Sucrose and Malic Acid as the Compounds Exported to the Apical Bud of Pea following $^{14}CO_2$ Labeling of the Fruit[1]

NO EVIDENCE FOR A SENESCENCE FACTOR

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ABSTRACT

The G2 line of peas (*Pisum sativum* L.) displays senescence and death of the apical bud only in long days and in the presence of fruit. As the removal of fruit prevents senescence, one possible mechanism by which fruits induce senescence is that the fruits produce some 'senescence factor' under long day conditions, which is then transported to the apical bud. Allowing developing fruits to photosynthesize in the presence of $^{14}CO_2$ results in the recovery of label in the apical bud. In order to determine the chemical nature of this radiolabeled material, fruits of G2 peas, growing under long days, were exposed to $^{14}CO_2$ at the time when the first senescence symptoms start to appear. The radiolabeled material from apical buds was then extracted, purified, and identified. Using HPLC and GC-MS the major labeled compound found in the apical bud following exposure of pea fruits to $^{14}CO_2$ was identified as sucrose, while malic acid was identified as the major ethyl acetate-soluble compound. These compounds accounted for about 73 and 16%, respectively, of the radioactivity in the apical bud. No other compounds were present in significant amounts. As neither of these chemicals is likely to have any kind of senescence effect, we report no evidence for a senescence factor.

In most monocarpic annual plants the presence of fruits is needed for senescence of the whole plant to take place. This has been shown to be a property of the developing seeds. One hypothesis to account for this phenomenon is that the developing seeds produce a 'senescence factor' which is exported from the fruit to the rest of the plant, resulting in its demise (14). Chemical substances exported from fruits have in fact been suggested to be responsible for several correlative phenomena observed during fruit growth (19). The application of ^{14}C-containing compounds to the fruit of numerous plant species has been shown to result in the export of small amounts of radiolabel back into the plant (9). While the amounts of radiolabel exported are nutritionally insignificant, it is nevertheless clear that fruits are capable of producing and exporting substances to other parts of the plant. There have been no firm reports of the chemical identification of endogenous substances exported by fruit in an intact plant.

Pisum sativum, line G2, displays a photoperiodically sensitive decrease in the growth rate of the apical bud, followed by apical senescence, when grown in LD in the presence of developing seeds (18). In SD senescence does not occur and growth continues. In peas, apical senescence is the prelude to the senescence and death of the whole plant. When G2 pea pods are treated with ^{14}C sucrose or allowed to photosynthesize in $^{14}CO_2$ there is a movement of radiolabeled material from the fruit to the apical bud (7). In two previous papers (9, 10) we have characterized the export of radiolabeled material from G2 pea fruits treated with $^{14}CO_2$. About 1% of the fixed ^{14}C is exported from the fruit and of this 10% can be found in the apical bud (9). The pathway of movement of the radiolabeled material out of the fruit is in the xylem, driven by the water potential gradient between the fruit and the leaves (10).

Does the radiolabeled material which reaches the apical bud represent a senescence factor? Gianfagna and Davies (7) showed that a radioactive fraction isolated from apical buds was unique to plants grown in LD, and was not evident in those grown in SD, suggesting a connection with the LD-induced apical senescence. An attempt to identify the transported material resulted in the identification of two novel plant compounds (5), but did not uncover the identity of the radiolabeled material which was recovered from the apical buds of treated plants (7).

The work presented in this paper is a continuation of the attempt to elucidate the nature of the material transported from the fruit to the apical buds. In addition, we have looked for compounds secreted from seeds in *in vitro* culture.

MATERIALS AND METHODS

Plant Material. *Pisum sativum* L. line G2 plants were grown as previously described (9). LD plants were grown with 18 h of light while SD plants were given 9 h of light. The oldest pod on a plant was labeled with CO_2 by the addition of $NaH^{14}CO_3$ and 0.3 N HCl in an enclosing plastic bag 8 to 10 d after anthesis as previously described (9). Leaves were labeled in the same manner as pods. Apical buds selected for use consisted of all the tissue from the apex down to and including the most recently expanded leaf.

Isolation of Exported Material. After the appropriate treatment, plant parts were harvested and either used immediately or frozen at $-80°C$. Tissues were ground in a Sorvall Omni-Mixer (Newton, CT) in 80% methanol (MeOH), shaken for 3 to 6 h at 4°C and filtered. The residue was reground in fresh 80% MeOH, refiltered, and the filtrates combined. The MeOH was removed *in vacuo*, and much of the Chl precipitated by the addition and subsequent evaporation of a one-third volume of hexanes. The aqueous solution was filtered, brought to pH 3 with acetic acid (HAc), and partitioned five times against ethyl acetate (EtOAc). Residual amounts of EtOAc were removed from the aqueous fraction by evaporation under a stream of nitrogen. The EtOAc

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fraction was dried over anhydrous $MgSO_4$, brought to dryness under a stream of nitrogen, and taken up in 20% MeOH for subsequent injection onto HPLC.

HPLC. Samples for chromatography were dissolved in the appropriate solvent and then filtered through a 0.2 μm filter attached to a 10 mL glass syringe (Durapore, Millipore, Bedford, MA), or centrifuged through a 0.2 μm filter (Nylon 66, Rainin, Woburn, MA). The HPLC equipment consisted of two Altex model 110A solvent metering pumps run by an Altex model 420 controller/programmer (Beckman, Berkeley, CA). Solvent gradients used are described in the text and figure captions.

HPLC columns used included a 10 × 150 mm PRP-1 preparative column (Hamilton, Reno, NV), a 4.6 × 150 mm PRP-1 analytical column, and a 4.6 × 250 mm 5 micron spherical particle C18 analytical column (Microsorb C18, Rainin). Flow rates were 2 ml/min through the preparative PRP-1 column, and 1 mL/min through all other columns.

Radioactivity was detected by a Packard (Downers Grove, IL) "Trace" 7140 radioactivity flow monitor equipped with a flow cell packed with insoluble scintillation beads (170 μL void volume). Efficiency at 1 mL/min was typically 6 to 8% for ^{14}C. An automatic peak detection circuit controlled an LKB (Bromma, Sweden) fraction collector which routinely collected >96% of every peak. As this radioactivity detector does not require addition of fluor to the effluent, the entire sample could be used for further analysis.

Gas Chromatography—Radioactivity Counting. Samples to be injected were placed in a 1 mL Reactivial (Pierce, Rockford, IL) and dried *in vacuo* overnight over P_2O_5. Five to 10 μL of pure, dry pyridine were injected into the vial to dissolve the sample, followed by an equal volume of Sylon BFT trimethylsilylating reagent (bistrifluoroacetamide [BSTFA] + 1% trimethylchlorosilane [TMCS], Supelco, Bellafonte, PA); 0.5 μL of the sample was then immediately injected onto a 0.53 mm × 10 m RSL-300 (50% methyl, 50% phenyl bonded phase) capillary column (Alltech, Deerfield, IL) in a Hewlett Packard (Allentown, PA) model 5830 gas chromatograph equipped with an effluent splitter (SGE, Victoria, Australia). N_2 carrier gas was regulated to 30 cm/s (approximately 3 mL/min) and N_2 make-up gas was added after the column to produce a flow of 40 mL/min at the flame ionization detector. Operating conditions were isothermal at 115°C for 1.5 min, and then temperature programmed at 2°C/min to 250°C. The temperatures of the injector, detector, and transit line to the collector were held at 280°C. Radioactivity was detected by splitting the effluent 1:1 between the flame ionization detector and an outlet. Material was collected at the outlet in 2 mm capillary tubes containing 2 cm of OV-17 packing material which had been chilled on dry ice. The collection tubes were changed every 30 or 60 s. After collection, the condensed material was eluted with 1 mL of EtOAc, and radioactivity was detected by using an aliquot for scintillation counting. For ease of description, this column and chromatograph will be referred to as GC system 1.

GC-MS. For GC-MS analysis, samples were run on a 0.25 mm × 30 m RSL-300 (50% methyl:50% phenylsilicone bonded phase, Alltech) capillary column, or a 0.20 mm × 12 m HP-1 (100% methylsilicone bonded phase, Hewlett Packard, Palo Alto, CA) capillary column in a Hewlett Packard model 5890 gas chromatograph connected to a Hewlett Packard model 5970B mass selective detector (MSD). Temperature programming was as above. The MSD had an ionizing potential of 70 eV, and the mass range scanned was 0 to 500 mass units resulting in 0.86 scans/s. This GC-MS system will be referred to as GC system 2, and reference will be made to which of the two columns was being used. (The first column used in this system, *i.e.* RSL-300, was chosen in order to reproduce the patterns of peak elution of GC system 1. The second column, *i.e.* HP-1, was chosen to match the column available in the chemical ionization GC-MS system used for obtaining the mass ion.) The GC injector was set in either the 'splitless' or 'split' mode; the former, used for samples, vented all of the injected material onto the column, and the latter, used for standards, vented some to waste. Split injection routinely produces sharper peaks than splitless.

For chemical ionization GC-MS, the sample was run on a 0.75 mm × 30 m SPB-1 (100% methyl silicone bonded phase) column (Supelco, Bellefonte, PA) in a model 3300 Finnegan (Sunnyvale, CA) GC-MS with methane as the carrier gas (source pressure 600 microns, giving a flow rate of about 30 mL/min), at 115°C for 1.5 min and then temperature programmed at 6°C/min. Ions were scanned from 60 to 500 at approximately 4 scans/s.

Identification of Carbohydrates by HPLC. Polar HPLC fractions were collected and any MeOH present was removed *in vacuo*. Aqueous fractions were then deionized by passing them through a 5 ml glass column (Econo-column, Bio-Rad, Richmond, CA) containing 1 mL Amberlite IRA-45 anion exchange resin (free base form, Sigma) overlain by 1 mL of Dowex 50W cation exchange resin (hydrogen form, Sigma). The aqueous eluent was condensed *in vacuo*, passed through a 0.2 micron filter, and run on a Sugar Pak I carbohydrate column (Waters, Milford, MA) heated to 90°C, and the peaks were detected by a model R-140 Refractive Index detector (Waters). This HPLC was run with water as the mobile phase at a flow rate of 0.5 mL/min (4).

Seed Exudate Experiments. Pods selected for seed exudate experiments were labeled as usual unless noted. The pods were harvested and seeds were removed from the pod in a sterile hood after surface sterilization of the pod for 3 min in a 5% sodium hypochlorite solution. Seedcoats were removed with a razor blade and 6 to 8 embryos were placed in 10 ml of sterile nutrient solution composed of 0.5 mM KCl, 0.5 mM NaCl, 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 1.0 mM MeS (pH 6), and 150 mM sucrose (16). The embryos were shaken gently at room temperature for 24 h. For very young seeds (less than 8 d after anthesis) whole seeds rather than embryos were used. After 24 h the bathing solution was decanted and either used as recovered or condensed by rotary evaporation. Portions were removed and filtered for analysis by HPLC.

RESULTS

Analysis of Apical Extracts. Plants were grown in both LD and SD to determine if there were any qualitative differences in the types of radiolabeled materials exported to, and subsequently recovered from, the apical bud. Apical buds were harvested 24 h after a 6 h exposure of a pod to $^{14}CO_2$. The tissue was extracted in 80% MeOH, the MeOH removed *in vacuo*, made basic (pH 9) and partitioned against hexanes, then made acidic (pH 3) and partitioned against EtOAc. The results were similar between LD and SD, with about 6 to 7% of the total radioactivity partitioning into the hexane fraction, about 20 to 23% partitioning into the EtOAc fraction, and the rest remaining in the aqueous fraction. Thus, the majority of the radioactivity was found to move into the aqueous fraction in this study, unlike previously reported results (7) with several partitioning systems which indicated that the bulk of the radioactivity recovered from a methanolic extraction of apical buds would partition into an EtOAc phase from an acidic (pH 3) aqueous phase.

After extraction of the apical buds in 80% MeOH and subsequent partitioning against EtOAc, equal amounts of radioactivity from aqueous and EtOAc samples from both LD and SD were run on preparative HPLC. Each sample was injected onto a PRP-1 preparative column with a flow rate of 2 mL/min and programmed from 0.1 N HAc—100% MeOH over 15 min, producing the chromatographic profiles shown in Figure 1, A to D. The aqueous fractions from both LD and SD plants were similar and

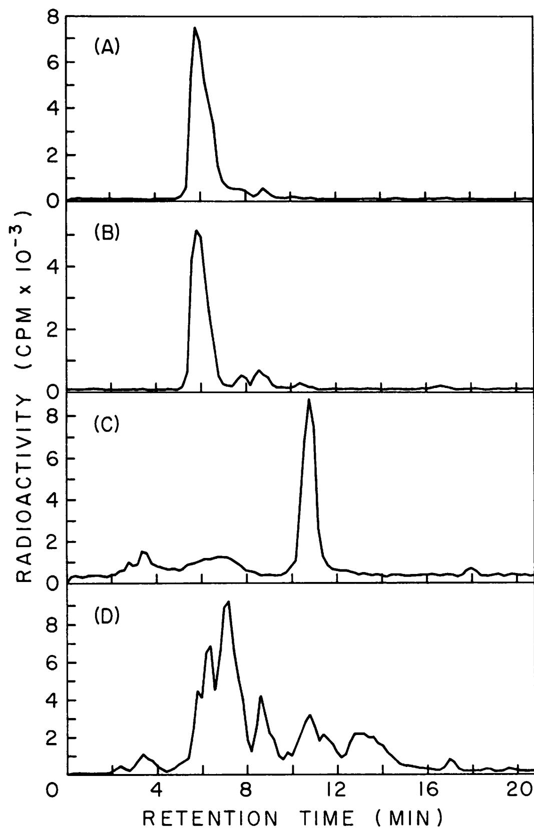

FIG. 1. Reverse-phase HPLC traces of the radioactive aqueous (A and B) or EtOAc (C and D) fraction of the extract from the apical buds of plants grown in LD (A and C) or SD (B and D) conditions. Solvents were 0.1 N acetic acid to 100% MeOH over 20 min at 2 mL/min on a PRP-1 preparative column.

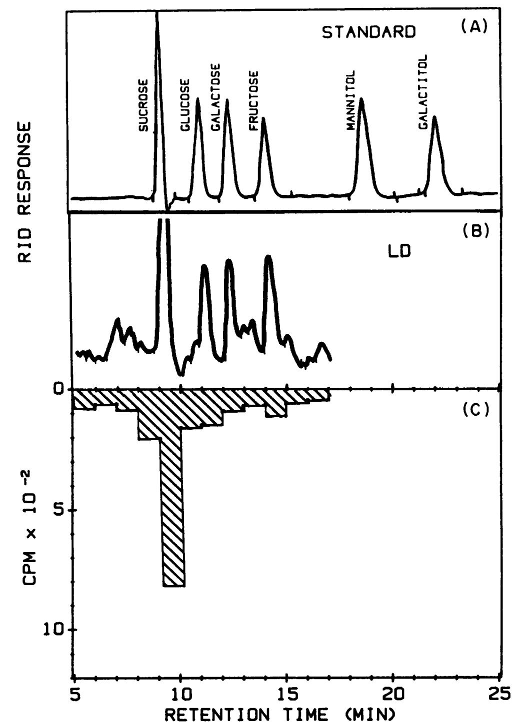

FIG. 2. Refractive index (B) and radioactivity traces (C) from HPLC of the large radioactive peak from the LD aqueous fraction shown in Figure 1 compared to standard sugars run under identical conditions (A). The sample was run in water at 0.5 mL/min on a Sugar Pak I carbohydrate column. Samples were collected at 0.5 min intervals and aliquots were used for scintillation counting as described in the text. The trace from the SD aqueous fraction was almost identical.

showed a single large polar peak (Fig. 1, A and B). There was, however, a difference in the profiles of the EtOAc fractions. The HPLC profile of the SD apical bud showed several peaks of radioactivity, while that of the LD apical bud showed most of the radioactivity incorporated into a single peak at retention time 10.8 min (Fig. 1, D and C).

Analysis of the Aqueous Fraction. As preliminary TLC results suggested that the large aqueous peak might be a carbohydrate, the radioactive peaks from both the LD and SD extracts were collected, the volume reduced *in vacuo*, and the sample prepared for carbohydrate HPLC as described in "Materials and Methods." The HPLC trace of both the LD and SD fractions showed the bulk of the radioactivity incorporated into a peak co-eluting with sucrose, with lesser amounts incorporated into peaks coeluting with fructose and glucose (Fig. 2). Invertase treatment of a portion of the LD sample identified the first peak as sucrose; this treatment resulted in a virtual elimination of the sucrose peak with a sharp increase in the glucose and fructose peaks, as well as the amount of radioactivity associated with them (data not shown).

Analysis of the Ethyl Acetate Fraction. The EtOAc fraction from the apical buds grown in LD was taken with the goal of identifying the large peak which is not present in SD. To increase the total amount of radioactivity recovered from the apical bud, a developing pod was treated as usual, but with 1 mCi of $NaH^{14}CO_3$ added to the treatment bag. The radioactive sample was initially purified on a PRP-1 preparative column as described above. The radioactive peak was collected, reconcentrated and run on a PRP-1 analytical column with a gradient programmed from 100% H_2O (made pH 3 with HAc) to 10% MeOH (pH 10) over 15 min. The peak was again collected and rerun through an analytical C18 column (Rainin Microsorb C18) programmed from 0.1 N HAc—10% MeOH over 15 min. Finally, the reconcentrated peak was run again through an analytical PRP-1 column with water adjusted to pH 3 with HAc. The radioactivity remained in a single peak throughout these preparative procedures (data not shown). Samples of the radioactive fraction from HPLC were derivatized for GC injection as described in "Materials and Methods."

As organic acids are likely candidates for extraction by the procedures mentioned here, the radioactive fraction partitioning into EtOAc was tested for the presence of a carboxylic acid moiety. Derivatization of the radioactive peak from HPLC with ethereal diazomethane (specific for its reaction with acid groups) produced multiple peaks of radioactivity, thus proving inconclusive. As a result, trimethylsilyl (TMS) derivatization was selected for volatilization for GC.

An untreated parallel sample was also prepared using 25 apical buds from nontreated plants grown in LD. A small amount of the previously isolated radioactive fraction was used to spike the large extract to follow it through the identical HPLC purification steps. Injection of 1 μL of a spiked sample onto a 0.53 mm × 10 m RSL-300 (Alltech) GC column, combined with collection and scintillation counting of a split fraction (GC system 1), resulted in the chromatogram shown in Figure 3. The bulk of the radioactivity can be seen to be associated with a peak at retention time 9.85 min. Once the radioactive peak was determined, an equivalent nonspiked sample was run on an equivalent column (0.25 mm × 30 m RSL-300 column) in the gas chromatograph equipped with a mass spectrometer as a detector (GC system 2) using the splitless injection mode. While the retention times were not the same, due to the different sizes of the columns, the

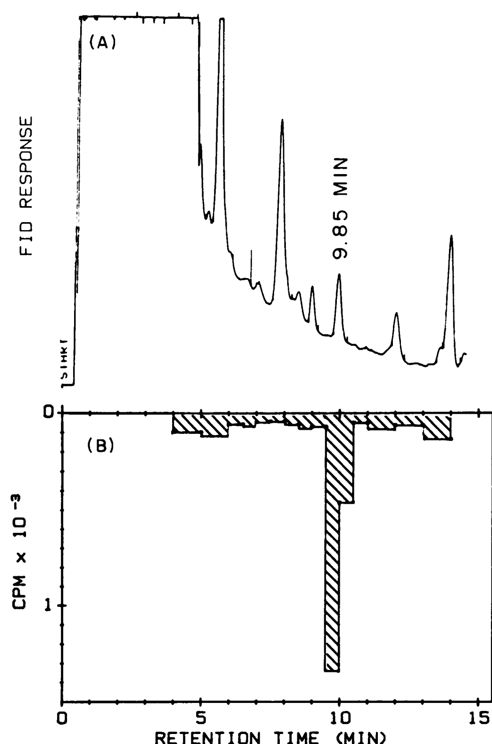

FIG. 3. Flame ionization (A) and radiocounting (B) trace from a GC separation of the purified, TMS-derivatized EtOAc sample from apical buds grown in LD. The sample was run on a RSL-300 capillary GC column maintained isocratically at 115°C for 1.5 min, then temperature-programmed at 2°C/min to 250°C. Fractions were collected at 1 min intervals between minutes 4 to 6 and 11 to 14, and at 0.5 min intervals between minutes 6 to 11, and aliquots were used for radioactivity counting as described in the text.

pattern of peak elution remained the same between the two columns, and a mass spectrum of the peak, earlier shown to be radioactive, could be obtained (Fig. 4A). Algorithmic matching of the resulting spectrum indicated the compound was probably a TMS-derivatized dicarboxylic acid.

To increase the amount of material in the peak of interest, fractions corresponding to the peak were collected from several injections of the spiked sample, using the gas chromatograph equipped with the effluent splitter (GC system 1), and the collected material was added back to the nonspiked sample. The pooled material was then rederivatized. Chemical ionization GC-MS of this sample run on an SPB1 column revealed an $(M+1)^+$ peak at m/z 351, corresponding to TMS-derivatized malic acid and with a similar retention time to that of a malic acid standard (data not shown). The rederivatized pooled sample was then run on GC system 2 equipped with an HP-1 column (methylsilicone), with the injector set in the splitless mode. The resulting chromatogram and mass spectrum (Fig. 4B) showed that the principal radiolabeled material in the apical buds, that partitions into EtOAc, was indeed malic acid. A TMS-derivatized malic acid standard was also run on this system and is shown in Figure 4C.

The sample peak in Figure 4B appears to be slightly contaminated, showing a slight shoulder on the leading edge of the peak. However, the ions found to be associated with the shoulder were not seen in the earlier sample.

Seed Exudate Experiments. To determine if developing seeds exude substances into the seed coat apoplast, intact seeds were labeled by one of several means, harvested from the plant, removed from their seed coats, and incubated in a bathing solution designed to simulate the osmolarity and chemical make-up of the apoplastic space (8, 14). After incubation for 24 h, the bathing medium was analyzed for exuded radiolabeled metabolites.

Pods and seeds were initially labeled with $^{14}CO_2$ via the bag technique described previously. Bathing medium was partitioned against EtOAc as described for tissue extracts, but no material was found to partition into the EtOAc fraction. A 100 μL sample of the bathing medium was analyzed at 2 mL/min on a PRP-1 10 × 150 mm preparative reverse phase HPLC column programmed from 0 to 10% over 10 min with a 0.1 M HAc/MeOH gradient. Radioactivity detection revealed one relatively polar peak of radioactivity, sometimes accompanied with a much smaller, less polar, peak (Fig. 5). The main peak co-chromatographed with sucrose in this system, so it was collected, deionized, and chromatographed on carbohydrate HPLC as described. The effluent was collected and aliquots were used for scintillation counting. The radioactivity was found to co-chromatograph with sucrose in this system (Fig. 6). Other sugars were present in the sample, but were not found to be radiolabeled.

The presence and size of the second peak of radioactivity found in the bathing medium was extremely variable and could not be associated with either seed size, seed age, or bacterial contamination of the medium. Its significance must therefore be considered questionable.

It is possible that the method of radiolabeling employed here was not adequate to uniformly label the seeds. Attempts were made to label the seeds either by radiolabeling the subtending leaf with $^{14}CO_2$ and harvesting the pod after 24 h, or by adding [^{14}C]sucrose directly to the bathing medium with unlabeled seeds for 6 h, and then placing the seeds in fresh medium to look for the presence of exuded metabolites. In all cases HPLC analysis of the bathing medium after a 24 h incubation showed only one radiolabeled fraction which corresponded with sucrose.

DISCUSSION

The objective of this research was to identify the chemical nature of the organic materials exported from developing fruits of pea, to the apical buds, in order to determine whether a fruit-produced senescence factor might be the cause of apical senescence and the subsequent senescence of the whole plant. We have now identified the major compounds, representing 89% of the recovered labeled material, as sucrose and malic acid. These two compounds account for the principal compounds, and the vast majority of the total amount, exported from fruits to the apical buds of G2 peas in LD under which senescence takes place. In these experiments the radiolabel corresponding to the malic acid peak in LD consisted of approximately 78% of the radioactivity which partitioned into the EtOAc fraction. This accounts for approximately 16% of the total radioactivity found in LD apical buds, while label recovered as sucrose constituted approximately 73%. Thus, 11% of the label recovered from the apical bud remains unidentified, but this either represented numerous compounds in quantities too small to give distinct peaks on HPLC, or a general spread of radioactivity in the analytical methods used, as no other single peak of material was observed in either the aqueous or EtOAc fractions. Of the clearly defined peaks sucrose constituted 82% and malic acid 18%.

There may still be a chance that an undetected amount of physiologically active material is exported from G2 pea fruit, but we must conclude that there is no evidence from any of the experiments described here to support the hypothesis that a senescence factor is exported from the pods of G2 peas grown in LD.

It is highly unlikely that a molecule as ubiquitous as malic acid is a senescence factor or a growth regulator responsible for a decrease in growth rate. There are especially high levels of phosphoenolpyruvate carboxylase (PEP-carboxylase) in fruit

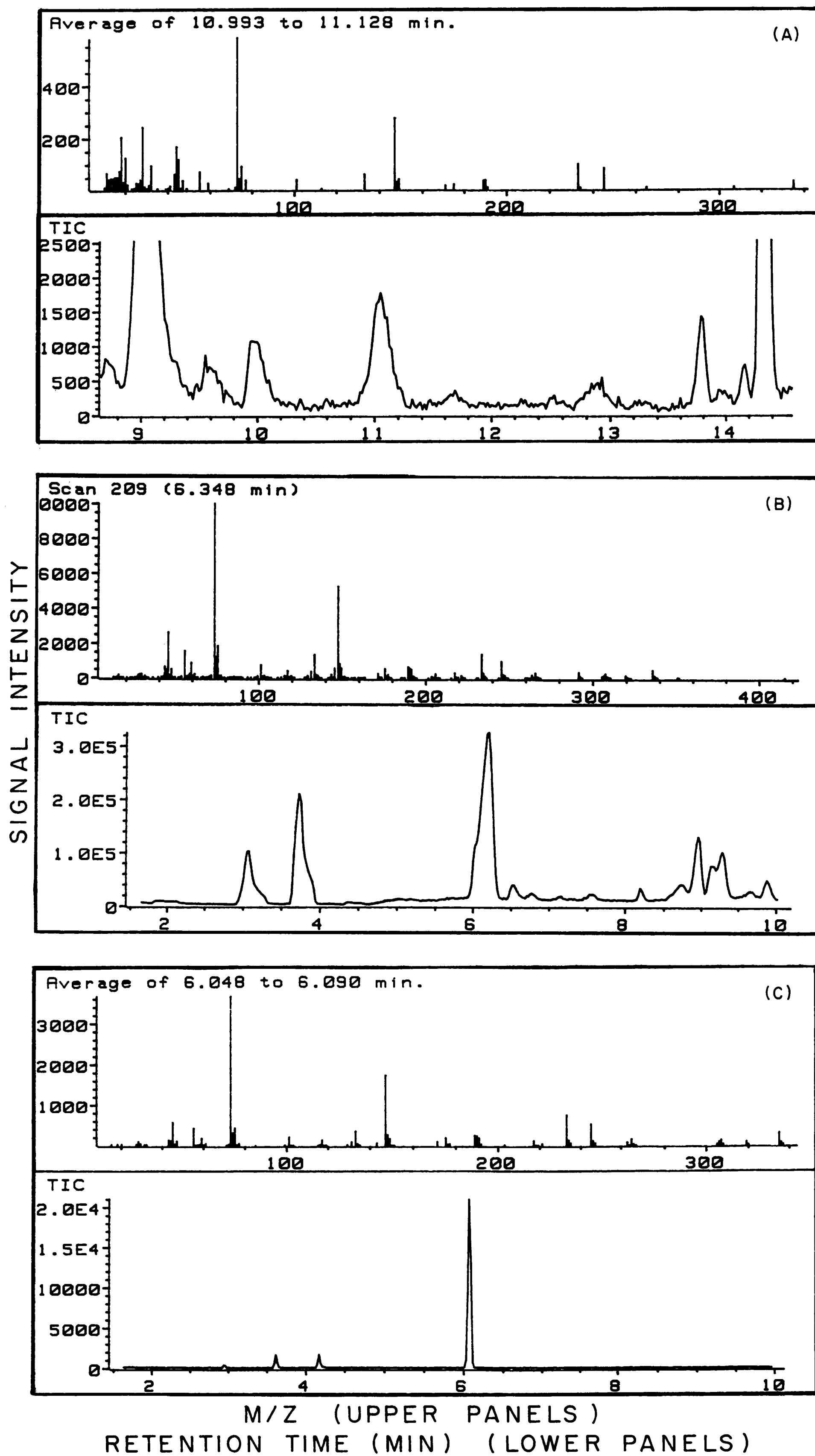

FIG. 4. Total ion current (TIC) traces from GC-MS (lower panels) and mass spectra (upper panels) of specific peaks in the TIC trace of: (A) purified, TMS-derivatized EtOAc sample for apical buds grown in LD; (B) as (A) plus added material collected as described in the text; the spectra (upper panels) in (A) and (B) are of the peak which corresponds to the radioactive peak at 9.85 min in Fig. 3; (C) TMS-derivatized sample of malic acid. Analytical conditions were as follows: (A) RSL-100 capillary column maintained isocratically at 115°C for 1.5 min and temperature-programmed at 2°C/min to 250°C; (B) HP-1 capillary column maintained isocratically at 115°C for 1.5 min and temperature programmed at 2°C/min to 250°C; (C) as B except that the sample was injected while the GC injector was set in the split mode.

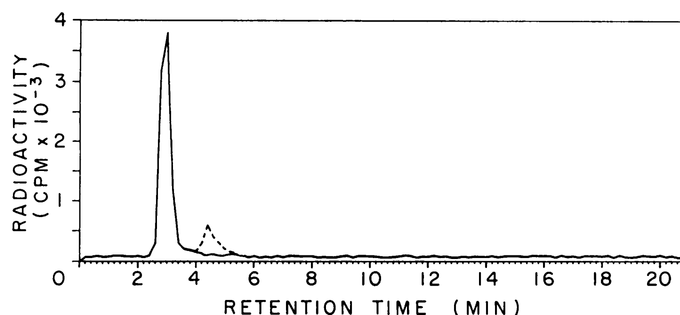

FIG. 5. Reverse-phase HPLC trace of the radioactive aqueous medium recovered from the seed exudate experiment described in the text. HPLC conditions were as in Figure 1. The dotted line indicates a peak whose appearance was not reproducible.

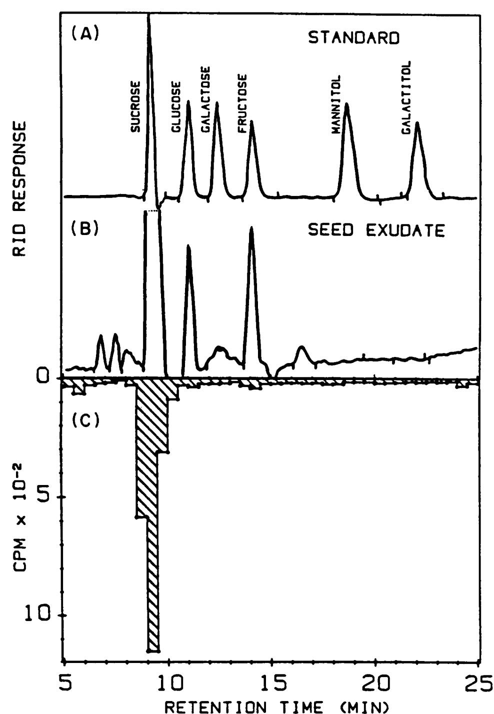

FIG. 6. Refractive index (B) and radioactivity trace (C) from HPLC of the radioactive aqueous fraction from the seed exudate experiment shown in Figure 5. Standard sugars run under the same conditions are shown in (A). HPLC conditions were as in Figure 2.

walls of legumes, as well as in seed coat and cotyledonary tissues (11). The major function of this C4 type of carbon fixation in legume carpels is widely thought to be the refixation of respired seed CO_2 (11), and malate is often a major product of C4 carbon fixation, especially when it occurs in nominally C3 plants (1). It is not unusual, therefore, to find that much of the label has become incorporated into malate after $^{14}CO_2$-treatment of pea pods. What may be unusual is that malic acid has apparently been exuded from the pods or seeds in enough quantity to be recoverable in other parts of the plant. Malic acid is known to be exported by leaf mesophyll cells of those C4 plants which use malic acid to shuttle carbon to bundle sheath cells. However, 'typical' C4 plants display Kranz-type morphology necessitating this type of carbon shuttle. Pea pods do not display any unusual morphology related to carbon shuttling, so the presence of malate in the apoplastic solution is enigmatic. Malic acid has been found in the phloem of several species (15, 20), and there is some evidence for its transport in the xylem (3).

Evidence presented in an earlier paper (10) indicates that various materials can be exported from surgically exposed seed coat tissue. It is possible, therefore, that at least some of the material exported from the intact pod could originate from this tissue. This could occur *in vivo* if developing pea seeds exude materials into the xylem-accessible apoplastic space which exists between the embryonic cotyledonary tissue and the maternal seed coat tissue. Sucrose was identified as the major radiolabeled material exuded into a bathing medium for 24 h after various labeling treatments (Fig. 6).

It is interesting to note that the extraction of the seed exudate medium with EtOAc resulted in no radiolabel migrating into the EtOAc fraction. Since the evidence points to the export of malate from intact whole fruit, it is probable that the exported malate does not derive from the seeds, but from the pod wall. The presence of PEP-carboxylase in the pod wall, coupled with the knowledge that export has been shown to occur from carpel tissue (17), suggests that the export of malate originates from the carpel rather than the seed. This could occur either by entry of malate directly into carpel wall xylem, or by its delivery to the seed coat apoplast, from which export has already been shown to occur (2, 10). Pea seeds also are known to have high levels of PEP-carboxylase but they would not likely be exposed directly to $^{14}CO_2$ in these experiments.

The reason that a large peak of malate is recovered from apical buds in LD and not SD is not readily obvious. Pea seeds have been shown to have a more rapid rate of growth in LD than SD (6), so it is possible that the PEP-carboxylase activity in LD pods is increased commensurately. Another possibility for a decreased percentage of labeled malate recoverable from plants grown in SD is the more rapid growth rate of the apical bud as compared to those grown in LD. Labeled malate reaching an actively growing SD apical bud would likely be metabolized relatively quickly. In addition, any labeled sucrose reaching the same apex would also be metabolized quickly, possibly to other EtOAc-soluble compounds, diluting the total percentage of malate. Thus, it is difficult to conclude that the peak of malate recoverable from plants grown in LD represents a preferential export of malate to the apical bud in LD. It is possible that malate was exported in a complex with another molecule(s), but uncombined malate was the major EtOAc-soluble molecule recovered from apical buds.

Previously, we had ruled out nutrient drain by the seeds as the cause of whole plant senescence, as the growth of seeds in G2 peas in SD, when senescence did not occur, could exceed that in LD when senescence occurred (6). We have therefore inclined to favor the possibility of a fruit-produced senescence factor, on the basis that export of organic compounds from fruits did occur (7). Our current work on the quantitative export from fruits (9), and the identification of the exported material as sucrose and malic acid, now leads us to abandon the concept of a senescence factor. Nonetheless, it is well documented that fruit removal delays or prevents whole plant (monocarpic) senescence (14). If nutrient drain to the seeds is not the absolute cause of plant senescence (6), then we seem to be left without an explanation for the effect of fruits in promoting senescence. The likely explanation is a complex of factors. As plants enter the reproductive phase there is a change in the physiology of the plant so that more photosynthate and other nutrients are diverted to the developing reproductive structures (13, 14). This takes place very early in reproduction before the presence of seeds (13, 14). As a result of this shift in nutrient partitioning, less nutrients are available for continued vegetative growth. As seeds develop, the nutrient demand of the seeds leads to a further reduction in nutrients available for continued growth. This is not the cause of senescence (6), but magnifies the already existing nutrient depletion of vegetative tissue, so that senescence takes place. Removal of the developing fruits frees nutrients for continued

growth, but, at least in peas, this continued growth is accompanied by very rapid growth and development of the new fruits, providing a further indication of the shift in partitioning in favor of the reproductive structures. G2 peas do not senesce in SD because under these conditions they maintain a juvenile type of nutrient partitioning (13), which continues to support primarily vegetative growth of the apical bud and gives a slow rate of growth of the reproductive structures (6, 12).

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LITERATURE CITED

1. Basra AS, CP Malik 1985 Non-photosynthetic fixation of carbon dioxide and possible biological roles in higher plants. Biol Rev 60: 357–401
2. Bennett AB, BL Sweger, RM Spanswick 1984 Sink to source translocation in soybean. Plant Physiol 74: 434–436
3. Benioni A, Y Vaadia, SH Lips 1971 Nitrate uptake by roots as regulated by nitrate reduction products of the shoot. Physiol Plant 24: 288–290
4. Boersig MR, FB Negm 1984 Prevention of sucrose inversion during preparation of HPLC samples. HortScience 20: 1054–1056
5. Gianfagna TJ, PJ Davies 1980 Isolation and identification of *N*-benzoylaspartate and *N*-phenylacetylaspartate from pea seeds. Phytochemistry 19: 959-961
6. Gianfagna TJ, PJ Davies 1981 The relationship between fruit growth and apical senescence in the G2 line of peas. Planta 152: 356–364
7. Gianfagna TJ, PJ Davies 1983 The transport of substances out of developing fruits in relation to the induction of apical senescence in *Pisum sativum* line G2. Physiol Plant 59: 676–684
8. Gifford RM, JH Thorne 1985 Sucrose concentration at the apoplastic interface between seed coat and cotyledons of developing soybean seeds. Plant Physiol 77: 863–868
9. Hamilton DA, PJ Davies 1988a Export of organic materials from developing fruits of pea and its possible relation to apical senescence. Plant Physiol 86: 951–955
10. Hamilton DA, PJ Davies 1988b Mechanism of export of organic material from the developing fruits of pea. Plant Physiol 86: 956–959
11. Hedley CL, DM Harvey, R Keely 1975 Role of PEP-carboxylase during seed development in *Pisum sativum*. Nature 258: 352–353
12. Kelly MO, PJ Davies 1986 Genetic and photoperiodic control of the relative rates of reproductive and vegetative growth in peas. Ann Bot 58: 13–21
13. Kelly MO, PJ Davies 1988 Photoperiod and genetic control of carbon partitioning in peas and its relationship to apical senescence. Plant Physiol 86: 978–982
14. Kelly MO, PJ Davies 1988 The control of whole plant senescence. Crit Rev Plant Sci 7: 139-173
15. Kursanov AL 1963 Metabolism and the transport of organic substances in the phloem. Adv Bot Res 1: 209–274
16. Lichtner FT, RM Spanswick 1981 Electrogenic sucrose transport in developing soybean cotyledons. Plant Physiol 67: 869–871
17. Pate JS, MB Peoples, AJE van Bel, J Kuo, CA Atkins 1985 Diurnal water balance of the cowpea fruit. Plant Physiol 77: 148–156
18. Proebsting WM, PJ Davies, GA Marx 1976 Photoperiodic control of apical senescence in a genetic line of peas. Plant Physiol 58: 800–802
19. Tamas IA, CJ Engels, SL Kaplan, JL Ozbun, DH Wallace 1981 Role of indoleacetic acid and abscisic acid in the correlative control by fruits of axillary bud development and leaf senescence. Plant Physiol 68: 476–481
20. Ziegler H 1975 The nature of transported substances, *In* MH Zimmerman, JA Milburn, eds, Transport in Plants I, Phloem Transport. Encyclopedia of Plant Physiology, New Series VI. Springer-Verlag, Berlin, pp 59–136