). Carbonate-free sand in the size range 0.5–1.5 mm was used. Water content was adjusted to 60% of water-holding capacity by adding 96 mL of distilled water to the loamy soil + loess (hereafter called Loess) and 84 mL to the loamy soil + sand (hereafter called Sand). The water content of Loess and Sand was kept at 60% of water holding capacity during the whole experiment by weighing the pots and adding water when needed.

Fruits of *B. arvensis* were pre-germinated in the dark on wet filter paper. When plant height was around 1 cm they were transplanted into the growth pots, the lids closed, and placed into a growing chamber at 25–27°C with a 14-hr photoperiod and a $180 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity.

Labeling Procedure

Labeling started one week after the first flowers developed and was repeated five times over a one-month period thereafter. Labeling was applied to either the roots or the shoots. In both cases, 200 kBq of ^{14}C in the form of $\text{Na}_2^{14}\text{CO}_3$ solution was used at each labeling occasion. The applied ^{14}C activity for labeling was several orders of magnitude higher than natural abundance of ^{14}C in plant organs or soils. Hence, the initial ^{14}C activity of plant organs or soils had no effect on the results of labeling. Before starting the procedure, the space between the stem and the main opening in the lid was filled with cotton and covered with petroleum jelly to provide an air-tight seal, which was maintained for the one-month period. The three small openings were only closed for the few hours of each labeling procedure, using tight-fitting plastic pins. Separation of the root and shoot atmospheres during the labeling procedure was necessary to prevent dissolution of $^{14}\text{CO}_2$ in the soil solution while labeling the shoots, and to avoid photosynthetic assimilation of labeled $^{14}\text{CO}_2$ that might be released from the soil solution during root labeling (Amiro and Ewing 1992; Cramer and Richards 1999).

For shoot labeling, the pots were placed in an air-tight labeling chamber made of Plexiglas ($0.5 \times 0.5 \times 0.6 \text{ m}^3$), which was fitted with four connections and a fan for circulating $^{14}\text{CO}_2$. To produce $^{14}\text{CO}_2$, 5 mL of 2.5 M $\text{Na}_2^{14}\text{CO}_3$ was acidified by addition of H_3PO_4 . The $^{14}\text{CO}_2$ was pumped into the chamber using 2 inlets. After 1 hr the chamber was connected via the 2 outlets to a glass bottle with 20 mL of 1 M NaOH to trap unassimilated $^{14}\text{CO}_2$. The trapping period was also 1 hr. Afterwards, the plants were returned to the normal conditions outside the labeling chamber and the plastic pins were removed.

For root labeling, 3 mL of 0.002 M $\text{Na}_2^{14}\text{CO}_3$ solution was injected deeply into the soil in each pot via the three small openings in the lids (1 mL each) (Figure 1, left). This fairly low concentration of sodium carbonate had no effect on plant growth or fruit production compared to the shoot-labeled plants.

^{14}C Analyses

One week after the 5th labeling, ^{14}C activity was measured in plant organs (shoots, roots, and fruits), bulk soil and soil solution. After collecting the fruits, the plant stems were cut at the base and soils were washed with distilled water to separate the roots and to collect soil solution. To wash the soils, 1000 mL of distilled water was used for Loess and 880 mL for Sand. The bulk soils, shoots, roots and fruits were dried overnight at 40°C to determine dry weights. Afterwards, ^{14}C was measured in a subsample of each material.

^{14}C in fruits was measured separately in carbonate and organic components. The fruits were acidified with H_3PO_4 and the released CO_2 was trapped in 1 M NaOH solution. The alkali solution was mixed with scintillation cocktail (Rotiszint EcoPlus, Carl Roth, Germany) and ^{14}C was measured after decay of chemiluminescence with an Automatic TDCR liquid scintillation counter (HIDEX 300 SL, Turku, Finland). The acidified fruits were washed again with distilled water, dried at 40°C and weighed again to determine the weight lost from carbonates. The weight loss after acidification was taken as the fruit carbonate content. The remaining fruit material (i.e. the organic part) was combusted at 900°C using a biological oxidizer (OX 400) to yield CO_2 . The produced CO_2 was trapped in NaOH and ^{14}C activity was measured as described above.

^{14}C measurement in the bulk soil was similar to that for fruits. For soil acidification, 0.1 g of Loess and 2 g of Sand were used. ^{14}C measurements of shoots and roots were performed in the same way as for the organic parts of fruits, but as finely ground powders. ^{14}C in soil solution was measured after addition of scintillation cocktail. To differentiate between dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC), a part of the solution was acidified before addition of scintillation cocktail. This provided a ^{14}C determination in DOC. The difference between ^{14}C activity of total dissolved carbon and that of DOC was the ^{14}C activity in DIC.

Calculation of Carbon Incorporation into Plant Organs and Age Overestimation

The C amounts incorporated into plant organs (mg) were calculated based on the C content of the labeling solution (mg) added to each pot, total ^{14}C activity applied to each pot, and the ^{14}C activity measured in plant organs (i.e. fruit carbonates, fruit organics, roots, shoots) (see Kuzyakov et al. [2006] for more details).

To calculate the age overestimation because of incorporated HCO_3^- carbon, we used the usual ^{14}C decay equation (Bowman 1995)

$$T = -8267 \cdot \ln(A_{\text{SN}}/A_{\text{ON}}) \quad (1)$$

where A_{SN} is the normalized number of measured ^{14}C atoms in a given sample and A_{ON} is the initial normalized number of ^{14}C atoms at the beginning of decay and T is the time elapsed since the beginning of decay. Assuming a constant atmospheric ^{14}C concentration over time,

$$A_{SN} = A_{ON} \cdot e^{-\lambda T} \quad (2)$$

where $\lambda = 1/8267$. This law remains true as long as no new fractions of ^{14}C or radiometrically dead C are added to a sample. If a portion P of radiometrically dead carbon is added to a sample, the ^{14}C concentration in such a sample becomes lower by a factor $1/(1+P)$, which modifies Equation 2 in the following way:

$$A_{SN} = A_{ON} \cdot e^{-\lambda T} \cdot \frac{1}{1+P} \quad (3)$$

Combining Equations 1 and 3, we obtain a formula for the measured age T' of a sample with a portion of radiometrically dead carbon P

$$T' = -8267 \cdot \ln \left[\left(A_{ON} \cdot e^{-\lambda T} \cdot \frac{1}{1+P} \right) / A_{ON} \right] \quad (4)$$

It is further apparent that

$$T' = -8267 \cdot \ln \left(\frac{e^{-\lambda T}}{1+P} \right) = T + 8267 \cdot \ln(1+P) \quad (5)$$

Equation 5 can provide the offset between the measured age of a sample with admixtures of dead carbon and its true age ΔT under stable ^{14}C atmospheric concentration

$$\Delta T = T' - T = 8267 \cdot \ln(1+P) \quad (6)$$

As it follows from Equation 6, this offset does not depend on time and is only determined by the quantity of dead carbon admixture.

Statistics

Mean values and standard errors were calculated for 6 replicates of each treatment. The significance of differences between shoot- and root-labeled plants was assessed using the post-hoc Fisher LSD test at $\alpha = 0.05$ significance level. Statistical analyses were done in STATISTICA 10 (StatSoft Inc., Tulsa, USA).

RESULTS

The ^{14}C distribution via shoot- and root labeling showed obvious and significant differences ($p < 5\%$) between various organs (Table 1). ^{14}C specific activity after shoot labeling was the highest in shoots, followed by fruit organics and roots. In contrast, the highest ^{14}C activity after root labeling was recovered in the roots, followed by DOC and DIC. ^{14}C fraction recovered in shoots was around 6 times higher (43–47%) after shoot labeling than root labeling (7–8%). Recovery after shoot labeling was also about 9 times higher in fruit organics, but around 3 times lower in roots.

Total incorporation of C from shoot labeling by Loess-grown plants was 90.6 mg, lower ($p < 5\%$) than for the Sand-grown plants (92.1 mg). Incorporated C from root labeling was 74.1 and 103 mg for Loess and Sand, respectively (Table 2). Fruit carbonate had greater

Table 1 Percentage of ^{14}C label recovered in different plant organs and soils via photosynthesis (shoot-labeling) or taken up by roots (root-labeling). Standard errors are shown in parentheses.

Labeled fractions	Shoot-labeling		Root-labeling	
	Sand	Loess	Sand	Loess
Fruit carbonate	0.16 (0.01)	0.15 (0.01)	0.08 (0.01)	0.06 (0.01)
Fruit organics	25.1 (0.98)	30.4 (1.19)	4.38 (0.70)	2.46 (0.23)
Shoots	46.8 (2.64)	43.3 (1.17)	7.88 (0.47)	6.93 (0.39)
Roots	23.6 (2.26)	20.3 (1.66)	69.7 (0.90)	49.8 (3.15)
Dissolved organic carbon	2.86 (0.11)	3.54 (0.05)	13.2 (0.39)	20.4 (1.20)
Dissolved inorganic carbon	1.17 (0.04)	1.14 (0.08)	3.81 (0.34)	9.18 (0.95)
Soil carbonate	0.25 (0.02)	1.17 (0.10)	1.00 (0.05)	11.2 (1.24)

Table 2 Amounts of incorporated labeled carbon (mg) in plant organs after shoot or root labeling of Sand- or Loess-grown plants. Standard errors are shown in parentheses.

Labeled fractions	Shoot-labeling		Root-labeling	
	Sand	Loess	Sand	Loess
Fruit carbonate	0.15 (0.01)	0.14 (0.01)	0.10 (0.01)	0.08 (0.01)
Fruit organics	24.2 (0.94)	29.3 (1.14)	5.47 (0.88)	3.07 (0.29)
Shoots	45.1 (2.54)	41.6 (1.12)	9.85 (0.59)	8.66 (0.49)
Roots	22.7 (2.17)	19.6 (1.60)	87.1 (1.12)	62.3 (3.93)
Total	92.1 (0.13)	90.6 (0.17)	103 (0.90)	74.1 (3.62)

incorporation from shoot labeling than from root labeling: 1.5 times higher in Sand and 1.9 times in Loess (Table 2).

DISCUSSION

The soil properties (Loess vs. Sand) and the labeling approach (shoot vs. roots) had no effect on total plant growth or individual organs. Therefore, we can directly compare the label incorporation and distribution between the soils and labeling conditions.

The obvious differences in ^{14}C activity of various plant organs after root labeling compared to shoot labeling reveal that HCO_3^- carbon was taken up by *B. arvensis* roots (Table 1). To determine the amount of HCO_3^- carbon incorporated by *B. arvensis*, the total incorporated C via shoot labeling in Loess and Sand were compared. If we assume no re-uptake via HCO_3^- , there should be no difference between the incorporated C from $^{14}\text{CO}_2$ in Sand- and Loess-grown plants following shoot-labeling. The comparison, however, reveals 1.51 mg less photo-assimilated C in Loess than in Sand (Table 2). CaCO_3 solubility in distilled water is 13.1 mg L^{-1} at 25°C (Aylward 2007). Therefore, in Loess with approximately 700 mL water², 9.1 mg CaCO_3 can be dissolved. According to the C mass proportion in CaCO_3 (12 mg C

²Cumulative amount of water added to the pots to keep the water content of Loess at 60% of water holding capacity during one month labeling.

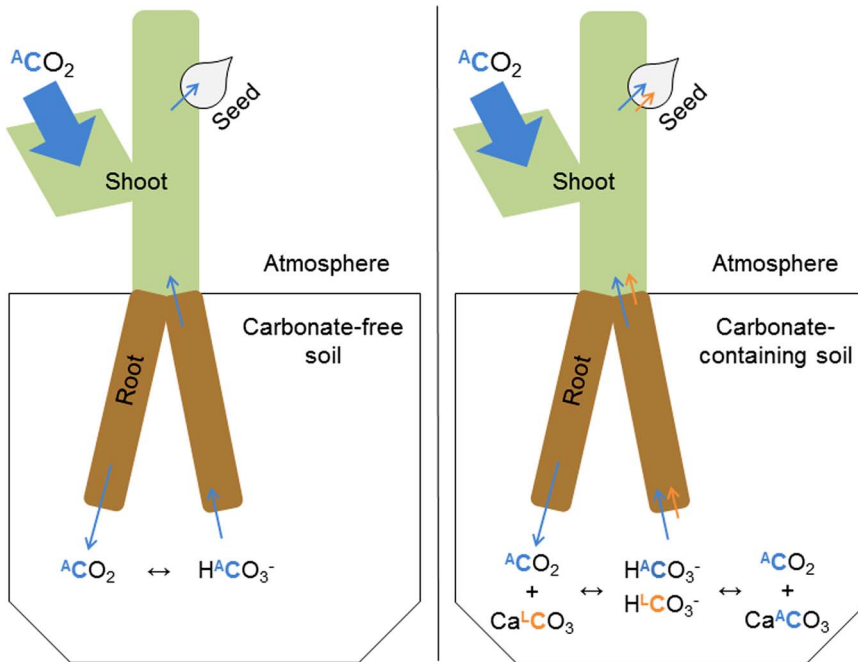


Figure 2 Dilution of ^{14}C content of plant organs by dissolved inorganic C (HCO_3^-) taken from 2 sources. In carbonate-free soils, the only source of HCO_3^- is dissolution of root- and rhizomicrobially respired CO_2 originally from the atmosphere ($^{14}\text{CO}_2$). In carbonate-containing soils, the dissolution of lithogenic carbonates ($\text{Ca}^{14}\text{CO}_3$) is a second source. The HCO_3^- from root-respired CO_2 is diluted by the HCO_3^- from lithogenic carbonates (Kuz'yakov et al. 2006; Gocke et al. 2011). For shoot-labeled plants, this process leads to a reduction of the ^{14}C activity in the re-absorbed HCO_3^- .

$100\text{ mg}^{-1}\text{ CaCO}_3$), this amount of dissolved CaCO_3 contains 1.1 mg C (fairly equal to the solubility of CaCO_3). Root-respired CO_2 can dissolve in soil solution and be reabsorbed by roots (Ford et al. 2007). However, root-respired CO_2 is diluted in Loess solution before re-uptake (Figure 2). Hence, total incorporated C in Loess plants was lower than in Sand plants. In conclusion, the so-called reservoir effect, i.e. incorporation of ^{14}C -depleted carbon from soil into biologically formed carbonates which has already been proven for other types of biogenic carbonates, such as land-snail shells (Pigati et al. 2004; Pigati et al. 2010 and references therein) also takes place in fruit carbonate of *B. arvensis*.

Since the incorporated C from soil carbonate is ^{14}C dead, this may lead to overestimations of ^{14}C ages based on biogenic carbonates (Goodfriend 1987). Considering the total weight of C in fruit carbonates (8.08 mg C, based on 20 fruits) and the difference between HCO_3^- incorporation into fruit carbonate in Sand and Loess after shoot labeling (0.012 mg C) approximately 0.15% of C in fruit carbonate—after 10-hr labeling—originated from soil solution. The total HCO_3^- incorporated into the whole plant amounted to 1.6% of dry weight. However, fruit carbonate in Loess after shoot labeling showed 7.6% more HCO_3^- than in Sand (Table 2). Furthermore, extrapolating the 10-hr labeling period to the full month of this study (420-hr photoperiod) indicates around 6.3% of fruit carbonate in Loess is derived from lithogenic carbonates. A 6.3% share of lithogenic HCO_3^- leads to ^{14}C ages overestimated by 505 ^{14}C yr (Equation 6), based on fruit carbonate of Loess-grown *Buglossoides arvensis*.

In this connection, it is important to note that too-old ^{14}C ages on fruit carbonate were reported in literature (Pustovoytov et al. 2004, 2010; Pustovoytov and Riehl 2006). One of the ways to explain the discrepancy between an age measured on the carbonate fraction of fruits and the true age of the sample could be the uptake of inorganic carbon from the soil by root systems. Regarding the suitability of fruit carbonate for dating purposes, an age overestimation of order of 500 ^{14}C yr, though persistent with increasing sample age, becomes insignificant against the measurement uncertainties in relatively old samples (such as 11,000 yr and older, i.e. after 2 ^{14}C half-lives).

Some of the other findings may also deserve particular attention. As expected, the distribution of C from soil CaCO_3 decreases with the distance of plant organs from the roots (Brix 1990) (Table 2). The HCO_3^- distribution in plant organs has usually been attributed to passive uptake with transpiration flow (Stolwijk and Thimann 1957; Amiro and Ewing 1992). This means that HCO_3^- moves with water from roots towards stomata (Amiro and Ewing 1992). However, the different ^{14}C activities in various organs following shoot labeling in Sand and Loess, arising from the dilution effect of lithogenic HCO_3^- , suggest the selective incorporation of HCO_3^- carbon in specific organs (Ford et al. 2007). After shoot labeling, there was 3.20 mg more labeled C in roots and shoots and 0.012 mg more labeled C in fruit carbonate of plants grown in Sand than of those in Loess (Table 2). At the same time, Sand-grown plants had 5.11 mg less labeled C in fruit organics. The higher difference indicates a higher dilution effect by soil carbonate and higher incorporation of HCO_3^- . Therefore, the highest HCO_3^- amount was retained in roots and shoots, followed by fruit carbonate, while fruit organics showed the lowest HCO_3^- incorporation. This may suggest some active uptake processes (Vuorinen et al. 1989; Ford et al. 2007) enhancing fruit carbonate compared to the fruit organics, since these components are the same distance from the roots. The apparent lower HCO_3^- incorporated in Loess- compared to Sand-grown plants after root labeling, on the other hand, is partly due to substitution of added $\text{Na}_2\text{CO}_3\text{-C}$ with Loess $\text{CaCO}_3\text{-C}$ (Figure 1) (Kuz'yakov et al. 2006).

CONCLUSIONS

1. *Buglossoides arvensis* takes up dissolved inorganic carbon (HCO_3^-) from the soil via roots under laboratory conditions. The source of HCO_3^- can be dissolution of carbonate minerals (radiometrically dead, e.g. loess carbonate) and dissolution of root-respired CO_2 (recent C) in soil solution;
2. The HCO_3^- uptake is mostly passive; however, HCO_3^- can be preferentially incorporated into organs such as fruit carbonate, which are formed at specific plant development stages;
3. The incorporated HCO_3^- taken up by roots may contribute more than 6.0% of fruit-carbonate C in plants growing on a carbonate-containing soil. Therefore, an age overestimation of approximately 500 yr is possible. Inflated ages based on fruit carbonate can be attributed to HCO_3^- uptake by roots during fruit development. This calls for further investigation of possible effects of calcareous substrates on the outcome of ^{14}C -dating of the fruit carbonate fraction;
4. The age overestimation because of lithogenic HCO_3^- incorporation in fruit carbonate, however, is insignificant in relatively old samples, approximately after 2 ^{14}C half-lives.

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