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Inhibition of Loading of ^{14}C Assimilates by *p*-Chloromercuribenzenesulfonic Acid[1]

Localization of the Apoplastic Pathway in *Vicia faba*

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ABSTRACT

The apoplast of mature leaves excised from broadbean (*Vicia faba* L.) plants was infiltrated with 2 millimolar *p*-chloromercuribenzenesulfonic acid (PCMBS) via the transpiration stream, and the ability of the tissues to take up sugars was tested. An infiltration time of 75 minutes was sufficient to obtain a maximal (75%) inhibition of exogenous [^{14}C]sucrose (1 millimolar) uptake. This infiltration affected neither CO_2 assimilation nor the transmembrane potential difference of leaf cells but strongly inhibited phloem loading of endogenous [^{14}C] assimilates. The study of the symplastic relations between the different cell types of the mature leaf showed that the density of the plasmodesmata is generally very low in comparison with other species investigated so far, particularly when considering the mesophyll/bundle sheath and the bundle sheath/phloem cells connections, as well as the connections of the transfer cell-sieve tube complex with the surrounding cells. These three successive barriers therefore strongly limit the possibilities of symplastic transit of the assimilates to the conducting cells. The comparison of the densities of plasmodesmata in an importing and an exporting leaf suggests that the maturation of the leaf is characterized by a marked symplastic isolation of the phloem, and, within the phloem itself, by the isolation of the conducting complex. As a consequence, these physiological and cytological data demonstrate the apoplastic nature of loading in the mature leaf of *Vicia faba*, this species undoubtedly presenting a typical model for apoplastic loading.

Twelve years ago, on the basis of various considerations, several groups hypothesized that the mechanism of phloem loading was a proton-sucrose cotransport energized by a proton electrochemical gradient. In this model which was supported by work conducted mainly with two species, *Beta vulgaris* and *Vicia faba*, (4, 13 and references therein), the apoplast is supposed to be the main route for the assimilates moving from the mesophyll to the companion cell-sieve tube complex or from the phloem parenchyma to the conducting complex.

The concept of apoplastic loading was developed starting from the seventies. Again, the main evidence substantiating this concept stemmed from a very limited number of species, mainly *B. vulgaris* and *V. faba*. The evidence obtained was different for these two species; work with *B. vulgaris* demonstrated (a) the ability of the mesophyll cells to leak assimilates in the leaf apoplast (16), (b) a strong osmotic gradient between the sieve tube/companion cell complex and the surrounding cells (11), and (c) an inhibition of the loading of photosynthates by infiltration of PCMBS[3] in the leaf apoplast (12). Work with *V. faba* showed (a) a transformation of the companion cells into transfer cells, which results in an increase of the surface area available for exchanges between the apoplast and the symplast at the level of the conducting complex (14, 15), (b) diurnal variations of the sucrose concentration in the apoplast of the leaf, *i.e.* increase during the day, decrease during the night (6), and (c) a relationship between the blocking or deblocking of phloem transport and apoplastic sucrose concentration (22).

Until recent years, the concept of apoplastic loading was generally accepted, although its basis was grounded on experiments limited to a few species, and although its proponents never pretended to extend it to all the Angiosperms (3, 13). Recently, however, on the basis of some structural and physiological data, this agreement has been questioned, and the results of some works suggest that phloem loading occurs via a symplastic pathway (29 and references therein). The best evidence favoring the symplastic pathway has been obtained in *Ipomea tricolor*. In this species, the distribution of the plasmodesmata between the different cell types suggests a symplastic continuity from the assimilating parenchyma up to the sieve tube/companion cell complex (18). This symplastic continuity seems physiologically functional since Lucifer Yellow, a fluorescent probe moving in the symplast, reaches the phloem after being injected in a leaf parenchyma cell (18), and since PCMBS does not affect the loading of endogenous sugars (17).

The evidence favoring either the apoplastic or the symplastic pathway of phloem loading is sometimes equivocal. Thus, the changes in the sucrose content of the leaf apoplast as a function of the day and night time and as a function of the blocking or deblocking of phloem transport (22) may indicate that this compartment is a transitory reservoir without necessarily being a pathway in the phloem region. On the other hand, the entry of Lucifer Yellow in the sieve tube/companion

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[3] Abbreviation: PCMBS, *p*-Chloromercuribenzenesulfonic acid.

cell complex has not yet been demonstrated, and this probe, once it has reached the veins, may move in two opposite directions (29), contrary to the assimilates. Thus, the presence of plasmodesmata at the interface between two cell types does not necessarily mean that these symplastic connections are functional (3).

Consequently, either one of these two concepts (symplastic and apoplastic loading) is not valid, or phloem loading occurs via the symplastic pathway in some species and via the apoplastic pathway in other species. The opposite results obtained with PCMBS in *B. vulgaris* on the one hand (12) and in *I. tricolor* on the other hand (17) agree with the latter possibility. The pattern of vein labeling during rinsing after a short incubation on exogenous [^{14}C]sucrose suggests rather well, but does not really demonstrate, that the mechanisms of loading are different in *Pisum sativum* (possible apoplastic loading) and in *Coleus blumei* (possible symplastic loading) (28). It is therefore obvious that this topic deserves further investigation. To clarify the debate, it is necessary to search for confirmations via new arguments, by using plant species already partially studied, and liable to represent typical models of phloem loading. The present work represents a contribution to this clarification.

As a first approach, we studied the effect of PCMBS on the loading of endogenous sugars in *V. faba*. PCMBS is a good tool since, in this species, this non- (or slowly) permeant thiol reagent blocks the sucrose carrier without effect on the proton pump of the plasmalemma, at least for the short term treatments (5, 20). Another advantage is that this compound does not affect the symplastic connections (18). Then, we studied the frequency of plasmodesmata between the various cell types and we compared the evolution of this frequency when the leaf switches from an importing to an exporting stage. The results of this physiological and ultrastructural investigation, summarized in a preliminary communication (1), demonstrate that phloem loading does occur from the apoplast in the leaf of *V. faba*. The results also allow us to localize the sites where the apoplast is the obligatory pathway for the sugars before the loading step. Finally, they suggest a dramatic regression of the symplastic connections between the phloem and the mesophyll during the import/export transition.

MATERIALS AND METHODS

Plant Material

The conditions used for growing the plants (*Vicia faba* L. cv Aguadouce) have been described elsewhere (5). The plants were used when they possessed four mature bifoliate leaves.

Infiltration of the Leaf Apoplast with PCMBS

The mature leaves (fourth bifoliate leaf) were excised, and their petiole was immediately dipped into a solution containing 0.5 mM $CaCl_2$, 0.25 mM $MgCl_2$, buffered at pH 5.0 with 5 mM Na_2 citrate/10 mM Na_2 phosphate (standard medium), with (treated samples) or without (control) 2 mM PCMBS. The leaves were put under light (35 W m^{-2}) in a growth cabinet (temperature: 25 ± 1°C; RH: 55 ± 2%), under conditions allowing an efficient infiltration of the leaf apoplast by PCMBS. Depending on the sets of leaves, this infiltration lasted 30 to 180 min, and its efficiency was checked by studying the rates of uptake of exogenous [^{14}C]sucrose (1 mM) by leaf discs without lower epidermis sampled in the different batches. [U-^{14}C]sucrose (specific activity: 19.98 GBq $mmol^{-1}$) was purchased from Amersham France (Les Ulis).

Loading of Endogenous ^{14}C Assimilates

After 75 min infiltration of the leaf apoplast by a medium with or without PCMBS (Fig. 1), the petiole of each excised leaf was dipped into standard medium. The distal parts of the leaves (Fig. 1) were allowed to assimilate $^{14}CO_2$ generated from 925 KBq NaH[^{14}C]O_3 (specific activity 2.09 GBq $mmol^{-1}$) by addition of 1 mL 1% H_2SO_4. The assimilation chamber (41 × 26 × 2 cm) permitted the study of six leaves simultaneously (three controls + three treated). Light intensity, temperature, and RH were the same as the ones indicated above. After assimilation of CO_2 for 5 min, unreacted $^{14}CO_2$ was flushed into KOH solution for 5 min and the tissues were collected.

Autoradiography and Radioactivity Measurements after [^{14}C]Sucrose Uptake or $^{14}CO_2$ Assimilation

The tissues were immediately frozen on dry-ice and lyophilized. Autoradiographs were prepared according to standard procedures (5). The tissues were then combusted to $^{14}CO_2$ in an oxydiser (Oxymat IN 4101, Kontron), and radioactivity was counted by liquid scintillation spectroscopy.

Electrophysiological Measurements

Eighty-five and 180 min after the beginning of apoplast infiltration by 2 mM PCMBS or by the standard medium, leaf pieces without lower epidermis were floated on standard medium. Electrophysiological measurements were made in a Faraday cage, with the equipment previously described (21). Briefly, a glass micropipette (tip diameter < 1 μm, tip resist-

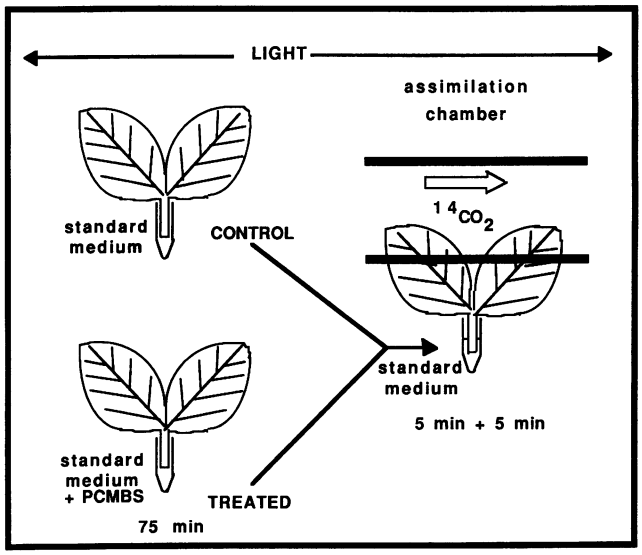

Figure 1. General procedure for infiltration of the leaf apoplast of *V. faba* with 2 mM PCMBS and for $^{14}CO_2$ assimilation by the distal part of the leaflets.

ance 15–20 mΩ) and a reference micropipette (diameter 1 mm, filled with 3 M KCl in 1% agar) were connected to an electrometer-amplifier (model M707, WP instruments, New Haven, CT). The output signal of the amplifier was monitored with an oscilloscope (DM 64, Telequipement, Tektronix UK Ltd, London, UK) and a chart recorder (Kipp and Zonen, Delft, The Netherlands). After 20 min incubation, the reference electrode was dipped into the bathing medium, and the glass micropipette was inserted into a mesophyll cell with a mechanical micromanipulator. This 20 min incubation period on the standard medium is necessary for the tissues to recover from the isolation shock. During the incubation, the PCMBS remains fixed to the plasmalemma proteins (20).

Estimation of Symplastic Connections between the Main Cell Types of the Leaf

This estimation was made both on exporting (fourth bifoliate leaf counted from stem basis, fifth order vein) and importing (eighth bifoliate leaf, 12 to 15 mm-long, third order vein) leaves. Pieces of leaf blade (about 1 mm^2) were fixed for 4 h at room temperature (about 20°C) in 2% glutaraldehyde in 0.05 M cacodylate (pH 7.2), and postfixed in 2% OsO_4 overnight in the refrigerator. The samples were dehydrated in absolute ethanol at 4°C. Embedding and inclusion of the tissues were performed in an epon-araldite mixture at room temperature. The samples were usually sectioned with a glass knife and the sections obtained (about 90 nm) were used for counting of plasmodesmata frequency. In some cases, thinner sections (about 70 nm) were made with a diamond knife, and they were used for observation of the details and photography. The sections were contrasted with uranyle acetate and lead citrate and viewed with a Jeol 100 C or a Jeol 100 S electron microscope. Plasmodesmata were counted only if they extended more than half-way across a cell-wall interface.

RESULTS AND DISCUSSION

Effect of PCMBS in the Apoplast on the Uptake of Exogenous Sucrose

After 30 min infiltration, the uptake of 1 mM [^{14}C]sucrose was already decreased by 50% (Fig. 2). The inhibition reached 75% after 60 min infiltration and remained almost unchanged for longer times (Fig. 2). This inhibition, similar to that observed in *Beta vulgaris* (24) resulted in a nearly complete disappearance of vein labeling (Fig. 3). Residual uptake occured mainly in the mesophyll (Fig. 3). As expected (19), dithiothreitol (20 mM) fully released the tissues from PCMBS inhibition (Fig. 2).

Infiltration of the leaf apoplast for 1 h is therefore sufficient to obtain maximal inhibition of uptake of sugars present in this compartment. This is the reason we chose to infiltrate the tissues for 75 min to test the effect of PCMBS on the loading of endogenous sugars. We verified that such a treatment did not affect basic processes in the tissues, particularly the assimilation of CO_2 and the transmembrane potential difference driving the influx of sugars in the conducting cells.

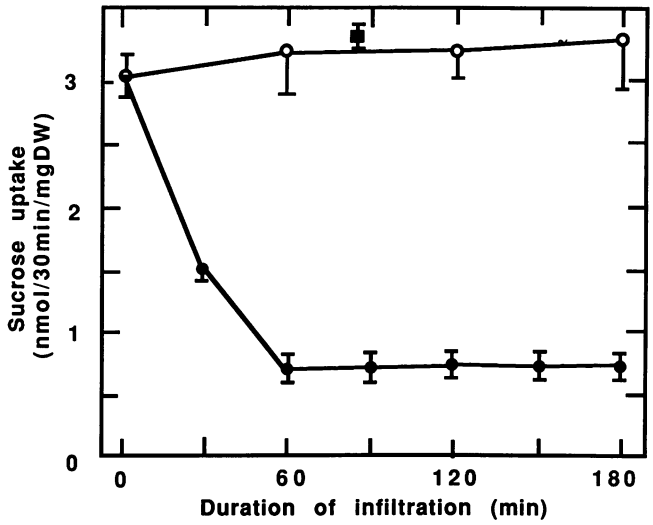

Figure 2. Uptake of [^{14}C]sucrose by leaf discs as a function of duration of apoplast infiltration by standard medium with (●) or without (○) 2 mM PCMBS. After infiltration, the lower epidermis was peeled and leaf discs were excised and floated onto a solution containing 0.5 mM $CaCl_2$, 0.25 mM $MgCl_2$, 5 mM Na_2-citrate/10 mM Na_2-phosphate (pH 5.0) (standard medium) and 1 mM [^{14}C]sucrose. Thirty min after the beginning of uptake, the discs were rinsed three times 2 min each on standard medium, frozen, and lyophilized. Data are means of 12 samples ± SE. (■), Reversal of PCMBS inhibition by dithiothreitol. After infiltration of the leaf apoplast by 2 mM PCMBS for 85 min, leaf discs without lower epidermis were sampled and floated on standard medium + 20 mM dithiothreitol for 20 min, rinsed two times 2 min each, and floated on standard medium +1 mM [^{14}C] sucrose according to the procedure described above (M ± SE, n = 20). DW, dry weight.

Effects of PCMBS on $^{14}CO_2$ Assimilation, Transpiration, and Transmembrane Potential Difference

The lack of effect of PCMBS on various physiological processes, demonstrated in previous works, suggests that this thiol reagent does not penetrate into the cell. Giaquinta (12) showed that incubation of sugar beet leaf tissues for 25 min in the presence of 10 mM PCMBS did not inhibit the rate of $^{14}CO_2$ assimilation during the 90 min following the treatment. Similarly, infiltration of broad bean leaf tissues with 2 mM PCMBS for 75 min did not affect $^{14}CO_2$ assimilation. Ten min after the beginning of $^{14}CO_2$ assimilation, the radioactivity of the assimilating zone was almost the same in both series of leaves: 6794 ± 835 dpm mg^{-1} dry weight, in the control, 7037 ± 247 dpm mg^{-1} dry weight in the treated sample (M ± SE, n = 12).

PCMBS only slightly affected transpiration: after 75 min infiltration, the amount of water taken up by each broad bean leaf was decreased by 10% in the presence of PCMBS (0.335 ± 0.011 mL in the control; 0.305 ± 0.010 for the treated leaves; M ± SE, n = 40). The apoplast volume of a *Vicia faba* leaf is about 2.3 μL cm^{-2} (2). Since the mean surface of the leaves used was 62.9 cm^2, this amount of water represented 2.3 and 2.1 times the volume of the apoplast in the control and in the treated samples, respectively. In spite of the PCMBS accumulation which may occur in the vicinity of guard cells as a result of transpiration, the functioning of the stomatal

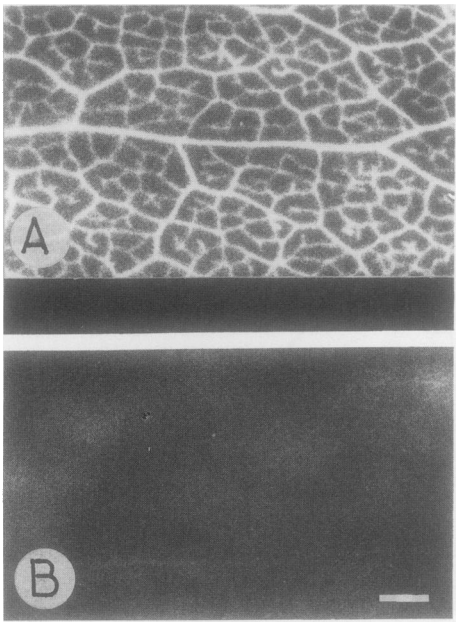

Figure 3. Autoradiographs from leaf discs incubated on [^{14}C]sucrose as indicated in the legend of Figure 2. Before excision of the discs, the leaf apoplast was infiltrated for 75 min with standard medium (A) or standard medium + 2 mM PCMBS (B). The radioactivity appears in white. The exposure time was 1 week. The scale represents 1 mm.

apparatus did not seem markedly altered. In the phloem apoplast, which is not or only very slightly implicated by the evaporation phenomena (lack of lacunae, small meatus limited to the phloem/bundle sheath interface), the PCMBS concentration was expected to rise slowly up to 2 mM. In various tissues, particularly in broad bean leaf, PCMBS blocks the sucrose carrier without affecting the proton-pumping ATPase of the plasmalemma (5, 20). The transmembrane potential difference maintained by this ATPase is insensitive to 0.5 mM PCMBS, at least for short time treatments (30 min or less), while the penetrating thiol reagent NEM decreases strongly and immediately the electrical component of the proton motive force (20). Under our experimental conditions, which involved a long exposure of the cell surface to PCMBS, this inhibitor had no effect on the transmembrane potential difference: -137.6 ± 6.2 mV for the control *versus* -126.7 ± 7.4 mV for the treated samples (M ± SE, $n = 5$) 85 min after the beginning of infiltration and, -165.7 ± 6.1 mV for the control *versus* -165.0 ± 8.3 mV for the treated samples (M ± SE, $n = 5$) 180 min after the beginning of infiltration. This lack of effect on the transmembrane potential difference shows not only that the functioning of the proton-pumping ATPase was not altered, but also that the integrity of the ATP generating processes was not affected. Overall, these data show that PCMBS did not penetrate into the cell under our experimental conditions, even if it was present for several h in the apoplastic compartment. Therefore, infiltration of the leaf apoplast by PCMBS fed via the transpiration stream provides a reliable experimental model to study the effect of this inhibitor on the loading of the assimilates (this paper), as well as on their export out of the leaf blade.

Effect of PCMBS in the Apoplast on the Loading of ^{14}C Assimilates

Ten min after the beginning of $^{14}CO_2$ assimilation, the ^{14}C assimilates began to accumulate in the veins of the control tissue (Fig. 4A) while they stayed in the parenchyma in the treated sample (Fig. 4B). These results, which are similar to those obtained with *B. vulgaris* (12), suggest that the assimilates are loaded into the phloem from the apoplastic compartment. This complete inhibition of assimilate loading by PCMBS was maintained when the duration of the experiment was extended for several hours (PCMBS infiltration in the leaf apoplast for 75 min, infiltration of the leaf apoplast with standard medium and $^{14}CO_2$ assimilation for 30 min, exudation and collection of sieve sap for 3 h). Therefore, the export of ^{14}C-assimilates out of the leaf blade was blocked (data not shown).

Density of Plasmodesmata between the Main Cell Types of the Leaf

In the mature leaf of *V. faba*, the mean frequency of plasmodesmata is, overall, very low. The frequency of plasmodesmata of the mesophyll (Table I) was similar to that observed in *B. vulgaris* (0.09 plasmodesmata μm^{-1} of interface (7), which is equal to 1.29 plasmodesmata μm^{-2}, the mean thickness of the sections being 70 nm). This frequency is also close to that noted in *Coleus blumei* (2.00, 1.41, and 2.12 plasmodesmata μm^{-2}) depending on the interface (8) but always smaller than that measured in *Populus deltoides* (0.19 and 0.34 plasmodesmata μm^{-1}) depending on the interface (23), which is equal to 2.72 and 4.86 plasmodesmata μm^{-2}.

However, the main difference between *V. faba* and the other species lies in the relationships between the bundle sheath and the surrounding cells. Thus, the density of the

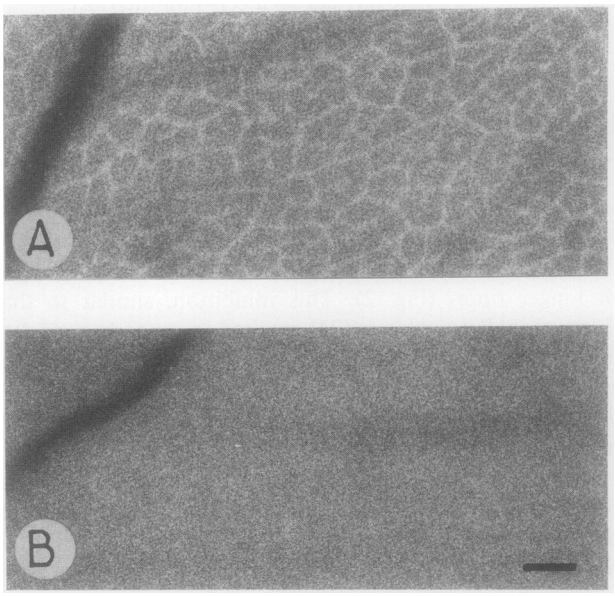

Figure 4. Autoradiographs from leaves infiltrated and labeled as indicated in Figure 1, and frozen 10 min after the beginning of $^{14}CO_2$ assimilation. A, control; B, infiltration with 2 mM PCMBS. The exposure time was 8 h. The scale represents 1 mm.

Table 1. *Frequency of Plasmodesmata between the Cell Types of Fifth Order Veins (Mature Leaf) or of Third Order Vein (Importing Leaf) and the Surrounding Tissues of V. faba*

BS, bundle-sheath cell; CC, companion cell (importing leaf); PL, palisade parenchyma cell; PP, phloem parenchyma cell; SP, spongy parenchyma cell; ST, sieve tube member; TC, transfer cell (mature leaf).

Mature Leaf	Importing Leaf
PL-PL 0.66 ± 0.23[a]	PL-PL 3.66 ± 0.14
PL-SP 1.50 ± 0.31	PL-SP 4.61 ± 0.30
SP-SP 1.24 ± 0.16	SP-SP 4.96 ± 0.43
SP-BS 0.08 ± 0.05	SP-BS 2.40 ± 0.31
BS-BS 0.84 ± 0.21	BS-BS 3.09 ± 0.29
BS-PP 0.11 ± 0.05	BS-PP 3.61 ± 0.24
BS-ST 0.00	BS-ST 0.00
BS-TC 0.07 ± 0.07	BS-CC 0.96 ± 0.20
TC-PP 0.05 ± 0.03	CC-PP 1.71 ± 0.20
TC-TC 0.22 ± 0.10	CC-CC 2.16 ± 0.28
TC-ST 0.40 ± 0.07	CC-ST 1.04 ± 0.18
ST-ST 0.03 ± 0.03	ST-ST 0.46 ± 0.26
ST-PP 0.18 ± 0.04	ST-PP 0.19 ± 0.11
PP-PP 0.57 ± 0.08	PP-PP 1.99 ± 0.20

[a] Number of plasmodesmata μm^{-2} of interface, mean ± SE, $n = 20$ to 50.

plasmodesmata at the interface of the spongy parenchyma/bundle sheath (0.08 plasmodesmata μm^{-2}) was about 15, 25, 50, and 200-fold lower than in *B. vulgaris*, *C. blumei*, *P. deltoides*, and *Amaranthus retroflexus*, respectively (7, 8, 9, 23). In the same way, the frequency of the plasmodesmata at the bundle sheath/phloem interface was extremely low (Table I; Fig. 5, A and B) and was also smaller or dramatically smaller than that noted usually in other species (7–9, 18, 23, 26, 27). Indeed, in *V. faba*, the symplastic connections are almost absent at the bundle sheath/sieve tube interface and at the bundle sheath/transfer cells interface. At the interface between the bundle sheath and the parenchyma cells, they were, for example, 15-times less numerous than in *B. vulgaris* [0.13 plasmodesmata μm^{-1} interface, which is equal to 1.86 plasmodesmata μm^{-2} (7)], and about 50-fold less numerous than in *P. deltoides* [0.43 plasmodesmata μm^{-1} interface, which is equal to 6.15 plasmodesmata μm^{-2} (23)].

In the mature leaf of *V. faba*, the phloem thus presents very limited symplastic connections with the surrounding tissue contrary to the 'plasmodesmogramm' of this species recently drawn by Van Bel *et al.* (30). In this relatively isolated system, the transfer cells (Fig. 5, A and B) possess symplastic connections only with the sieve tubes since the frequency of plasmodesmata at the interface of the transfer cells/parenchyma cells was as low as at the interface of the transfer cells/bundle sheath cells (Table I). The plasmodesmatal frequency between the transfer cell and the sieve cell was low (0.40 pore μm^{-2} interface on the sieve cell side, 0.65 pore μm^{-2} interface on the transfer cell side), but the pore diameter was large (170 nm) (Fig. 5B).

Additional experiments were made with the eighth bifoliate leaf of the same plants. These leaves, only 12 to 15 mm long, are fully importing, and their minor veins network is not yet differentiated. Consequently, the study of the frequency of plasmodesmata involved the secondary veins. These data are

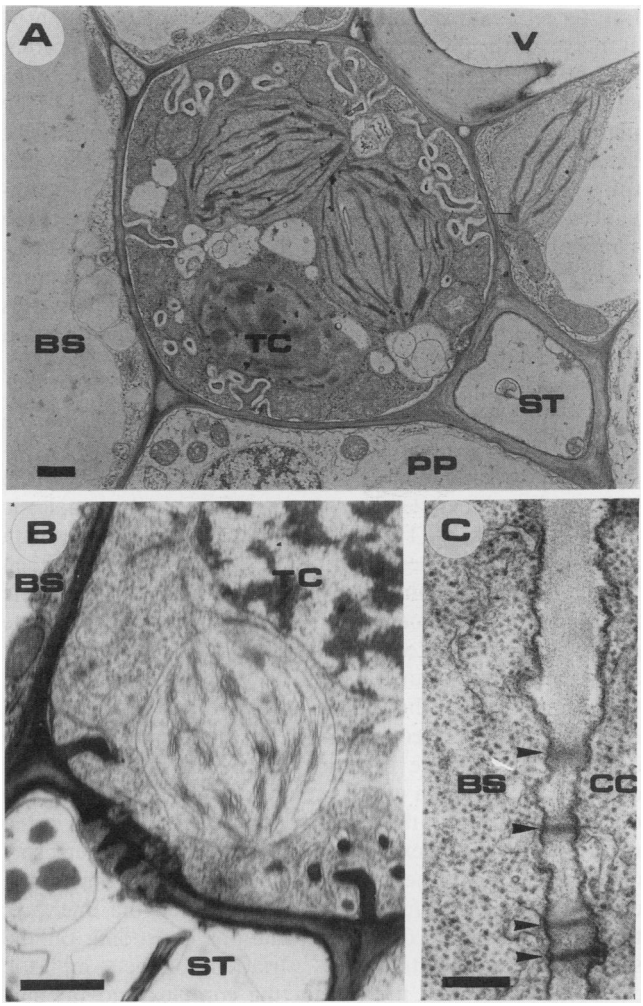

Figure 5. Structure of the minor veins of *V. faba* leaf. A, Principal cell types of a fifth order vein in a mature leaf; B, symplastic relations between a sieve tube cell and a transfer cell in a mature leaf; C, symplastic relations between a companion cell and a bundle sheath cell in a very young leaf (third order vein). BS, bundle sheath; CC, companion cell; PP, phloem parenchyma; ST, sieve tube; TC, transfer cell; V, vessel. Plasmodesmata are shown by arrows. The scale bar represents 1 μm (A and B) and 0.25 μm (C).

thus not directly comparable with the previous ones since they concern neither the same leaf nor the same category of veins. However, they showed considerable differences in the symplastic connections of the phloem with the surrounding tissues, between an importing vein and an exporting vein (Table I).

From a general standpoint, the density of the plasmodesmata was markedly higher in the very young leaf than in the exporting leaf. This density was three- to five-fold higher for several kinds of interface (palisade parenchyma/palisade parenchyma, palisade parenchyma/spongy parenchyma, spongy parenchyma/spongy parenchyma, bundle sheath/bundle sheath, phloem parenchyma/phloem parenchyma). Furthermore, for some particular interfaces, the plasmodesmata were 15 times (bundle sheath/companion cell or transfer cell) or 30 times (spongy parenchyma/bundle sheath, bundle sheath/phloem parenchyma, phloem parenchyma/companion cell or

transfer cell) more numerous (Table I; Fig. 5C). Consequently, at this stage, the phloem of the importing vein possesses rather well developed connections with the surrounding tissues, in contrast with what occurs in the minor exporting veins of the mature leaf. These results give support to the hypothesis of Schmalstig and Geiger (25) since they suggested that the transition from the importing to the exporting stage is accompanied, in some species at least, by a dramatic decrease of the symplastic connections between the phloem and the assimilating parenchyma. The evolution of the symplastic relationships between the different cell types and of the relationships between the apoplast and the symplast during the maturation of the leaf will be detailed later (S Bourquin, J-L Bonnemain, unpublished data).

In conclusion, the physiological (effect of PCMBS on the loading of endogenous sugars) and the cytological data (density of the plasmodesmata between the different cell types) indicate that phloem loading occurs mainly via the apoplastic pathway in *V. faba*. The marked paucity of plasmodesmata at two successive interfaces (bundle sheath-mesophyll, then bundle sheath-phloem cells) results in an almost complete isolation of the phloem symplast. To gain access to the phloem, the assimilates must therefore transit in the apoplast at the level of the bundle sheath. From this compartment, they accumulate in the transfer cell-sieve cell complex since the symplastic connections between this complex and the surrounding cells are extremely weak. In addition, when compared to the data of the literature (7–10, 18, 23, 26, 27) our results show that the density of the plasmodesmata in the mesophyll generally exhibits little variation in different species, while for the mesophyll/phloem interface and for the companion (or transfer) cell/phloem parenchyma interface, this density exhibits dramatic variations depending on the species. The species to species variations of the symplastic connections between the conducting complex and the surrounding cells have already been clearly outlined in various Angiosperms (10). Consequently, it is obvious that our conclusions concerning the pathway of phloem loading in the veins of *V. faba* cannot be extended to all vascular species. Indeed, our results exclude neither the possibility of symplastic loading in some species, *Ipomea tricolor* for example (17, 18), nor that of a contribution of both pathways in other species, *Commelina benghalensis* for example (30).

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