Transfer of phloem-mobile substances from the host plants to the holoparasite *Cuscuta* sp.

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Abstract

During the development of the haustorium, searching hyphae of the parasite and the host parenchyma cells are connected by plasmodesmata. Using transgenic tobacco plants expressing a GFP-labelled movement protein of the tobacco mosaic virus, it was demonstrated that the interspecific plasmodesmata are open. The transfer of substances in the phloem from host to the parasite is not selective. After simultaneous application of $^3$H-sucrose and $^{14}$C-labelled phloem-mobile amino acids, phytohormones, and xenobiotica to the host, corresponding percentages of the translocated compounds are found in the parasite. An open continuity between the host phloem and the *Cuscuta* phloem via the haustorium was demonstrated in CLSM pictures after application of the phloem-mobile fluorescent probes, carboxyfluorescein (CF) and hydroxypyrene trisulphonic acid (HPTS), to the host. Using a *Cuscuta* bridge $^{14}$C-sucrose and the virus PVYN were transferred from one host plant to another. The results of translocation experiments with labelled compounds, phloem-mobile dyes and the virus should be considered as unequivocal evidence for a symplastic transfer of phloem solutes between *Cuscuta* species and their compatible hosts.

Key words: CF, *Cuscuta*, HPTS, plasmodesmata, symplastic transport, virus.

Introduction

All species of the genus *Cuscuta* (Convolvulaceae) are holoparasites. Their geographical distribution ranges from 64° N latitude (Schmucker, 1959) to 47° S latitude (Kuijt, 1969). The morphology of *Cuscuta* species is quite simple. Plants are characterized by rootless green, yellow or white stems with scale-like leaflets. Some species, in particular *Cuscuta reflexa*, contain small amounts of chlorophyll and have the ability to photosynthesize. However, they cannot live without being attached to a host (Hibberd *et al.*, 1998; Van der Kooij *et al.*, 2000; Birschwilks *et al.*, 2001). The plants are completely dependent on the host for a supply of assimilates and water. 99% of the carbon that *Cuscuta* uses comes from the host plant (Jeschke *et al.*, 1994a).

During host infection the parasite produces a haustorium that penetrates the host tissue. Searching hyphae grow between and through the host cells (Dörr, 1968, 1969; Vaughn, 2003). Upon reaching cells of vascular bundles, the searching hyphae undergo a remarkable differentiation. Terminal hyphae cells adjacent to xylem elements differentiate to xylem elements. Terminal hyphae cells adjacent to sieve tubes differentiate into xylem elements. Terminal hyphae cells adjacent to sieve tubes differentiate into the so-called absorbing hyphae that closely attach to the host sieve tube (Dörr, 1972). Afterwards parenchyma cells in the haustorium also differentiate into phloem or xylem elements. When the histological connection between the vascular bundles of the host and the parasite is complete; *Cuscuta* becomes a very strong sink, competing with the host sinks for assimilates (Jacob and Neumann, 1968; Wolswinkel, 1974; Jeschke *et al.*, 1994a). In addition, the parasite induces an increase...
in the photosynthetic rate of the host (Jeschke et al., 1994b, 1997; Jeschke and Hilpert, 1997). Some Cuscuta species are a serious problem affecting crop production, particularly in the subtropics and Mediterranean countries, but also in North America and Central Europe (Dawson et al., 1994). The transport of water and minerals from the host to the parasite xylem requires no transfer through a membrane. Between the xylem of the host and the parasite a continuous open connection exists (Kollmann and Dörr, 1987; Dörr, 1990; Dawson et al., 1994). In contrast to transport in the xylem; the transfer of substances between the plasmodesmata and sieve pores between absorbing hyphae and the sieve tubes of the host seem to be absent, necessitating an apoplastic transfer of solutes (Dörr, 1972; Tsivion, 1978; Wolswinkel and Ammerlaan, 1983; Wolswinkel et al., 1984; Kollmann and Dörr, 1987; Jeschke et al., 1994b). On the other hand, Cuscuta is known as an effective vector for the transmission of viruses and phytoplasmas (Bennett, 1944; Hosford, 1967; Heintz, 1989; Kaminska and Korbin, 1999), but their transport, as well as the demonstrated transfer of GFP (Haupt et al., 2001) requires a symplastic connection between host and parasite. Nevertheless, there is doubt about the symplastic pathway of the transfer. According to Christensen et al. (2003) the transport of GFP seems to be specific only for the investigated host–parasite system Nicotiana tabacum–Cuscuta reflexa. In their opinion it is not possible to generalize this result. In the study presented here, the transport of sucrose, xenobiotics, phloem-mobile fluorescent dyes, and viruses was investigated in different host–parasite systems. The results are further evidence for an open connection between the plasmodesmata of the host and the parasite.

Materials and methods

Plant material

Stock cultures of Cuscuta reflexa, Cuscuta platyloba, and Cuscuta odorata parasitizing on Pelargonium zonale were cultivated in a greenhouse at 18–22 °C and 60–80% RH (relative humidity) with a daily light regime of 16 h light (daylight, supplemented from November to March with Hg-fluorescence lamps) and 8 h darkness. Nicotiana tabacum cv. Samsun NN, and Nicotiana tabacum cv. xanthi were grown from seeds in a mixture of soil and sand in a greenhouse at 18–22 °C under a 15/9 h light/daylight, supplemented from November to March with Hg-fluorescence lamps) and 8 h darkness. Pelargonium zonale cv. 'C255' was cultivated in soil in a greenhouse at 20 °C under a 15/9 h light/daylight regime.

Host–parasite systems

To establish the host–parasite systems, well-grown Cuscuta shoots of 20 cm in length were cut from the stock culture and with their apical region (2 cm in length) carefully fixed with a tape on the stem (Nicotiana and Cuscuta) or on the petiole (Pelargonium) of the host plant (‘parallel mode of induction’). 16 d after infection with Cuscuta, the host–parasite systems were used for the experiments. Rapid growth of Cuscuta (generally more than 4 cm d−1) and the exudation of sugar by nectaries on the stem of the parasite gave visible signs of a successful connection between host and parasite.

To investigate the translocation of viruses and sucrose from one plant to another, two host plants were connected with a Cuscuta stem (‘bridge-system’). In these experiments the first host plant (donor plant) was infected with a detached Cuscuta shoot on the petiole of a source leaf (Nicotiana) or on the stem (Vicia). After 14–16 d the apical region of the parasite was fixed on the stem of a second host plant (acceptor plant).

Translocation experiments with labelled compounds

24 h before translocation experiments, successfully parasitized leaves of Pelargonium were harvested by cutting of the petioles from the stem. The cut surface was immediately immersed in MES-EDTA (10 mM 2-[N-morpholino] ethanesulfonic acid, adjusted with NaOH to pH 5.0–10 mM EDTA, disodium salt). After 30 min the medium was replaced by 10 mM MES-buffer, pH 5.0. During translocation experiments the petioles were kept in this solution.

24 h prior to use for translocation experiments, explants were prepared from successfully parasitized Vicia faba plants. The explants consisted of the parasitized internodium with the well-growing parasite and the source leaf above it. Similar to the Pelargonium leaves, the lower surface of the internodium was immersed at first in MES-EDTA solution for 30 min and afterwards in 10 mM MES-NaOH buffer, pH 5.0.

In experiments using ‘bridge-systems’, both Vicia faba plants were prepared in the same manner.

Application of 14CO2 and labelled compounds

To study the distribution of 14CO2-derived assimilates between host and parasite; detached parasitized leaves of Pelargonium zonale were transferred to a growth chamber (HPS 500, Heraeus Vötsch, Balingen Germany) with 22/15 °C day/night temperature and 60–80% RH. Photosynthetic photon flux on the leaf blade enclosed in a Perspex chamber was 165 µEm−2 s−1 during a 16 h photoperiod. 14CO2 was released from 14C-Na2CO3 by addition of excess 5% perchloric acid in a small tube connected to the leaf chamber. A 14CO2-pulse of 1.85 kBq of 5 min was applied to the leaf blade of the host.

The feeding solutions were made up in 10 mM MES-buffer, pH 5.0. The substances were applied to Pelargonium leaves by injecting 10 µl solution into the petiole parenchyma directly below the leaf blade (Grimm et al., 1995). The distance between the place of injection and the haustorium of the parasite was always more than 3 cm.

In the case of Vicia explants 20 µl of substances were applied to the upper surface of the leaves. To improve the absorption, the epidermis was slightly abraded with fine sandpaper and the treated area repeatedly moistened with buffer.

Translocation experiments were stopped by cutting the host–parasite systems into defined parts. To remove non-absorbed substances from the surface, the Vicia leaves were carefully rinsed. The plant samples were extracted in methanol.

Measurements of 3H and 14C

Radioactivity of methanol extracts and buffer solutions was counted in a liquid scintillation spectrometer (LS 6000, Beckman Instruments Inc., Fullerton, USA) using a cocktail of 4.0 g 2,5-diphenyloxazole (PPO) and 0.24 g 1,4-bis (5-phenyloxazoyl)benzene (POPOP) in 1.0 L toluene. During measurement the tissue samples were retained in the probes.

To image the distribution of labelled substances in the haustorial region, free-hand longitudinal sections were freeze-dried and placed on a storage phosphor screen for 12–96 h (Molecular Dynamics). The exposed screen was scanned using a PhosphoImager (Molecular Dynamics).
Translocation of phloem-mobile fluorescent dyes
Fluorescent probes of 5,6-carboxyfluorescein (CF) and 8-hydroxy-pyrene-1,3,6-trisulphonic acid (HPTS) were applied in their ester forms 5,6-carboxyfluorescein diacetate (CFDA) and 8-acetoxy-pyrene-1,3,6-trisulphonic acid (HPTSA) to the host leaves. Prior to application the surface of the leaves was carefully abraded with fine sandpaper. To prevent evaporation of the dyes and to ensure an even coverage across the leaf surfaces, the treated leaves were covered with a thin polyethylene film (Roberts et al., 1997). The plants were imaged after translocation in the light for between 2–3 h.

Prior to confocal imaging the parasitized area of the host–parasite system was cut free-hand into longitudinal or transverse sections. To prevent efflux and redistribution of the dyes, the sections were mounted immediately in silicon oil, covered with a cover slip, and imaged.

Fluorescence microscopy
For visualizing CF and HPTS a Zeiss Axiovert 100 M (Carl Zeiss Jena, Germany) combined with the confocal laser scanning system Zeiss LSM 510 was used. Both probes were excited by the 488 nm line produced by a krypton/argon laser. For detecting the fluorescence a 505–530 nm emission filter was used.

In the case of Pelargonium the fluorescence was visualized with an epifluorescence microscope (Axioskop, Zeiss, Jena, Germany) using the proper filter. Micrographs were taken by a CCD camera (Sony, Tokyo) and processed through the Adobe Photoshop (Adobe Systems, Mountain View, CA).

Electron microscopy
Haustoria-bearing regions of the host–parasite systems were prefixed with 3% (v/v) glutaraldehyde/phosphate buffer pH 7.4 (2 h), fixed with 1% (w/v) OsO4/Palade buffer (1 h) and dehydrated in series of acetone. After embedding in ERL, ultrathin sections were stained with Pb and viewed using a transmission electron microscope (EM 912 OMEGA LEO Elektronenmikroskopie, Oberkochen Deutschland).

Infection of tobacco plants with potato virus Y isolate N (PVYN) and immunological detection of the virus
To investigate the transfer of viruses, a bridge-system of two tobacco plants (Nicotiana tabacum cv. Samsun NN) was used. The donor plant was parasitized for 14 d with Cuscuta reflexa then the source leaves were infected with PVYN (Herbers et al., 1996) and the parasite was fixed immediately to the acceptor plant.

Cuscuta was allowed to parasitize the acceptor plant for 18 d. Afterwards the host–parasite system was dissected and the virus in defined parts immunologically detected. Extraction procedures and ELISA were performed as described by Herbers et al. (1996).

Results
Translocation of 14C-derived assimilates
Both Pelargonium zonale and Vicia faba are compatible hosts for Cuscuta reflexa, C. platyloba, and C. odorata. Between 10 d and 14 d after fixing of Cuscuta sp. to its host the parasite had attached, i.e. the functional vascular connection between host and parasite was established. That was apparent by an extraordinary increase in the translocation rate and in the amount of 14C-derived assimilates translocated into the parasite. In the following days the translocation rate and the rate of 14C-derived assimilates in Cuscuta remained nearly constant (Fig. 1). In all the investigations 14–16-d-old host–parasite systems were used. The translocation experiments were carried out with detached leaves of Pelargonium infected on the petiole with Cuscuta and with explants of Vicia faba, consisting of the parasitized internodium with the well-growing parasite stem and one source leaf above it (Fig. 2).

By removing the free growing stem and the haustorium-bearing stem region of Cuscuta the distribution patterns of assimilates were influenced. In comparison to the complete host–parasite system in the reduced systems, a higher amount of substances was found in the petiole above and below the infection site. However, in all experiments Cuscuta remained the dominant sink. Even when all parts of the parasite outside the host were removed and only the haustoria in the host tissue were left, the amount of translocated assimilates measured in the infected part of the petiole (Table 1) was still 60%.

The transfer of assimilates is locally restricted to the haustoria. In the PhosphorImager the maximum of radioactivity was detected in this area (Fig. 3B).

Translocation of 3H-sucrose, 14C-amino acids, 14C-phytohormones and 14C-xenobiotics
To compare the distribution pattern of sucrose, the main translocated compound in the phloem, with that of other phloem-mobile substances, 3H-sucrose and a 14C-labelled phloem-mobile compound were applied simultaneously to the host (Table 2). In the host–parasite system Pelargonium...
zonale–Cuscuta reflexa the substances were injected into the non-parasitized parenchyma below the leaf blade. In the host–parasite system Vicia faba-Cuscuta reflexa the substances were applied to the leaf blade of the host. Three h after application, no significant differences in the distribution patterns of sucrose and the other phloem-mobile compounds in both host–parasite systems could be detected (Table 2).

Translocation of phloem-mobile fluorescent probes: To demonstrate the pathway in the phloem from the source leaf of the host to the sink Cuscuta, the translocation of the phloem-mobile fluorescent dyes 5,6-carboxyfluorescein (CF) and 8-hydroxypyrene-1,3,6-trisulphonic acid (HPTS) were studied. Two hours after application of the dyes in their ester forms to the surface of the host leaves, CF and HPTS could be detected in the parasite using confocal laser scanning microscopy (CLSM). A continuous fluorescent labelling between host and parasite across the haustorium was visible for both dyes (Fig. 3A, C). Corresponding histological and CLSM pictures show that the fluorescence was restricted to the phloem (Fig. 3C). These results indicate an open continuity between the phloem of host and parasite.

Plasmodesmata between searching hyphae and host parenchyma cells: During haustorium development searching hyphae grow between and through host parenchyma cells, and are connected to the host cells by interspecific plasmodesmata (Fig. 4). To investigate whether the interspecific plasmodesmata are open, transgenic tobacco plants expressing a GFP fusion to the movement protein of tobacco mosaic virus (TMV MP:GFP plants), which specifically labels branched plasmodesmata (Atkins et al., 1991; Ding et al., 1992; Oparka et al., 1996a, b; Padgett et al., 1996) were parasitized with Cuscuta reflexa for 14 d. CLSM pictures of sections through the parenchyma cells of the tobacco plants show green fluorescent spots in the connecting cell walls, indicating the plasmodesmata. Figure 3D represents a magnification of a searching hypha growing through a host parenchyma cell. Green fluorescent spots are not only prominent in the cell walls between parenchyma cells of the transgenic plants expressing the tobacco mosaic virus movement protein, but are also visible between the host parenchyma cell and the searching hypha.

Transfer of viruses between two host plants by Cuscuta: In order to investigate the translocation of viruses, two plants of Nicotiana tabacum cv. Samsun NN, were connected with a Cuscuta reflexa stem (‘bridge-system’). The first host plant (donor plant) was infected with a Cuscuta shoot attached to the petiole of a source leaf. After 14 d the source leaves of the host plant were inoculated with the PVYN virus and the apical region of the parasite was immediately fixed on the stem of a second host plant (acceptor plant). The growth of the parasite was not influenced by the inoculation of the host with the virus. Very rarely the apical region showed a brown discoloration and a cessation of growth. In those cases many lateral shoots developed simultaneously. After 14 d sink leaves of some acceptor plants showed chlorotic and necrotic strips caused by the PVYN virus infection. Independently of the occurrence of visible disease symptoms the virus protein was detectable by ELISA in all investigated sink leaves of the acceptor plants indicating a successful transport from the first tobacco plant by Cuscuta. A smaller amount of the virus was found in the tissue of the donor and acceptor plants containing the haustorium and in the free-growing Cuscuta stem. The content of virus in the Cuscuta bridge was always very small (Fig. 5). In haustoria-free tissues at the infection site of acceptor plant, no virus protein was detectable by ELISA (data not shown).

Transport of 14C-sucrose from the parasite into the host: In addition to viruses, sucrose is transferred from the parasite to the host. For these experiments, a bridge-system consisting of two explants of Vicia faba connected with a Cuscuta stem were used. To establish a continuous functional phloem connection between host plants and the parasite, the explants are prepared a minimum of 14–16 d after inoculation of the second plant. Figure 6B represents the distribution patterns of the 14C-labelled compounds 24 h after application of 14C-sucrose to the leaf of the first host. 45% of the translocated labelled compounds are detected in the free-growing Cuscuta stem and 12.7% in the bridge between the two plants. With regard to the second host plant, the parasitized intermedium with the haustorium and the areas above and below the haustorium showed significant amounts of compound upon measurement, indicating a transport from the parasite to the host.
Table 1. Distribution pattern of 14C-derived assimilates in the host–parasite system Pelargonium zonale–Cuscuta reflexa after gradual shortening of the parasite

<table>
<thead>
<tr>
<th>Parasite in the system consisted of</th>
<th>% Translocated 14C-labelled compounds in the plant part after a translocation duration of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 h</td>
</tr>
<tr>
<td>Haustoria in the host-tissue, haustoria-bearing stem region, and free-growing stem (complete parasite)</td>
<td>Host petiole above infection site 6.5±2.4</td>
</tr>
<tr>
<td></td>
<td>Host petiole with haustoria 5.9±1.1</td>
</tr>
<tr>
<td></td>
<td>Host petiole below infection site 0.8±0.7</td>
</tr>
<tr>
<td>Cuscuta</td>
<td>86.8±2.9</td>
</tr>
<tr>
<td>Haustoria-bearing stem region</td>
<td>Host petiole above infection site 10.8±2.8</td>
</tr>
<tr>
<td></td>
<td>Host petiole with haustoria 29.8±5.8</td>
</tr>
<tr>
<td></td>
<td>Host petiole below infection site 2.3±2.3</td>
</tr>
<tr>
<td>Cuscuta</td>
<td>57.1±8.5</td>
</tr>
<tr>
<td>Haustoria in the host tissue</td>
<td>Host periole above infection site 29.2±5.8</td>
</tr>
<tr>
<td></td>
<td>Host petiole with haustoria 56.2±6.8</td>
</tr>
<tr>
<td></td>
<td>Host petiole below infection site 14.6±1.2</td>
</tr>
<tr>
<td>Cuscuta</td>
<td>–</td>
</tr>
</tbody>
</table>

The percentage of translocated labelled compounds in this parts was amounted to 11.5%.

The measured labelled substances in the second plant are a result of the lateral scanning from the haustoria-bearing stem region (Fig. 6). Obviously, this part with the haustoria in the second host is an additional sink of the parasite shoot. The concentration of labelled compounds in this part and in the second host (31±9 dpm mg⁻¹ FW) is higher than in the adjacent 10 cm of the parasite shoot (4±1 dpm mg⁻¹ FW). The value is comparable with the concentration in the other sink region, the apical 10 cm of the Cuscuta shoot. For this part 24±7 dpm mg⁻¹ FW were calculated.

Translocation of CF from the parasite into the host: CFDA was injected into the parenchyma of the Cuscuta bridge between the two Vicia explants. After 2–3 h the plants were examined by confocal laser scanning microscopy (CLSM). In sections through the haustoria region, the dye was detected not only in the bundles of the parasite, but also in the haustorium and in the bundles of the acceptor plant (Fig. 3E). Corresponding pictures were also obtained after cutting the bridge and application of CFDA to the cut surface. In both experiments the distance between the point of dye application and the examined parasitized interodium measured 10–15 cm (Fig. 7).

Discussion

An important prerequisite to study and quantify the transfer of substances from the host to the parasite was the establishment of an experimental system with clear source–sink relations. When Cuscuta is parasitizing a whole plant, a complex network of phloem connections between different sinks exists. All sinks compete with each other for assimilates translocated in the phloem from the source leaves. The import rate in the parasite depends not only on the sink strength of the parasite, but also on the sink strength of growing parts of the host, on the site of parasite infection and on the source leaf of the host. In our experimental systems consisting of detached leaves of Pelargonium zonale or explants of Vicia faba parasitized with Cuscuta, these difficulties were omitted. The phloem stream is directed only basipetally to the lower cut surface of the petiole or the internodium inserted in a MES solution. By setting the cut surfaces in an EDTA solution, a closure of the sieve tubes by callose was prevented (King and Zeevaart, 1974). Fixing the parasite with a tape to one side of the petiole or the internodium (‘parallel mode of induction’; Ihl and Wiese, 2000) the natural twinning around the host was suppressed. In contrast to the natural mode of induction, all haustoria undergo a simultaneous development and it is easy to separate the tissues of host and parasite for extraction.

In both host–parasite systems Cuscuta was the dominant sink. In all cases the content in the parasite apex was higher than in the haustoria-bearing stem region (results not shown). The intensive transfer of substances from host to parasite was only slightly reduced by the cutting of the stem parts of the parasite outside of the host.

In the pictures of the PhosphorImager the highest concentration of radioactivity was detected in the haustorium and in the vascular bundles adjacent to the haustoria, indicating an extensive unloading of the host sieve tube and an intensive uptake by the bundles of the parasite.

Until now two mechanisms of transfer from the host phloem to the haustorium of the parasite were discussed, an apoplastic and a symplastic transfer. The apoplastic transfer comprises three steps with two inevitable membrane passages. (i) The release of substances from the host phloem...
into the apoplast between the sieve tube of the host and the absorbing hyphae. (ii) The transfer through the apoplast. This could be connected with some metabolic conversions of the released substances, for instance, sucrose hydrolysis by extracellular invertase. (iii) The uptake of the released substances and/or their metabolites into the absorbing hyphae. According to the two membrane passages for substances with different uptake mechanisms, differences in transfer rate should be expected, too. The first step appears to correspond to an apoplastic unloading, which was described for petioles of celery (Keller and Matile, 1989) and stalks of sugarcane (Thom and Maretzki, 1992). However, the release of substances in these plants occurs into typical sink organs, which are programmed by their ontogenetic development. The attack by *Cuscuta* is, more or less, accidental; it is induced by the presence of the absorbing hyphae of the parasite. Usually the transported substances should be completely retained in the sieve tubes during translocation along the path. An apoplastic leakage for sucrose is compensated by a specific carrier-mediated influx, which is qualitatively similar to apoplastic phloem loading accomplishing by proton co-transport (Minchin and Thorpe, 1987; Patrick, 1990). For xenobiotics the retention in the sieve tube is given either by the hydrophilicity of the substances with optimum membrane permeability like glyphosate (Tyree et al., 1979) or by an ion-trapping mechanism at the pH of the phloem sap for substances with acidic properties such as MCPA and 2,4-D (Neumann et al., 1985; Kleier, 1988; Grayson and Kleier, 1990). In contrast to biotic compounds, there are no specific carriers for xenobiotics; their uptake is accomplished by diffusion of the undisassociated moiety. A substantial release of sucrose from the sieve tube induced by *Cuscuta* could possibly be caused by an inhibition of the carrier responsible for the re-uptake of sucrose into the phloem. Wolswinkel and Ammerlaan (1983) concluded from their results that the release of sucrose is under metabolic control. On the other hand, the mechanism of an increased leakage of xenobiotics is quite unclear. For acidic compounds it seems to be possible by a local increase in the pH value in the apoplast and the following change in the dissociation equilibrium, but there is no evidence of such a local pH

### Table 2. Translocation of $^3$H-sucrose and $^{14}$C-labelled amino acids, phytohormones, and xenobiotics in host–parasite systems

<table>
<thead>
<tr>
<th>Host–parasite system</th>
<th>Applied substances</th>
<th>% Translocated substances in the parasite</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pelargonium zonale</em>–<em>Cuscuta reflexa</em> detached leaves</td>
<td>$^3$H-sucrose</td>
<td>66.9±3.1</td>
</tr>
<tr>
<td></td>
<td>$^3$H-sucrose</td>
<td>74.8±3.9</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-alanine</td>
<td>68.9±4.7</td>
</tr>
<tr>
<td></td>
<td>$^3$H-sucrose</td>
<td>64.9±4.0</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-leucine</td>
<td>62.4±3.8</td>
</tr>
<tr>
<td></td>
<td>$^3$H-sucrose</td>
<td>73.4±3.1</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-2,4-D</td>
<td>70.2±3.3</td>
</tr>
<tr>
<td></td>
<td>$^3$H-sucrose</td>
<td>63.6±3.6</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-2,4-D</td>
<td>65.0±5.2</td>
</tr>
<tr>
<td></td>
<td>$^3$H-sucrose</td>
<td>63.6±3.9</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-2,4-D</td>
<td>71.0±5.5</td>
</tr>
<tr>
<td></td>
<td>$^3$H-sucrose</td>
<td>58.9±7.7</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-MCPA</td>
<td>60.8±4.4</td>
</tr>
<tr>
<td></td>
<td>$^3$H-sucrose</td>
<td>63.9±5.2</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-MCPA</td>
<td>58.0±5.7</td>
</tr>
<tr>
<td></td>
<td>$^3$H-sucrose</td>
<td>57.1±4.3</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-glycophorad</td>
<td>64.3±2.4</td>
</tr>
</tbody>
</table>

| *Vicia faba*–*Cuscuta reflexa* explants | $^3$H-sucrose | 35.6±2.4 |
|                                          | $^3$H-sucrose | 33.1±2.4 |
|                                          | $^{14}$C-MCPA | 28.6±2.4 |
|                                          | $^3$H-sucrose | 37.3±6.2 |
|                                          | $^{14}$C-glycophorad | 32.6±5.1 |

Fig. 3. (A) *Pelargonium zonale–Cuscuta reflexa*. Translocation of carboxyfluorescein (CF). Fluorescence picture of a free-hand cross-section through the haustorial region 2 h after the application of the dye to the host. A continuous fluorescence labelling is visible between host (Pz) and parasite (Cr) via haustorium. Note that CF is only restricted to the phloem (arrows). Bar=100 μm. (B) *Pelargonium zonale–Cuscuta reflexa*. Localization of the labelled compounds in the haustorial region. Free-hand longitudinal section through the haustorial region of the petiole 3 h after application of $^{14}$CO$_2$ to the leaf of the host. (Ba) Phosphorimager picture. (Bb) Computer-assisted overlay of the Phosphorimager picture and the transmitted light picture showing that the maximum of radioactivity is localised in the two haustoria complexes (ha) and the parasitized vascular bundles of the host (vb). Bar=1 mm. (Ca) *Vicia faba–Cuscuta reflexa*. Translocation of carboxyfluorescein (CF). Free-hand cross-section through the haustorial region 3 h after applications of CF to the host leaf. (Ca) CLSM image of the cross-section. (Cb) Transmitted light picture of the same region showing the longitudinal section of the haustorium with the central xylem and the two adjacent phloem strands and the cross-section of the bundles in the host and parasite. (Cc) Computer-assisted overlay of the CLSM and the transmitted light picture showing a continuous fluorescence labelling between the bundles of the host and the parasite and the restriction of the dye to the phloem in the haustorium. Bar=50 μm. (D) *Nicotiana tabacum–Cuscuta reflexa*. Magnification of a searching hypha growing through the parenchyma cell of the transgenic *Nicotiana* plant expressing a GFP-labelled movement protein of the tobacco mosaic virus. (Da) CLSM image. (Db) The corresponding transmitted light picture. (Dc) Computer-assisted overlay of the CLSM and the transmitted light picture. Green fluorescent dots are not only prominent in the cell walls between host parenchyma cells but also visible between the MP-GFP expressing plant parenchyma cells (PC) and the searching hypha (SH) indicating the secondary intersectoric plasmodesmata. Bar=10 μm. (E) *Vicia faba–Cuscuta reflexa*. Transfer of carboxyfluorescein (CF) from the parasite to the host. Free-hand cross-section through the haustorial region in the internodium 3 h after the application of CF/DA to the parasite. The distance from the injection site to the host plant was 15 cm. (Ea) CLSM image of the cross-section. (Eb) Corresponding transmitted light picture. (Ec) Computer-assisted overlay of the CLSM and the transmitted light picture showing that the fluorescent dye was translocated from the parasite via haustorium to the host phloem. In the haustorium the dye is transported exclusively in the phloem. In the central xylem no fluorescence is visible. Bar=100 μm. C/Cr, Cuscuta/Cuscuta reflexa; CPh, Cuscuta-phloem; CX, Cuscuta-xylem; H, host; ha, haustoria complex; HPh, host-phloem; HX, host-xylem; PC, parenchyma cells; Pz, Pelargonium zonale; SH, searching hypha; vb, vascular bundles.
shift. However, the efflux of glyphosate cannot be influenced in this way. Its permeability is pH-independent (Grimm et al., 1995). Assuming that the second membrane transfer, the uptake from the apoplastic into the absorbing hyphae of the parasite, is also accomplished by different mechanisms with different velocities, (a carrier-mediated uptake of sucrose and diffusion of xenobiotics) then different rates of transfer of substances from host to the parasite would be expected. The distribution of two different labelled substances in the same plant was investigated under absolutely identical conditions. In addition, the possibility of a poisonous effect of xenobiotics on phloem transport (self-limitation) as described for glyphosate (Gougler and Geiger, 1984) by the simultaneous application of the substances could be monitored. Nevertheless, no differences could be found in the distribution patterns of sucrose or any other translocated substances belonging to amino acids, phytohormones and xenobiotics (Table 2). All substances translocated in the phloem of the host were transferred to the phloem of the parasite, too. There were no hints for any selectivity in the transport. In Cuscuta parasitizing on Genista acantholada, Lupinus albus or Digitalis sp. alkaloids and glycosides synthesized in the host are transported into the parasite (Grimmer et al., 1958; Bäumel et al., 1993, 1995; Wink and Witte, 1993; Rothe et al., 1999). These results are not compatible with an apoplastic transfer. On the contrary, they rather correspond to an open symplastic connection between the phloem of host and parasite. The symplastic connection was also demonstrated by translocation experiments using the fluorescent dyes CF and HPTS. These hydrophilic dyes, which were translocated together with the assimilates in the phloem and unloaded symplastically into the sinks, are regarded as unambiguous markers for a symplastic continuity (Grignon et al., 1989; Oparka, 1991; Wang and Fisher, 1994; Wang et al., 1994; Wright and Oparka, 1996). In all investigated host–parasite systems with Cuscuta reflexa, C. platyloba, C. odorata (results for C. platyloba and C. odorata not shown) 3 h after application of the ester forms of CF and HPTS the dyes were detectable in the parasite. In both the host and the parasite the fluorescence was restricted only to the phloem (Fig. 3C).

Further evidence of a symplastic connection was given by the successful transfer of virus from host to parasite. In source leaves, virus can be loaded via plasmodesmata into the sieve tubes and translocated with the assimilate to the sinks. In the sinks the virus is unloaded symplastically through plasmodesmata (Nelson and van Bel, 1998; Oparka and Santa Cruz, 2000). According to the experiments of Roberts et al. (1997), the distribution patterns of CF and viruses are identical. In our experiments Cuscuta parasitized on the host plant for at least 14 d, when the source leaves were inoculated with the PVY^N^ virus. Immediately after the inoculation the parasite was attached to the second host. In the following days it produced new haustoria. These represent a strong sink which competes with the apex for the phloem solutes translocated from the first host plant. Together with assimilates, viruses are transferred from the first host to the parasite. As the CF, viruses can only be transferred symplastically between the sieve tubes at the interface. During a short period of haustorium development in the second host plant, functional plasmodesmata exist between the searching hyphae and the host parenchyma cells. In the later stages, with a functional phloem and xylem connection, these plasmodesmata are closed by cell wall depositions (Kollmann and Dörr, 1969). At least in the early stages of haustorium development a virus transfer to the second host via plasmodesmata cannot be excluded. In this way a local infection in the parenchyma cells can...
be triggered. For transport to the sink leaves, indicated by the observed disease symptoms and the results of the ELISA, a subsequent loading of the virus from parenchyma cells into sieve tubes of the stem via plasmodesmata is necessary. Until now there has been no evidence for the presence of plasmodesmata at this region. According to these results, transport through an open connection between the phloem of parasite and host is more likely. Applying CF to the parenchyma, as well as to the cut end of the parasite clearly demonstrated this possibility. As investigated in experiments with detached inflorescence stalks of *Yucca* (Tammes et al., 1973) or detached leaves of *Cyclamen* (Grimm et al., 1995), an uptake of phloem-mobile substances in the phloem takes place after their application to the apoplast. Afterwards, they are translocated together with assimilates to the sinks. After application of CF to the parasite, CLSM pictures showed a symplastic continuity from the phloem of the parasite to the phloem of the host through the haustorium.

The results of the CF transfer confirm the translocation studies using $^{14}$C-sucrose. In the investigated bridge systems *Cuscuta* was allowed to parasitize the second host plant for at least 14 d. The majority of assimilates (45 ± 6.7%) translocated from the first host plant, were found in the free-growing stem of *Cuscuta*. In the parasitized stem of the second host and in the stem parts below and above the parasite 10.5% of the translocated

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**Fig. 6.** Distribution patterns of $^{14}$C-sucrose in a ‘bridge-system’. (A) Preparation of the bridge-system for measurement of the radioactivity. (B) Percentage of the translocated $^{14}$C-compounds in the parts of the bridge-system. The explants were prepared 24 h before the translocation experiments. To avoid a closure of the phloem, the lower cut surfaces were immediately immersed in MES-EDTA (10 mM 2-[N-morpholino] ethanesulphonic acid, adjusted with NaOH to pH 5.0+10 mM EDTA, disodium salt). After 30 min the medium was replaced by 10 mM MES-buffer, pH 5.0. During translocation experiments the explants were kept in this solution. *Cuscuta* was allowed to parasitize the first plant for 28–30 d, and the second plant for 14–18 d. $^{14}$C-sucrose was applied to the upper surface of the leaf blade of the first *Vicia* plant. After 24 h the systems were divided into the defined parts for measurement of radioactivity by LSC. For calculation of the distribution patterns the measured radioactivity in each part was expressed as a percentage of the total radioactivity measured outside the leaf blade. Each value represents an average of nine measurements ± SE, V, *Vicia*; C, *Cuscuta*.

**Fig. 7.** ‘Bridge system’ of two *Vicia* plants connected by *Cuscuta reflexa*. Preparation for the application of 5,6-carboxyfluorescein diacetate (CFDA) to the parasite stem. The application sites of the dye are marked by a rectangle (injection) and by a scissor (application to the cut end).
14C-compounds were measured, indicating a lateral transport through the bundles in the haustorium. The extent to which transport of assimilates from the second host to the parasite in this stage of development takes place (if indeed it does) will be investigated in further experiments.

Nevertheless, the results presented here of translocation experiments with labelled compounds, phloem-mobile dyes, and virus are an unequivocal evidence for a symplastic transfer of phloem solutes by an open phloem connection between Cuscuta species and their compatible hosts.

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