DISTRIBUTION OF ASSIMILATED CARBON AND 14C-Glyphosate IN QUACKGRASS (Agropyron repens) RHIZOMES

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ABSTRACT

At any given time, the tissues of a rhizome of quackgrass (*Agropyron repens*) may be dormant and importing little, actively importing but not expanding in size, or actively importing and expanding. To characterize sink activity of rhizome tissue, sink-related processes were examined by measuring several physiological parameters in apical segments, axillary buds, and internodal tissues. Carbon partitioning was studied by steady-state labeling of whole plants with $^{14}$CO$_2$. The distribution of imported $^{14}$C labeled photoassimilate which accumulated in various sinks and local sink activity were visualized by making autoradiographs of rhizomes. Sink activity was quantified by computing a growth coefficient which compared the amount of recently fixed carbon imported relative to the total amount of standing carbon in a given tissue. Respiratory activity was quantified by measuring O$_2$ uptake rate and expressing it per unit of carbon in rhizome tissues. Autoradiographic images and growth coefficients revealed differences in sink activity, both among individual rhizomes on the same plant as well as among the various tissues within a single rhizome. Sucrose levels were highest in those rhizome tips that were importing the greatest amount of recently fixed carbon. Carbon balance in rhizome tissues was studied by measuring carbon input by import and output by respiration. Growth coefficients were generally of the same order of magnitude as respiratory coefficients.
but in most tissues there was a slight to moderate positive carbon balance which resulted in some net accumulation of carbon. Tissues, such as apical segments, which had the highest rates of respiration also had the highest rates of carbon import and net accumulation and likely were expanding. Dual labeling of quackgrass plants by applying a solution containing $^{14}$C-glyphosate and $^3$H-sucrose to leaves was used to measure the accumulation of glyphosate relative to $^3$H-sucrose. This ratio was used to ascertain the ability of glyphosate to be translocated to various rhizome tissues relative to sucrose a major product of photosynthesis. Translocation and distribution of $^{14}$C-glyphosate generally was similar to that of $^3$H-sucrose in quackgrass rhizomes. However, in comparison to sucrose, glyphosate was preferentially accumulated in the rhizome apices, tissues which had the highest assimilate accumulation and metabolic activity. A means of classification is presented which categorizes the various rhizome tissues as maintenance sinks, storage sinks, or growth sinks based on their ability to import assimilate and their level of respiration. The classification, based on differences in carbon and glyphosate accumulation and respiration, may help predict the effectiveness of glyphosate for controlling quackgrass proliferation.
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Perhaps the greatest lesson I have learned as a master's student at UD is to pay attention to the process of learning, problem solving, and research rather than solely focusing on an endpoint or final goal. By learning from the process along the way one will not only achieve a desired goal but also learn the skills necessary to continue to learn and discover new things. Furthermore, the task of learning something new or seeking the answers to some unsolved problem will be much more rewarding and enjoyable if the person is able to explore the possibilities and opportunities that present themselves along the way, those that may be overlooked if one proceeds with blind determination to only "see the light at the end of the tunnel".

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INTRODUCTION

Quackgrass, *Agropyron repens*, is an aggressive perennial weed that is a nuisance to those engaged in agriculture. It is considered by many to be the most serious perennial weed of the northern temperate regions of the United States, Canada, and Europe, strongly competing with crop plants for available nutrients, light and water. Quackgrass possesses two inherent characteristics which contribute to its status as a serious noxious weed and which have stimulated much research. First, the species is cross pollinated and so there is a great variability among plants grown from seed. As a result of this striking variability there is no "standard" quackgrass or reproducible habit of growth (Holm et al., 1977) and, consequently, a variable response to control measures. Second, and more important, quackgrass possesses a vigorous rhizome system which allows the weed to propagate vegetatively. Quackgrass can readily spread through a field as it sends out rhizomes or underground lateral shoots that are capable of sprouting vertical shoots (tillers). Tilling enhances the process of quackgrass proliferation as fragments of the rhizomes are spread and axillary buds found along the length of the rhizome are released from dormancy and develop new vertical shoots.

Effectiveness of phloem-mobile herbicides such as glyphosate [N-
(phosphonomethyl)glycine] is based on their being imported by actively growing regions of the plant along with assimilated carbon. Previous reports show that, like assimilate, glyphosate is not distributed evenly within quackgrass rhizomes but accumulates preferentially in apical portions (Devine, 1989; Chandrasena and Sagar, 1986; Claus and Beherens, 1976; Harker and Dekker, 1988a). While potentially lethal amounts of glyphosate may reach the most actively growing regions of rhizomes, less active axillary buds may not accumulate sufficient glyphosate to reach phytotoxic levels. In fact, the axillary buds which survive the glyphosate treatment may then begin to actively grow as they have been released from apical dominance by the killing of the apical portion of the rhizome.

In order to address the problem of failure to kill less active buds in quackgrass rhizomes, it is important to understand better the physiological processes which occur in the apical segments, axillary buds, and internodal sections which govern the ability of rhizomes to import and accumulate materials exported from source leaves. There is a wealth of descriptive material concerning the influence of various environmental conditions on quackgrass rhizome bud growth and development and their resulting impact on the effectiveness of glyphosate. On the other hand, there is a paucity of information concerning the physiology and biochemistry of quackgrass rhizomes and their relation to glyphosate distribution.
LITERATURE REVIEW

Carbon Partitioning Among Sinks Regulation of carbon partitioning is an important process for maintaining balance between the source leaf processes of photosynthesis and export and sink processes of carbon import, incorporation, and use (Geiger and Fondy, 1991). A key component of this regulation is the ability of an individual sink to attract sufficient photoassimilate for it to carry on the needed level of maintenance, storage, and growth. The actual ability of a sink to import of assimilate is termed sink strength and is expressed as the amount of carbon imported by the part per unit time (Warren-Wilson, 1972, Ho 1988). The strength of a particular sink is a function of both its size and its activity. Sink activity is the ability of a unit amount of sink tissue to mobilize import of carbon and is expressed as the uptake of assimilates per time and unit weight of sink tissue (Warren-Wilson 1972, Ho 1988).

Sink activity is the result of complex interactions of a number of processes including phloem unloading into the apoplast or symplast, sugar conversions in either the symplast or apoplast and, finally, metabolic processes related to growth, storage, or maintenance (Ho, 1988). This parameter is useful for describing the intensity of growth or storage activity for a given sink and for assessing the relative ability of
these tissues to affect partitioning of recently fixed photoassimilate. Regulation of the diversion of fixed carbon into the metabolic pathways to support these sink activities involves the process of carbon allocation (Geiger, 1986). This latter regulation occurs at the cellular level by regulation of the activity and quantity of certain enzymes which function to control the flux of carbon through the various metabolic pathways (Ho, 1988). These cellular processes then determine the relative sink strength of the various sinks and ultimately influence carbon partitioning, the differential distribution of photoassimilates among sinks in the plant. The interplay among the processes regulating allocation and carbon metabolism establish a balance of carbon partitioning among sink organs (Geiger and Fondy, 1991). A key, unanswered question is what is the relative importance of regulation of enzymes for conversion of imported assimilate to forms usable in metabolism, enzymes for energy metabolism and enzymes involved in synthesis.

**Tracing Translocation and Partitioning** Labeling of assimilate with $^{14}$CO$_2$ has been used widely to study partitioning of recently fixed carbon. The $^{14}$CO$_2$ incorporated into exportable assimilates in photosynthesizing leaves serve as a tracer to study the partitioning of carbon to various plant parts and their subsequent redistribution (Hume and Criswell, 1973; Wien et al., 1976; Bidinger et al., 1977; Waters et al., 1980). To be a valid tracer of the fate of carbon fixed during a given period, labeling methods should trace all the carbon fixed during that period, what is immediately exported and what is temporarily stored (Geiger and Shieh, 1988). The latter usually is derived from transitory starch which may be reallocated to
sucrose at night to continue the supply of photoassimilate to sink regions of the plant while no photosynthesis is occurring. To allow calculation of the amount of tracer carbon entering or leaving a pool, the ratio of radioactive carbon to total carbon must be known (Geiger & Swanson, 1965; Geiger and Shieh, 1988). This criterion can be met by the use of steady-state tracer methods which essentially achieve isotopic equilibrium throughout the labeling period in the major carbon pools that supply material for export, accumulation, and growth (Geiger and Swanson, 1965; Webb, 1975; Geiger and Fondy, 1979; Kouchi and Yoneyama, 1984).

Whole-plant, steady-state labelling methods are used to measure the total carbon influx contributed from different sources (Shishido et al., 1987; Geiger and Shieh, 1988). Webb (1975) studied the dynamics of CO₂ uptake, translocation and carbon accumulation in several vegetative structures of Douglas fir seedlings by steady-state labeling whole seedlings for 4 days with ¹⁴CO₂. Using this method, the author determined that differences in accumulation rates among the various sinks can be ascribed to the individual carbon requirement for maintenance and synthesis of new tissue and differences in translocation times for a given tissue. New shoots of Douglas fir seedlings have a much higher accumulation rate than stems due to a higher metabolic activity per unit weight.

Recently, Geiger and Shieh (1988) and Geiger et al. (1989), used whole-plant steady-state ¹⁴CO₂ labeling to study carbon partitioning and quantify growth in the various organs of Phaseolus vulgaris. Their results supported the usefulness of whole-plant steady-state labeling and of comparing growth coefficients to study biochemical
and physiological mechanisms that regulate carbon partitioning. By deriving growth rate coefficients for the various structures under observation, one can compare the present partitioning of tracer carbon among organs with the carbon previously distributed to those organs to predict the future form of the plant as well as predict the rate of growth for a particular organ assayed (Geiger and Shieh, 1988). This method also allows for a quantitative comparison among various sinks within the same plant to describe competition among sinks for photoassimilate and display patterns of partitioning of carbon in the whole plant.

**Herbicide Translocation** Tyree, *et al.* (1979) developed a model, the intermediate permeability hypothesis, which successfully explained phloem mobility of some systemically-mobile xenobiotics that are not weak acids. The model was extended to include the factor of degree of acidity by Kleier, *et al.* (1988). The theory predicts that a substance, whose molecules are sufficiently lipophilic, will diffuse through membranes passively into the symplasm of the phloem and will be carried along with the assimilates. There is an optimum permeability, based on the degree of lipophilicity, which permits translocation of the substance through the symplasm as well as through the apoplast. The degree of permeability needs to be large enough to permit substantial passive permeation into sieve cells in the source leaf and yet is small enough to permit significant retention for phloem transport. As symplastic transport occurs, a certain amount of the chemical continuously leaks out of the symplasm into the apoplast and is swept along with the water in the xylem. Such substances are said to be ambimobile. The degree of permeability needed for
optimum movement is a function of the velocity of sap flow in sieve tubes, the radius of the sieve tube, the over-all length of the plant, and the length of the carbohydrate and concentration and location of the xenobiotic source (Tyree, et al., 1979).

Generally it is desirable that a xenobiotic chemical be distributed widely throughout a plant after a foliar spray. Ambimobile substances will travel down to the roots in the phloem and, depending on their properties, some may circulate back again in the xylem and, to some degree, the leaves and roots formed after the spray treatment. Redistribution is especially desirable for perennial weeds which often can propagate vegetatively by a subterranean shoot or root system after the initial treatment. It is not known to what extent redistribution of the initially applied herbicide will prevent regrowth from vegetative organs.

The translocation dynamics of such an ambimobile xenobiotic chemical are as follows: The xenobiotic enters the symplast because of its high concentration on the leaf surface and its ability to both remain in solution and penetrate the lipid membranes. The compound will move with increasing velocity in the leaf blade because water continues to enter the sieve elements of the minor veins as a result of active loading of sucrose. Velocity will be high in the larger veins of the leaf and in the sieve elements of the petiole and stem. In the root, the sap velocity decreases with distance along the sieve elements as solutes and water exit the sieve elements (Tyree, et al., 1974; Kleier, et al., 1988). This pattern of sap flow is in accordance with a Münch pressure-flow translocation model for a plant with short path length (Tyree et al., 1974). Since it is presumed that the shoot tip, or the rhizome tip in
this case, is a uniform sink for carbohydrate, water will flow out of the sieve tube across the plasma membrane as sugars are unloaded or metabolized. This water loss contributes to the decline in sap velocity and also tends to concentrate the xenobiotic faster than it is lost by passive permeation. The net effect is an accumulation in the sieve elements endings of roots and shoots, including rhizomes (Tyree, et al., 1979; Kleier, et al., 1988).

**Glyphosate a Phloem-Mobile Herbicide** Glyphosate, a nonselective post-emergence herbicide, has been shown to be phloem mobile in many species including quackgrass (Sprankle et al., 1975b; Harker and Dekker, 1988; Klevorn and Wyse, 1984a,b; McIntyre and Hsiao, 1982; and Coupland and Caseley, 1979). Using sugar beet plants, Gougler and Geiger (1981), provided evidence for a passive, nonfacilitated uptake of glyphosate into cells and its subsequent phloem transport. On the basis of calculated permeability of plant cell membranes to glyphosate, they suggested that translocation was well described by the intermediate permeability mechanism described earlier (Tyree, et al., 1979). The permeability constant calculated for glyphosate is such that slow entry and exit from the phloem will occur and allow accumulation and transport of glyphosate in the phloem (Gougler and Geiger, 1981).

Glyphosate has several ionizable groups and undergoes a transition from an overall charge of -1 to -2 at pH 5.6 to 5.8 (Sprankle, et al., 1975a; Wauchope, 1976). At physiological pH the herbicide exists as a zwitterion, and the extent of its charge at physiological pH would be expected to influence its ability to cross membranes to
enter or efflux from the symplasm. Consequently, it exists in a more polar form inside the symplasm and would be expected to efflux from the cell less readily than it enters. Gougler and Geiger (1981), however, found that while glyphosate displayed intermediary permeability, its uptake appeared to be relatively independent of pH.

Glyphosate was readily transported in sugar beet plants and its distribution was similar to that of $^3$H-sucrose indicating that the phloem is the primary means of distribution of glyphosate (Gougler and Geiger, 1981). A close correlation has also been found between assimilate movement and translocation of glyphosate in Canada thistle (McAllister and Haderlie, 1985). The movement of phloem-mobile herbicides depends on the processes that maintain phloem translocation. *Therefore, any changes that the herbicide causes in phloem translocation patterns will also affect its own transport* (Geiger and Bestman, 1990).

Glyphosate disrupts processes both in the source and sink regions that contribute the maintenance of phloem translocation. Gougler and Geiger (1984) showed that glyphosate exerts an independent effect on sink metabolism by slowing one or more sink processes that maintain translocation and so reduces assimilate import by a sink leaf. The observed effect is consistent with decreased use of imported sucrose for protein synthesis as a result of inhibited synthesis of aromatic amino acids, possibly by interference with synthesis of enzymes needed in sink leaf metabolism that maintains import (Geiger and Bestman, 1990). Another possibility is that glyphosate inhibits metabolism of the citric acid cycle in sink tissues and so interferes with energy metabolism (Killmer, *et al.*, 1981.)
With time, herbicide-induced slowing of translocation, due to disruption of the processes that maintain translocation, may result in leakage of herbicide from the phloem, and prevent accumulation of herbicide to phytotoxic levels in sink organs (Geiger and Bestman, 1990).

Glyphosate accumulates to a greater extent in the apical portions of quackgrass rhizomes than in other portions of the rhizome (Chandrasena and Sagar, 1986; Claus and Beherens, 1976; Devine, et al., 1983; and Harker and Dekker, 1988). Accumulation of a greater amount of glyphosate in the apices may account for the death of buds at the apex while those in more basal regions survive, a pattern reported by Claus and Beherens (1976; and Stoltenberg and Wyse (1986).

Differences between distribution of assimilates and glyphosate have been reported. In Canada thistle, glyphosate and photoassimilate distribution patterns were similar but relatively more glyphosate than assimilate was translocated into the shoot apex, and relatively less into branch roots (McAllister and Haderlie, 1985). A possible explanation for this pattern is that ambimobility resulted in movement in the xylem and subsequent reentry into the ascending phloem stream in the stem. Similar results were obtained in ivy leaf morning glory; more glyphosate was recovered in the shoot apex, and more assimilate in the stem (Dewey and Appleby, 1983). Glyphosate and assimilate distribution patterns were similar, although not identical in sugar beet (Gougler and Geiger, 1981). Different patterns may also be the result of self-limitation of herbicide transport by herbicide-induced effects on the translocation process (Devine, 1989; Geiger and Bestman, 1990).
Effectiveness of Glyphosate for Controlling Quackgrass  The persistence of quackgrass even after treatment with glyphosate has stimulated research into physiological mechanisms which may allow it to evade control even by systemic herbicides. Multiple rhizome buds contribute largely to the persistence of quackgrass (Werner and Rioux, 1977). They can escape herbicide treatment due to insufficient accumulation of herbicide, resulting in a sublethal concentration in the rhizome.

Studies have shown that the differential distribution patterns of photoassimilate and glyphosate in quackgrass rhizomes can result from several factors including soil and air temperature (Harker and Dekker, 1988), soil moisture (Klevorn and Wyse, 1984) and nutrient levels (McIntyre and Hsiao, 1982) as well as bud position on the rhizome as discussed for photoassimilate above (Harker and Dekker, 1988; McIntyre and Hsiao, 1982).

The apex is the most active sink of the rhizome, while the greatest sink strength among the axillary buds is usually in the distal buds of primary rhizomes (Harker and Dekker, 1988; McIntyre and Hsiao, 1982). Rhizome buds had no observed effect on import of glyphosate into the apex whereas the rhizome apex exerted some control over the amount of glyphosate imported into the axillary buds (Klevorn and Wyse, 1984b).

Tardif and Leroux (1990), used both assay with tetrazolium and growth of isolated buds to assess viability and metabolic state of quackgrass rhizome buds. These workers determined that for untreated buds, metabolic activity and growth rate measured in isolated buds decreased with increasing distance from the apex of the
rhizome. On the other hand, following glyphosate treatment, metabolic activity and ability to grow was approximately equal regardless of bud position (Tardif and Leroux, 1990).

Measurements taken a week after treatment revealed that sucrose and glyphosate did not have the same distribution in quackgrass rhizomes (Harker and Dekker, 1988). Compared with photoassimilate, glyphosate preferentially accumulates in areas of greatest metabolic activity in the rhizomes (Harker and Dekker, 1988; Klevorn and Wyse, 1984a). Experiments with water stressed plants indicate that photoassimilate and glyphosate do not accumulate in the same source to sink pattern under these conditions (Klevorn and Wyse, 1984a).

**Research Objectives** I have undertaken this study to understand of some physiological bases which affect control of quackgrass by the herbicide glyphosate. Topics of importance include photoassimilate partitioning, respiration in relation to sink activity and carbon balance among import, respiration and accumulation. An important object of the present study is to examine the effect of morphological features such as bud position on the distribution of glyphosate in quackgrass rhizomes. Accumulation of glyphosate relative to sucrose was compared by radiotracers in each of the various rhizome structures was to see if there were trends related to states of sink activity in the rhizome. A means of classifying the various rhizome structures as maintenance sinks, storage sinks, and growth sinks was devised. The system may help predict the effectiveness of glyphosate for controlling quackgrass proliferation based on differences in carbon and glyphosate accumulation and respiration.
MATERIALS AND METHODS

**Plant Material** Quackgrass plants, *Agropyron repens* (L.) Beauv., used for the experiments were from a clone derived from a single quackgrass rhizome obtained from the Monsanto Agricultural Products Co., St. Louis, Mo. For each experiment, pieces of rhizomes consisting of a single node were taken from a mature plant and transplanted into pots filled with a 1:1 mixture of sand and milled peat moss. Quackgrass starts were kept in a growth room for 3 weeks under a 14-h photoperiod with a light intensity of 800 \( \mu \)moles m\(^{-2}\) s\(^{-1}\) and a regime of 25°C day and 20°C night temperature. To induce rhizome formation, the plants were moved to a growth cabinet with a 10-h photoperiod under a regime of 15°C day and 9°C night temperature. Light intensity in the growth cabinet was 500 \( \mu \)moles m\(^{-2}\) s\(^{-1}\). Plants were watered three times daily each with 20 ml of 1/2 strength Snyder’s growth solution (Snyder, 1978) containing 100 ppm nitrogen. All experiments were performed with 3-month-old plants.

**Measuring Carbon Accumulation by Steady-State Labeling** Shoots of quackgrass plants were supplied with tracer \( ^{14} \text{CO}_2 \), sampled and assayed for radioactivity and carbon content as described by Geiger and Shieh (1988). Details for steady state labeling procedure are given in Appendix A. Whole plants were
placed in a 100-L plexiglass chamber and an atmosphere of air containing 350 ppm CO₂ with a specific radioactivity of approximately 1 nCi µg⁻¹ carbon during a 10-h photoperiod. The photon flux density was 800 µmol m⁻² s⁻¹ under a regime of 25°C day and 20°C night temperature. Plants were harvested 24 h after initiation of labeling.

For plant growth analysis, pieces of rhizome were freeze-dried, weighed, and reduced to a fine powder. Weighed aliquots of the powder were oxidized to measure the ¹⁴C and the carbon content of the structure (Bucholtz and Hess, 1983; Geiger and Shieh, 1988). See Appendix B for details of oxidation procedure. Alternatively, small pieces such as individual axillary buds and apices and internode pieces of less than 5 mg dry weight were oxidized directly, without first powdering. The radioactivity content of ¹⁴C labeled assimilate per unit tissue carbon was measured for the various sections of the rhizome and % growth coefficients were calculated to provide a quantitative representation of import rates both within and among the rhizomes. See Appendix C for details of growth coefficient calculations.

**Visualization of Accumulation of ¹⁴C-Labeled Photoassimilate** Prior to their being powdered and oxidized, rhizomes were flattened using a hydraulic press with operated at 36,000 psi to increase resolution. Kodak Industrex type M x-ray film was exposed to radioactivity in rhizomes pieces for a total of 6 to 8 million counts and processed as recommended by the manufacturer. Details of autoradiographic procedure are given in Appendix D.

**Respiration Rates of Rhizome Structures** Oxygen uptake was measured with
a Clark oxygen electrode and YSI model 53 oxygen analyzer (Yellow Springs, OH). Apical segments, axillary buds, and internodes from rhizomes were excised immediately prior to measurement. For measurements, one to three similar pieces were placed in 3.5 ml of a solution containing 200 mM sucrose, 10 mM KCl, 5 mM MgCl₂, 7 mM KH₂PO₄, 3 mM K₂HPO₄, and 10 mM tris-HCL, pH 7.2. Presence of sucrose was found not to affect the rate of respiration. The effect of respiration of microorganisms was not assessed but was assumed to be similar for the various rhizome pieces. Tabulated values for oxygen solubility in water at the assay temperature were used for calculating oxygen consumption rates. Respiration coefficients were determined along with growth coefficients to calculate net % growth coefficients from the same rhizome structure in order to estimate the actual accumulation of recently fixed photoassimilate. See Appendix E for calculation of respiration coefficients.

In these studies, the source of respired carbon, whether from recently fixed or reserve carbon, was not determined. Based on the assumption that in some tissues respiration may be derived not from tracer carbon but from stored carbon, net accumulation was estimated by calculating carbon balance. This parameter is computed by subtracting the respiration coefficient from the growth coefficient. Estimates of net carbon accumulation, obtained either by calculating the growth coefficient or from carbon balance obtained by subtracting the respiratory coefficient from the growth coefficient, are based on the rationale detailed in Appendix E.

**Carbohydrate Analysis** Individual rhizomes of a quackgrass plant were divided
on a gross level into tips, middle segments, and the basal regions of rhizomes. Each segment of the rhizome sample consisted of 3 to 4 expanded internodes.

Carbon in small samples of carbohydrates was measured by a modification of the assay described by Prioul, et al. (1990). Samples of approximately 10 mg of freeze-dried, powdered tissue were added to 300 μl of a mixture of methanol:chloroform:water (12:5:3) and homogenized with a teflon pestle for 1 min. After standing for 1-h, the homogenate was separated by centrifuge at 12,000g for 5 min. The supernatant was collected and the pellet re-extracted with the same mixture. The supernatants were combined and added to 300 μl of a solution of methanol:chloroform (1:1). A two-phase separation was produced after separation by centrifuge at 12,000g for 1 min. The water phase containing the soluble sugars was adjusted to 1 ml. The amount of glucose, fructose, and sucrose was determined spectrophotometrically by a coupled enzymatic assay (Jones, et. al. 1977). NADPH production was determined by measuring absorption at 340 nm with a 96-well microplate reader, model UVmax kinetic microplate reader (Molecular Devices Corporation, Menlo Pk, CA). First, glucose was determined by NADP⁺ reduction resulting from oxidation of glucose by the action of hexokinase and glucose-6-P dehydrogenase. Next, fructose was converted to glucose by the action of phosphoglucoisomerase and the additional glucose was measured. Finally sucrose was converted to glucose and fructose by adding invertase and the additional glucose was measured.

**Distribution of glyphosate relative to sucrose** To compare distribution of
assimilates and glyphosate, a solution containing $^3$H-sucrose and $^{14}$C-glyphosate was supplied to leaves of a quackgrass plant. A total of 275 $\mu$l of a solution containing 27.5 $\mu$Ci of $^3$H-sucrose (Amersham, Arlington Heights, IL) and 1.4 $\mu$Ci of [${^{14}}$C-methyl]glyphosate (Monsanto Agricultural Products) was applied to six abraded leaves of quackgrass over a total area of about 24 cm$^2$. The concentrations of components of the solution were 10 mM sucrose, 17 mM glyphosate, 5 mM KH$_2$PO$_4$ buffer at pH 6.5, 0.01% Tween 20 and 1.1% monoisoproplamine. Leaves were kept under light at a photon flux density of 800 $\mu$mol m$^{-2}$ s$^{-1}$ throughout a 10-h photoperiod. Strips of mylar were placed over the solution on each leaf. The areas were kept wet by the occasional addition of water during the experiment. After 24-h, rhizomes were harvested, lyophilized and prepared for analysis as described above. In-vial oxidation and dual-isotope scintillation spectrophotometry were used to measure the $^{14}$C and $^3$H in the rhizome structures. Concentrations of $^{14}$C-glyphosate and $^3$H-sucrose are expressed relative to concentrations in an actively growing apical segment of a rhizome importing material from labeled source leaves.

**Visualization of $^{14}$C-glyphosate accumulation** A total of 26.3 $\mu$Ci of [${^{14}}$C-methyl]glyphosate in 350 $\mu$l of solution was applied over 7 abraded leaves in the same manner as described above. After 24-h, the rhizomes were harvested, freeze-dried and pressed as before and autoradiographs were prepared.
RESULTS

Partitioning of photoassimilate in various rhizome structures of quackgrass.

Autoradiographic images of quackgrass rhizomes provided a qualitative indication of the levels of sink activity among rhizome apices, buds, and internodes. Generally, the apical segment along with the first one or two internodes back from the rhizome tip accumulated the greatest amount of assimilate (Fig. 1). Nearly all of the buds along the length of the rhizome accumulated substantial portions of the \(^{14}\text{C}\)-labeled photoassimilate. Accumulation of labeled assimilate in internodes decreased more rapidly with distance from the rhizome apex than did the label in the buds (Fig. 1). In cases where an axillary bud began to grow actively, the active bud accumulated a considerable amount of label while the apex accumulated less (Fig. 2).

The values for growth coefficients showed that the tips of rhizomes generally accumulated carbon at least twice as fast as the rest of the rhizome (Table I). Some rhizome tips imported carbon up to five times faster than others. Internodes 1 to 5 had relatively high growth coefficients, with a sharp decrease in their value at each position further away from the apex of the rhizome (Fig. 3). Internode segments 6 to 14 all showed comparatively low growth coefficients and little variation in import
Fig. 1  Partitioning of labeled photoassimilate in an actively growing quackgrass rhizome. Positive print of an autoradiograph prepared from a freeze-dried and pressed rhizome. Rhizomes were harvested after a 10-h night period following a 14-h period of steady state labeling of the entire shoot. This image is typical of the pattern observed in 10 autoradiograms of rhizomes of this type prepared from four plants.
Fig. 1
Fig. 2 Partitioning of photoassimilate in a quackgrass rhizome with an axillary bud that had recently broken dormancy. Preparation as in Figure 1. This image is representative of two autoradiographs showing rhizomes in which an axillary bud has been released from dormancy and is actively expanding. The accumulation of $^{14}$C-photoassimilate in portions of the rhizome distal to the active bud is too low to be visible.
Fig. 2
Table I. Comparison of total imported carbon and soluble carbohydrates in two rhizomes from a quackgrass plant. Data are for rhizomes with the highest and lowest growth coefficients among the five rhizomes present.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rhizome 1</th>
<th>Rhizome 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>base</td>
<td>tip</td>
</tr>
<tr>
<td>glucose (nmol mg(^{-1}) dry wt)</td>
<td>18.9</td>
<td>18.2</td>
</tr>
<tr>
<td>fructose (nmol mg(^{-1}) dry wt)</td>
<td>66.4</td>
<td>37.4</td>
</tr>
<tr>
<td>sucrose (nmol mg(^{-1}) dry wt)</td>
<td>48.7</td>
<td>129.2</td>
</tr>
<tr>
<td>growth coefficient (% d(^{-1}))</td>
<td>0.25</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Fig. 3  Net accumulation of recently fixed carbon per unit carbon in internodal
segments along the length of a whole rhizome.  A. Diagram of a rhizome
showing the various types of structures. Each internode segment is
represented by a number, increasing sequentially in the basipetal direction.
The corresponding numbered bud is at the base of the internode segment.  B.
Amount of recently fixed carbon per unit carbon in samples of internodes
determined by analyzing tracer present following oxidation of the tissue.
activity among them. The growth coefficients of the buds decreased at each node in a similar fashion to that just described for the internodes. Values for coefficients from the buds sometimes were two to three times higher than the values for the corresponding internodes (Fig. 4).

Apical segments, axillary buds, and internodes that were still actively growing, as evidenced by autoradiographs and values for growth coefficients, showed a high positive correlation, \( r^2 = 0.94 \), between dry weight and accumulation of photosynthate (Fig. 5). Structures that showed little activity displayed no correlation between dry weight and growth (data not shown).

**Growth and Respiration Coefficients and Carbon Balance** In general, the values for growth and respiratory coefficients for each individual rhizome piece were of the same order of magnitude (Fig. 6 and Table I). In portions of rhizomes where growth coefficients are high, these values were approximately twice as large as the corresponding the respiratory coefficients (Fig. 6, apical segment and axillary buds 4 to 6,11,12). Where growth coefficients were low, they were approximately the same value as the corresponding respiratory coefficients (Fig. 6, for example buds 9 and 10 and internodes 7 to 9.).

**Glyphosate partitioning relative to sucrose** Glyphosate was readily transported in quackgrass plants (Figs. 7, 8). The distribution of \(^{14}\)C-glyphosate resembled that for \(^{3}\)H-sucrose, which was used to represent the materials normally transported in the phloem. Differences in sink strength among rhizomes from a single plant were observed (Fig. 7). The distribution of sucrose in rhizomes of
Fig. 4  Net accumulation of recently fixed carbon per unit carbon in the various tissues along the length of a quackgrass rhizome. Coefficients are shown for the apical segment ( ), axillary buds ( ), and internodes ( ) for tip and the basal regions of the rhizome.
Fig. 4

![Graph showing growth coefficient (% d⁻¹) vs. node position on rhizome.]

- Y-axis: Growth coefficient (% d⁻¹)
- X-axis: Node position on rhizome
- Nodes 0 and 2 show higher growth coefficients compared to others.
Fig. 5 Relation of rate of growth per unit dry weight to organ dry weight in young, actively expanding buds at tips of rhizomes. Data are from apical segments and axillary buds within the first five nodes of the tip of rhizomes. \( n = 13 \).

Correlation coefficient, \( r^2 = 0.94 \).
Fig. 5

![Graph showing growth coefficient (%) vs. bud dry weight (mg).]
Fig. 6  Growth, respiration, and resulting carbon balance of rhizome tissues. Both growth (_growth_ ) and respiration ( _respiration_ ) coefficients were determined for each piece of tissue. Carbon balance ( _carbon balance_ ) was calculated from these values. Similar data were obtained from two other plants. See Appendix E for details.
Fig. 6

![Bar chart showing growth coefficient (% d⁻¹) for different node positions on a rhizome. The x-axis represents node positions: apex, b1-3, in1-3, b4-6, in4-6, b7-8, in7-9. The y-axis represents growth coefficient (% d⁻¹) ranging from 0 to 50.]
Fig. 7  Relative sink activity of rhizome organs and relative distribution of glyphosate compared with imported sucrose by rhizomes of a quackgrass plant. The numbers above the rhizome show relative sink activity, the ability of each apex and bud to import assimilate. This parameter is expressed as the relative level of \(^{3}\text{H}\)-sucrose imported by each rhizome structure per unit dry wt. The ability of these structures to import \(^{14}\text{C}\)-glyphosate, expressed relative to their ability to import exogenously applied \(^{3}\text{H}\)-sucrose, is shown by the values below each rhizome. Data are for a single quackgrass plant. Values for accumulation of \(^{3}\text{H}\)-sucrose and of \(^{14}\text{C}\)-glyphosate are expressed as quotient of the actual \(^{14}\text{C}\) or \(^{3}\text{H}\) present in a rhizome piece divided by the amount present in the structure that accumulated the most \(^{3}\text{H}\)-sucrose and \(^{14}\text{C}\)-glyphosate. In this case, this was the apical segment of rhizome 3. See Appendix F for details.
Fig. 7
Fig. 8 Comparison of distribution of glyphosate and sucrose imported into rhizome organs. Data for distribution of $^{14}$C-glyphosate and $^3$H-sucrose, which were applied to leaves, were obtained from a single plant and expressed in the manner described in Figure 7. Three rhizomes, differing in length, developmental stage, and the number of propagules per rhizome were analyzed. The figure shows representative data from one rhizome. A. The relative amount of $^3$H-sucrose accumulated by the apical segment, axillary buds, and internodes through the first five nodes. B. Ratios of $^{14}$C-glyphosate to $^3$H-sucrose accumulated in the same rhizome pieces as in A.
Fig. 8

A

Glycine accumulation

Node position on rhizome

0.8

0.6

0.4

0.2

1


apex in1 b1 in2 b2 b3 in4 b4 in5 b5

B

Glyphosate/sucrose ratio

Node position on rhizome

0.8

0.6

0.4

0.2

1


apex in1 b1 in2 b2 b3 in4 b4 in5 b5

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quackgrass was similar to that of assimilate derived from recently fixed \(^{14}\)CO\(_2\) (Figs. 1 to 4). The apical segment of a rhizome showed the greatest activity followed closely by the first internodal section and the first axillary bud. The \(^{14}\)C/\(^3\)H ratios, which indicate the relative degree of glyphosate accumulation by these portions of the rhizome were highest in these structures. This ratio was 20 to 40% lower in the rest of the rhizome. In general, more \(^{14}\)C-glyphosate accumulated per unit of \(^3\)H-sucrose in areas of the highest import and highest metabolic activity, such as the apices and was smallest where import was the least (Fig. 9).
Fig. 9  Regression analysis of the ratio of $^{14}$C-glyphosate to $^{3}$H-sucrose accumulation as a function of $^{3}$H-sucrose accumulated in actively growing portions of the rhizome. Only values for the apex and for the next five buds and internodes were used. Data are from one rhizome to avoid data with different slopes and ordinate intercepts but the regression is representative of the relationship seen in three rhizomes. The correlation coefficient = 0.84.
Fig. 9

Relative accumulation of $^{14}$C-glyphosate and $^{3}$H-sucrose versus sucrose accumulation.
DISCUSSION

Factors affecting carbon import and partitioning. Growth coefficient values describe the intensity of growth in dry wt for individual sinks and give a measure of their sink activity, the ability to influence partitioning of recently fixed photoassimilate (Geiger and Shieh, 1988). The observed patterns of growth coefficients (Figs. 3, 4), along with autoradiographs (Figs 1, 2) pointed out several factors, particularly developmental age, type of organ and point of origin of rhizomes, as important in affecting carbon import and partitioning.

Tips of rhizomes accumulated carbon up to five times as fast as the rhizome base (Table I). The apical segment, a composite of several developing structures which are expanding rapidly, was usually the structure with the highest rate of accumulation in the whole rhizome. The generally observed gradient in internode growth coefficients from high values in the tip to low values at the base of the rhizome (Fig. 3) illustrate the differences in rates of growth (Geiger and Shieh, 1988). The youngest portion of rhizomes, generally comprising the first five visible internodes, are expanding rapidly and accumulate carbon at a high rate (Fig 3). In this region of the rhizome, axillary buds are growing rapidly before reaching a critical stage when they cease expanding in size yet continue to actively import assimilate
until some environmental or developmental signal causes growth to resume. In this actively importing region, there was a close correlation between dry weight and growth coefficient (Fig. 5), as would be expected for young regions exhibiting near exponential growth. In some instances, where a rhizome bud away from the apex had begun to grow markedly, the apex was less intensely labeled than the active bud which appeared to be competing more effectively for the recently fixed photoassimilate (Fig. 2).

By contrast with developmentally active organs, internode segments 6 to 14 all showed comparatively low growth coefficients and little variation in import activity among them, suggesting that likely they were not growing. In these regions, there was virtually no correlation between growth and dry wt (data not shown). Growth coefficients also differ noticeably among the various organs (Fig. 4).

Metabolic activity as a determinant of carbon partitioning in and among rhizomes. The level of metabolic activity, as indicated by respiratory coefficient values, is an important predictor of carbon accumulation and partitioning among and within rhizomes (Fig. 6). Portions of the rhizome which were respiring at the highest rates generally accumulated the greatest amount of carbon. The general correspondence between the size of growth coefficients and respiration coefficients provides evidence that metabolism is an important factor in the regulation of import and accumulation of assimilate (Ho, 1988; Geiger and Fondy, 1991). Metabolism likely promotes import by preventing solute build up in developing sink cells (Ho, 1988; Geiger et al., 1989). However, the relative importance of the various metabolic
processes associated with conversion of imported assimilate, energy supply and synthesis remains to be determined.

Respiration represented a significant portion of carbon imported throughout the rhizome (Fig. 6). Relatively high rates of respiration and high levels of net accumulation of assimilate are needed to provide energy and material for growth. Structures that are actively expanding generally accumulate carbon approximately twice as fast as they respire it while obviously dormant organs have both low rates of respiration and import. The fact that growth coefficients are not markedly larger than respiratory coefficients in the most actively growing portions of the rhizome (Fig. 6) was a bit surprising at first. Apparently even in the tip sections where growth is strongly concentrated, growth is considerably less that in fruits or actively growing shoots of a bean plant (Geiger and Shieh, 1988) or new needles of Douglas fir seedlings (Webb, 1977). In the base where respiration is low, import would be associated with maintenance activity or a low storage activity. In these structures, rates of respiration and accumulation are nearly equal.

Other processes related to metabolism which also affect import and distribution of imported carbon include accumulation of starch or other storage materials, synthesis of solutes which affect turgor pressure and the osmotic gradient in sink tissues, transfer of sugars away from conducting tissue, uptake of sucrose from the apoplast, and changes in activities or amounts of enzymes for starch synthesis and sucrose degradation (Ho, 1988). Sucrose levels were highest in those segments that were importing the greatest amount of recently fixed carbon (Table I).
Analysis of dry weight, growth coefficient, and the carbon balance of the buds was used to assess the physiological status of individual rhizome buds. As discussed earlier, depending on the source of respiratory carbon, net accumulation of imported carbon in a given structure may be better described by growth coefficients or by carbon balance coefficients and so both will be used. Net accumulation of imported carbon, observed in the various structures, appears to be characteristic of a specific physiological state. Low values for dry weight, growth coefficient, and carbon balance indicate that the bud is neither importing much carbon nor expanding. On the other hand, high dry weight, growth coefficient, and carbon balance, demonstrate that the bud is currently actively importing assimilate and increasing in size. If an axillary bud has a high dry weight, but a low growth coefficient and carbon balance then we may infer that, while the bud has grown in the past, it is now dormant. Growth will resume when some event causes it to resume further development (Robertson et al., McIntyre, 1969; McIntyre, 1970a,b). Environmental factors such as soil moisture, temperature, light intensity and day length can serve as signals for resumption (McIntyre, 1967; McIntyre, 1970a).

By analyzing respiration and net growth in dry weight data gathered from different rhizome buds at various developmental stages one can infer the usual developmental sequence for a given rhizome bud. It appears that the growth and development of a rhizome bud resembles a typical logarithmic growth curve with an early phase of growth where little increase in size is observed followed by a phase of rapid, nearly exponential, increase in size and then a stationary phase where there
is little or no net increase in size of the structure. Thus, initially, the very young bud has a low dry weight, is not importing, and has a low level of respiration. Subsequently, import begins, securing reserves and substrates in preparation for growth. The ensuing high rate of respiration results in a lower net growth in dry weight. Later the bud achieves a high dry weight, continues to respire actively and attains a high positive carbon balance as a high import rate causes the rhizome bud to accumulate assimilate and increase in size. At this point the bud will either continue to grow and develop into a new rhizome propagule or a new shoot with leaves, or import slows, resulting in a low positive carbon balance, indicating onset of dormancy.

**Factors affecting glyphosate translocation.** Translocation of glyphosate generally follows the ability of individual sink structures to import assimilated carbon. Distribution of $^{14}$C-glyphosate both among and within rhizomes resembled that of $^{3}$H-sucrose (Fig. 7), which is in agreement with previous reports that the phloem is the primary means of distribution of glyphosate in plants (Gougler and Geiger, 1981; Klevorn and Wyse, 1984b). Accumulation of $^{3}$H-sucrose, or possibly a labeled metabolite thereof, as well as metabolic activity were highest in actively growing structures closest to the apex and decreased with distance toward the basal end of the rhizome (Figs. 8 and 9).

If glyphosate is translocated along with sucrose and no other factors come into play, the relative ratio $^{14}$C:$^{3}$H as devised in this study should have a value near 1. While values were generally near 1 among and within rhizomes, its value followed the
pattern: apex > buds > internodes. This quantitative pattern suggests that, while glyphosate generally follows the translocation path of sucrose exported from source leaves, glyphosate accumulation relative to imported sucrose is highest in the sinks with the highest demand for assimilate. These observations are consistent with those of other researchers who reported that photoassimilate and glyphosate are not distributed in an identical manner in plants (Gougler and Geiger, 1981; Devine, 1989; Chandrasena and Sagar, 1986; Claus and Beherens, 1976; Devine et al., 1983; Harker and Dekker, 1988; Klevorn and Wyse, 1984a; McAllister and Haderlie, 1985; Dewey and Appleby, 1983).

A possible factor associated with this phenomenon is that import and unloading of sucrose sometimes involves not only entry by way of the symplast through plasmodesmata but may also involve transport through the plasma membrane, possibly by a sucrose carrier (Devine and Hall, 1990). Glyphosate accumulates in terminal portions of sieve elements following entry by mass flow and passively diffuses out, both symplastically and apoplastically (Tyree et al., 1979). In less active sinks a greater proportion of sucrose will enter these sinks than glyphosate because unloading of sucrose is promoted by active unloading of sucrose, a mechanism that is not available to glyphosate. In more active sinks, rapid arrival of glyphosate will maintain a steep gradient between the vascular tissue and sink tissue for diffusion of glyphosate into the sink tissue.

Besides the physical limitations to entry of the herbicide into sink tissues, glyphosate, which is known to disrupt physiological and biochemical processes, may
interfere with processes that maintain phloem translocation. For instance, glyphosate is known to slow import of sucrose into some sinks within several hours after it is applied to source regions (Gougler and Geiger, 1984). Any changes which glyphosate causes in the patterns of phloem translocation of sucrose will also affect its own transport (Gougler and Geiger, 1984; Geiger and Bestman, 1990), possibly causing it to deviate somewhat from sucrose translocation patterns (Gougler and Geiger, 1984; Harker and Dekker, 1988).

Also, it is possible that some $^3$H-sucrose may be metabolized and the $^3$H label lost while glyphosate is not metabolized to any significant extent in quackgrass (Coupland, 1985). In this case, measured levels of $^3$H-sucrose accumulated in the more active sinks could actually be underestimating the amount of $^3$H-sucrose actually imported into this sink and show a preferential import of glyphosate.

**Effectiveness of glyphosate in controlling quackgrass.** The results of this study provide some bases to explain the effectiveness of glyphosate to control quackgrass or, conversely, the ability of quackgrass to remain viable following treatment with glyphosate. Tardif and Leroux (1990), using a tetrazolium assay and growth of isolated bud to assess viability and metabolic state of quackgrass rhizome buds, found that for untreated buds, metabolic activity and growth rate upon isolation increased with increasing distance from the base of the rhizome. The metabolic activity and ability of herbicide-treated buds to grow was approximately equal regardless of bud position, in contrast to the case with untreated rhizomes. This observation can be explained by the fact that the amount of glyphosate translocated to the buds, and

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resulting phytotoxicity, is in direct proportion to their initial metabolic activity. More active buds receive more herbicide and so are inhibited more while less active ones are inhibited less. As a result, the apical dominance which existed, is now removed and previously dormant buds will be released from the dormancy produced by the rhizome apex (Klevorn and Wyse, 1984b). New tillers will develop using stores of carbohydrate and will be relatively unaffected by the herbicide because only low levels of glyphosate had accumulated in these less actively metabolizing buds.

Apparently the glyphosate initially accumulated in the rhizome is not available in effective amounts for subsequent movement into rhizome buds and apices (Tardif and Leroux, 1990). As a result, the mobilizing of stored carbohydrates to support new growth does not result in sufficient mobilization of glyphosate to interfere with development.

The observed differential accumulation of glyphosate among buds from different rhizome systems in the same plant (Fig 7) appears also to be affected by the point of origin of the rhizome on the plant. Harker and Dekker (1988) found that plants treated with $^{14}$C-sucrose and glyphosate always accumulated more label in buds of the rhizome originating from the crown than from those from rhizomes originating from propagating rhizomes.

The effectiveness of glyphosate against quackgrass rhizomes appears to stem from two main factors. The rhizome buds that are less competitive sinks for assimilate may not import sufficient levels of glyphosate to induce a lethal response and this enables a number of potential growth sites to survive. Further, in contrast

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to accumulated assimilate, glyphosate has a relatively low ability to be redistributed in the event that growth resumes. As a consequence of these factors, some glyphosate-treated rhizome buds often survive and resume growth.

**Relation of sink activity and effectiveness of glyphosate.** Data from this study provided a basis for classifying sink activity of rhizome buds and to predict the effectiveness of glyphosate in killing rhizome organs. The physiological status of the sinks were described and classified in terms of their being maintenance sinks, storage sinks or growth sinks (Fig. 10). Sinks of each physiological type have several characteristic traits which also are related to the predicted effectiveness of glyphosate in killing that structure. These traits include whether or not the specific organ is expanding, what level of assimilate import occurs and the level of metabolic rates.

While the information presented here may offer help in explaining the basis of the difficulty in controlling quackgrass, it does not provide sufficient insight into the mechanisms of biochemical processes that regulate carbon partitioning and control of carbon balance. Neither does it adequately explain the mechanism of differential accumulation of glyphosate and assimilate in quackgrass rhizomes. Particularly important, the carbon metabolizing enzymes which control sink metabolism and allocation of carbon have not been identified.
Fig. 10  Classification of sink activity and the expected effectiveness of glyphosate in killing rhizome structures. The diagram relates the physiological parameters studied and predicts their relation to effectiveness of glyphosate for controlling quackgrass proliferation.
Fig. 10

Sink Activity

Photoassimilate partitioning

Carbon Balance

Glyphosate Accumulation Relative to Sucrose

Maintenance

fully expanded tissue
low import
low respiration
low 14C/3H

low effectiveness of glyphosate

Storage

fully expanded tissue
high import
low respiration
high 14C/3H

low effectiveness of glyphosate

Growth

expanding tissue
high import
high respiration
high 14C/3H

high effectiveness of glyphosate
CONCLUSIONS

Carbon Partitioning

1. Autoradiographic images and growth coefficients revealed that sink activities differ, both among individual rhizomes on the same plant as well as among the various structures within a single rhizome.

2. Sucrose levels are highest in portions of the rhizome which are importing the greatest amount of recently fixed carbon.

Carbon balance

1. Those organs which have the highest metabolic rates show a high level of import and high net accumulation of carbon, that is have a high positive carbon balance.

2. Generally apices have the highest positive carbon balance and basal internodes the lowest.

3. Portions of the rhizome with the highest respiratory metabolism have the highest nutrient status with respect to sucrose.
Glyphosate translocation.

1. Glyphosate generally follows the same partitioning pattern among the whole rhizome system of quackgrass as does photoassimilate, as well as showing differences in the level of accumulation among rhizomes.

2. In general, glyphosate is generally accumulated in sinks in direct proportion to the accumulation of imported sucrose in quackgrass rhizomes. Consequently its distribution is related to the level of metabolic activity of each rhizome as a whole and each part.

3. When compared to sucrose accumulation, glyphosate preferentially accumulates in the areas of highest metabolism and highest import.
Appendix A

Steady-State Labeling Method

At the start of the 10 h photoperiod, one or two, three-month-old quackgrass plants were placed in a 100-L plexiglass chamber, part of an apparatus to supply $^{14}$CO$_2$. The potted plants rested in a tray with 1 cm of water to prevent water stress during the labeling period. Aluminum foil was placed over the tray to prevent evaporation of the water as well as to reflect heat to prevent overheating of the roots or the labeling chamber. Two, 400-W metal halide and two 400-W high pressure sodium lamps above the chamber, provided a photon flux density of 800 $\mu$mol m$^{-2}$s$^{-1}$ at mid leaf level. Temperature was maintained between 20 to 25°C by circulating cooled antifreeze through a heat exchanger inside the chamber. Humidity was controlled by circulating air from the chamber over a chilled surface set to maintain the relative humidity at 70% ± 5%. An infrared gas analyzer connected to a controller regulated the CO$_2$ level to 350 ppm ± 30 ppm. The $^{14}$CO$_2$ of known specific radioactivity was generated as described by Geiger and Shieh (1988). Actual specific radioactivity of $^{14}$CO$_2$ in the enclosed atmosphere was monitored by withdrawing 20 ml of air from the chamber with a syringe at regular intervals.
throughout the labeling period. The gas was then slowly injected into 1.5 ml of 1 N KOH in a vial to trap the CO₂ and the amount of ¹⁴C present in the sample was counted with a liquid scintillation counter (Beckman model LS-3801, Fullerton, CA). Specific radioactivity of the ¹⁴CO₂ in the chamber was determined by dividing the amount of radioactivity measured by the total amount of carbon in the sample. The latter was calculated from the volume of the sample and the CO₂ concentration at the time of sampling. Assimilate fixed by the plants was labeled for the entire photoperiod. At night, the CO₂ level was maintained and no ¹⁴CO₂ was added to the system. Plants were harvested 24 h after the beginning of the labeling period to allow distribution of carbon from transient starch.
Appendix B

Determination of Radioactivity by Tissue Oxidation

1. **Harvesting of rhizomes.** Following labeling as described in Appendix 1, the quackgrass plants were removed from the chamber and the rooting medium was washed off of the roots and rhizomes. Usually, roots were removed from the rhizomes. Primary rhizomes originating from the culm of the plant were tagged and labeled as R-1, R-2, R-3, etc., and propagules from these primary rhizomes were labeled as R-1a, R-1b, R-1c, etc. The rhizomes were immediately frozen on dry ice and lyophilized.

2. **In-vial oxidation to measure $^{14}$C content of rhizome structures.** Apparatus and method were modified from methods previously described by Gupta (1966), Bucholtz and Hess (1983) and Geiger and Shieh (1988).

   a) Freeze-dried rhizome apical segments, axillary buds, or internodal segments were excised with a razor blade and weighed with a Cahn RG automatic electrobalance (Ventron Instruments Corp, Paramount, CA).

   b) Pieces of rhizome, weighing more than 5 mg dry weight, were powdered using
a Wiggle bug (Dentsply, Wilford, DL, USA). Pieces weighing less than 5 mg dry weight was oxidized intact.

c) Samples to be combusted were weighed in a paper cup made from cigarette paper. The maximum weight allowed for rhizome piece plus the cup is 20 mg, however, a combined weight of no greater than 10 mg is recommended to ensure complete oxidation.

d) The sample was then placed into a tapered nichrome basket wound on a lathe. The top diameter was 1 cm. the bottom 0.5 cm. A 1.5 ml portion of 1 N KOH was added to a 20-ml liquid scintillation vial for CO₂ absorption. If tritium only is being measured just add water. A neoprene stopper, with the basket containing the sample suspended from a small hook, was placed over the opening of the vial without sealing it.

e) The vial was flushed for 40 seconds with oxygen delivered from a 1 mm capillary tube at a rate of 200 ml min⁻¹. After being flush with oxygen, the vial was immediately stoppered.

f) Eight vials were prepared at one time in this manner and secured in a wooden rack with a wooden top and plexiglass sides. The cover was fasten down by tightening thumb screws after determining that all wires needed for heating the basket were aligned to exit through holes in the wooden cover.

g) The assembled box was paced in a hood designated for use with gaseous radioisotopes.

h) The voltage for heating the nichrome basket to ignite the sample was adjusted
with the variable transformer or variac, to eight volts AC.

i) Alligator clips from the variac were connected to the two wires from the basket in the first vial, making sure they do not touch. Power was then turned on until the sample burned with a bright flame, usually five to ten seconds, and then was turned off. This entire ignition procedure was repeated for each vial in the rack.

3. Counting samples to measure $^{14}$C or $^{3}$H content.

a) The samples in the secured box were allowed to stand for 30 minutes. Then the lid and front panel were removed and the stoppers were replaced with plastic screw caps.

b) Then 1 ml of water and 10 ml of Ecolume scintillation cocktail (ICN Biochemicals) were added to each vial. The vials were shaken vigorously on a vortex for one minute or longer until the solution was no longer milky but water clear.

c) To clean the baskets for the next use, each was dipped into 75% nitric acid and rinse with deionized water.

d) The radioactive content of each vial was then determined by counting with a liquid scintillation counter set for $^{14}$C or $^{3}$H or both as appropriate.
Appendix C

Growth Coefficient Calculations

Growth coefficients were calculated as described by Geiger and Shieh (1988). The growth coefficient is defined as the incremental carbon increase in a small time interval per total carbon content of a tissue sampled, or

\[ \alpha = \frac{\Delta c}{\Delta t^{-1} c} \]

The recently fixed and imported carbon is tracer carbon, that is it has the \(^{14}\text{C}:{^{12}\text{C}}\) ratio which is the same as that of the \(\text{CO}_2\) supplied. Since the specific radioactivity, \(s\), of the \(^{14}\text{CO}_2\) is known, then incremental carbon increase is

\[ \Delta c = \frac{\Delta c}{s} \]

where \(\Delta c^*\) is the amount of tracer carbon imported in the time measured. Thus

\[ \alpha = \frac{\Delta c^*/\Delta t}{cs} \]

1.) The specific radioactivity of each structure assayed was determined by
measuring the amount of $^{14}$C per mg dry weight of tissue as described for the in-vial oxidation procedure in Appendix B.

2.) The specific radioactivity of the gas, $s$, is determined by measuring the amount of $^{14}$C in a 20-ml sample of atmosphere removed from the plant chamber with a syringe. The $^{14}$CO$_2$ is trapped by injecting the gas into 1.5 ml of 1N KOH in a 20-ml liquid scintillation vial and counting the $^{14}$C with a liquid scintillation counter. The radioactivity contained in the sample is divided by the total amount of carbon in the gas to give the specific radioactivity of the gas. The total amount of carbon in the sample of gas removed from the chamber is calculated in the following manner:

$$ s = V \times \frac{ulCO_2}{mlair} \times \frac{12ugC}{24.5ulCO_2} $$

where V is the volume of the sample of gas withdrawn from the chamber.

The growth coefficient represents the net gain in carbon derived from the CO$_2$ supplied during the labeling period and exported from the source leaves during the 24-h observation period per unit of standing carbon in a given organ.
Appendix D

Autoradiography of Rhizomes

1. **Harvesting of rhizomes.** Following labeling as described in Appendix A, the quackgrass plants were removed from the chamber and the rooting medium was washed off of the roots and rhizomes. Usually, roots were removed from the rhizomes. Primary rhizomes originating from the culm of the plant were tagged and labeled as R-1, R-2, R-3, etc., and propagules from these primary rhizomes were labeled as R-1a, R-1b, R-1c, etc. The rhizomes were immediately frozen on dry ice and dried under vacuum in the inner chamber of a FTS Dura-Top TD-3 Tray Drying Chamber with a Dura-Dry Condenser Module FD-6-54 (FTS Systems, Inc. Stone Ridge, NY).

2. **Autoradiography.** Freeze dried rhizomes were placed between two stainless steel plates and put under 36,000 psi using a french pressure cell press (American Instruments Co. Inc., Silver Spring Maryland) at a setting of 2000. Kodak Industrex type M x-ray film was exposed to radioactivity for a total of 6 to 8 million counts. Film was developed using Kodak GBX developer and replenisher and fixer
and replenisher in formulations specified by the manufacturer. Film was developed without agitation for five minutes, rinsed with tap water for thirty seconds, and then placed in the fixer bath for 30 minutes. A final rinse in a water bath with at least a change of eight volumes of water per minute for two hours to complete the process.

Positive prints were made on Kodak Polycontrast III RC paper using a photographic enlarger. Prints were developed using an automatic resin developer for coated paper with Kodak S11 Activator. Prints were fixed for two minutes in a bath containing Kodak Rapid Fixer for films, plates, and paper in a formulation recommended by the manufacturer. The prints were then rinsed in a water bath for four minutes.
Appendix E

Calculation of Respiration Coefficients and Carbon Balance

1. **Respiratory Coefficient**  Respiration of rhizome structures was measured as described in the **Materials and Methods**. Rhizome pieces were placed in a vial containing 3.5 ml of assay solution at 27°C and equilibrated for three minutes prior to measurement. Measurements were taken for five minutes following equilibration with an oxygen electrode and electrode monitor (Yellow Springs Instruments, Yellow Springs, OH). The consumption of oxygen was monitored as the change in % full scale on the meter of the monitor per observation period, usually on the order of 5 min.

Using a tabulated value for oxygen concentration in solution at 27°C the number of μmoles of oxygen in 3.5 ml of the assay solution was calculated as

\[
V_g = 3.5 \text{ ml} \times 8.25 \mu g O_2 \text{ ml}^{-1} = 28.875 \mu g O_2
\]

\[
V_m = 28.875 \mu g O_2 \times (0.0312 \mu mol \mu g O_2^{-1}) = 0.9009 \mu mol O_2
\]

Oxygen uptake next was expressed as μmol O_2 d^{-1} per milligram^{-1} dry weight.

Calculation of the respiratory coefficient, RC, was based on the assumption
that the respiratory quotient, moles of CO\textsubscript{2} produced per moles of O\textsubscript{2} absorbed, was approximately one (Bidwell, 1974). This would be the case if carbohydrates are the main substrate for respiration in the plant tissue assayed.

\[ \Delta f.s. \; d^{-1} \; mg \; dry \; wt^{-1} \times (0.9009 \; \mu mol \; O_2) = \mu mol \; O_2 \; d^{-1} \; mg \; dry \; wt^{-1} \]
\[ \mu mol \; O_2 \; d^{-1} \; mg \; dry \; wt^{-1} = \mu mol \; C \; d^{-1} \; mg \; dry \; wt^{-1} \]
\[ [(\mu mol \; C \; d^{-1} \; mg \; dry \; wt^{-1}) \times mg \; dry \; wt] \times \mu mol \; C \; total = day^{-1} \]
\[ day^{-1} \times 100\% = Respiratory \; Coefficient \]

2. Carbon Balance. There are two boundary conditions which could describe the source of resired CO\textsubscript{2}. On the one hand, all of the carbon which is resired may come from recently fixed carbon or conversely some or nearly all of the carbon which is resired may come from reserve carbon.

In these studies, the source of resired carbon, whether from recently fixed or reserve carbon, was not determined. To distinguish this, one can measure the ratio of resired \textsuperscript{14}CO\textsubscript{2} to total resired CO\textsubscript{2} and compare the ratio to that for the labeled carbon in the CO\textsubscript{2} in the atmosphere for labeling photoassimilate. The ratio [resired C ratio:fixed C ratio] gives the proportion of carbon derived from newly fixed carbon. A value approaching 1 indicates that nearly all the resired carbon came from recently fixed carbon while a value near zero shows that the respiratory substrate was stored carbon fixed prior to the labeling period.

If nearly all of the resired carbon comes from the newly imported assimilate,
which is a good possibility, the growth coefficient itself is the true measure of carbon accumulation. In this case, subtracting the respiratory coefficient gives a false, markedly underestimated rate of carbon accumulation (compare bars for growth coefficient and carbon balance in Fig. 6)

Based on the assumption that in some organs respiration may be derived not from tracer carbon but from stored carbon, net accumulation was estimated by calculating the carbon balance coefficient. This parameter is computed by subtracting the respiration coefficient from the growth coefficient (Fig. 6).
Appendix F

Determining Relative $^3$H-Sucrose and $^{14}$C-Glyphosate Content in Rhizomes.

The amount of $^3$H-sucrose and $^{14}$C-glyphosate in each tissue was determined by oxidizing each tissue and then counting the nCi's of each isotope present using a dual-label scintillation spectrophotometer as per Materials and Methods. The amount of nCi's of $^3$H-sucrose in portions of the rhizome with the greatest accumulation of $^3$H-sucrose was divided by the same number to give a value of one. This value then represents the structure with the greatest import of $^3$H-sucrose in the whole rhizome system of the plant studied. The amount of nCi's of $^3$H-sucrose in all other organs assayed were divided by the nCi's present in the greatest importer to give a ratio of the amount of $^3$H-sucrose accumulated in each organ relative to the organ that was the greatest importer. The value of presenting the data this way is that it is easier to ascertain the relative sink strength of each individual structure in the rhizome system. In addition the values for the distribution of $^{14}$C-glyphosate are presented as a ratio of $^{14}$C-glyphosate/$^3$H-sucrose. First, relative amounts of $^{14}$C-glyphosate were determined in the same manner as $^3$H-sucrose, with the portion of
the rhizome that imports the greatest amount of glyphosate given a value of one. Then the relative values for $^{14}$C-glyphosate were divided by the relative values for $^3$H-sucrose to obtain the ratio presented in figures 7,8, and 9. This ratio then describes the distribution of glyphosate relative to $^3$H-sucrose. In theory, if glyphosate were to follow exactly the path of sucrose in plant tissues, one would expect that the ratio of $^{14}$C-glyphosate to $^3$H-sucrose to be one or very nearly one. In practice we find this relationship to be different.
LITERATURE CITED


Devine MD, Bandeen JD, McKersie BD (1983) Temperature effects on glyphosate
absorption, translocation, and distribution in quackgrass (*Agropyron repens*).

**Weed Sci** 31: 461-464


**Geiger DR, Shieh W-J, Saluke RM** (1989) Carbon partitioning among leaves, fruit,
and seeds during development of Phaseolus vulgaris Plant Physiol 91:291-297


Harker KN, Dekker J (1988) Temperature effects on translocation patterns of several herbicides within quackgrass (Agropyron repens). Weed Sci 36: 545-552


culture growth by glycolytic intermediates and organic and amino acids. Plant Physiol. 68: 1299-1302


McAllister RS, Haderlie LC (1985) Translocation of $^{14}$C-glyphosate and $^{14}$CO$_2$-labeled photoassimilates in Canada thistle (Cirsium arvense). Weed Sci 33: 153-159


McIntyre GI (1970a) Studies on bud development in the rhizome of Agropyron repens. 1. The influence of temperature, light intensity, and bud position on


Wauchope D (1976) Acid dissociation constants of arsenic acid, methylarsonic acid (MAA), dimethylarsinic acid (cactolyc acid), and N-(phosphonomethyl)glycine (glyphosate). J Agric Food Chem 24: 717-721
