## Overview on night tritium transfer from air to plants and conversion to OBT

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#### Introduction

- OBT generation in the darkness has already been observed by Moses and Calvin (1959) who exposed chlorella algae to HTO in their nutrient solution under conditions of light and darkness for 3min. The tritium incorporation into no exchangeable positions of the organic matter in the dark was one-third of that in the light. Thompson and Nelson (1971) exposed primary leaves of soybeans to HTO in the atmospheric humidity under conditions of light and darkness for 1 or 30min. Related to the same exposure time, the assimilation of tritium in the dark was only 10% of that in the light.
- While formed in leaves, OBT is translocated in the edible plant parts,, most of which are reproductive organs, and depends on the growth stage of the plant at the time of exposure. OBT concentration in edible plant part is highest in the generative period when the fruits grow (Arai *et al.*, 1985, Indeka, 1981)
- This overview will concentrate on night processes but this will be analyzed in relation with overall tritium transfer and conversion.
- The aim of this contribution is to point actual difficulties and need of further collaboration at international scale.

## Importance of Night OBT

- Night air concentration > day ( average factor 10, but can be > 40)
- Night HTO uptake by crops < day ( average factor 4; range 2-10)
- For same HTO in leaves, night OBT production is 1/10-2 from day one (exp. data)

## HYPO scenario EMRAS I

- Case 1 day Normalized by 6.10<sup>9</sup> Bq.s.m<sup>-3</sup>
- Case 3 night Normalized by 3.10<sup>11</sup> Bq.s.m<sup>-3</sup>





Diabate & Strack (paper in 1997, experiments in 1993-1994)

- The HTO concentration ratios under night conditions are reduced to 23% in leaves, to 25% in stems and 59% in ears, compared to those observed under high light conditions.
- There was no significant difference in the HTO uptake between spring wheat and winter wheat leaves
- In leaves, the initial relative OBT concentrations were typically ½ in night condition in comparison with high light conditions.
- It has been clearly demonstrated that there is a small but not insignificant OBT incorporation under night conditions in leaves, stems and ears, indicating that tritium can be incorporated into organic matter not only by photosynthesis but also by metabolic pathways independent of light
- In an extended night experiment, the OBT concentrations in the ears increased by a factor of 3 during the extended dark period. This indicates high rates of metabolic turnover in the ear, which does not result in *de-novo* synthesis of organic material.

Translocation index (TLI) The percentage of the OBT concentration in grain at harvest (Bq/ml' water of combustion) related to the TWT concentration in leaves (Bq/ml') at end exposure



#### interpretation

- The absolute value of TLI in figure is not relevant, as leaves maintain high HTO level for long time in the experiment and formation of OBT in leaves take more time that 2 hours (when leaf HTO is used for defining TLI). Important to note is that night values are close with the day ones!.
- The shape of the time dependence of the translocation index in figure 1 can be explained by general processes in wheat growth
- At the begin of grain filling, partition to grain is small and growth dilution effect is high (see ear at day 1 and harvest). This explains the low TLI. At the end of grain filing period, much of OBT formed in the leaves is used for maintenance respiration and few remains to be translocated to grain.

#### Diabate & Strack (unpublished, experiments in 1995-1996)



The conditions in the box (relative humidity , temperature) have been recorded as well as the photo-sinteticaly active radiation above

the box (PPFD) .The experimental data for the duration of HTO contamination in the box atmosphere are given in Table III. Reported are start hour, average temperature and relative humidity, PAR outside the box and day after flowering. Note that experiments in 1996 (bolded in table III) are of better quality as the level of Co2 in the box was maintained at natural values. Winter wheat, linear grain filling period, 1 hour exposure, conditions

		f3	f14	f7	f2	f4	f10	f15	f1	f9	f13	f5	f11	f6	f12
Start	н	7	7	8	9	10	11	11	14	15	15	20	20	23	23
Т	С	18	11	26	28	29	26	32	33	36	29	24	15	17	12
RH	%	76	93	76	76	63	75	63	70	70	72	84	89	89	93
PPFD	µmol/m 2s	160	179	370	644	1230	1160	1830	1180	1375	1170	54	86	0	0
DAA		18	22	24	17	18	14	28	15	12	21	22	20	22	20

#### The initial (1 h) uptake of HTO in the leaves, relative with the average HTO in air moisture in the box, is given in figure

140 120 leafTWTmeas 100 80 % 60 40 20 0 f-5 f-3 f-14 f-7 f-2 f-4 f-10 f-15 f-1 f-9 f-13 f-11 f-6 f-12 7 h 7 h 8 h 9 h 14 h 15 h 15 h 20 h 20 h 23 h 10 h 11 h 11 h 23 h

Leaf-TWT related to mean atmospheric HTO

The maximum relative TWT concentrations were reached in the leaves under conditions of strong sunlight when stomata were open (mean =  $73 \pm 19\%$ ). The uptake was only slightly reduced in senescing leaves. In the night experiments, a diminished uptake into TWT of leaves, stems and ears was observed because of the closure of the stomata (mean =  $18 \pm 1\%$ ).

#### Half-Lives of TWT Concentration in Wheat within 1 h after the End of Exposure to HTO.

		TWT half-lives (min)						
Plant parts	Exposure at dawn (3 exp.)	Exposure at day-time (6 exp.)	Exposure at dusk (2 exp.)	Exposure at night (2 exp.)				
Leaves	40-60	25-49	230-660	110-170				
Stems	45-49	20-26	130-320	60-190				
Ears	79-91	50-126	210-330	150-920				
Total plant	50-72	27-60	220-340	100-250				

Dynamics of OBT in leaves and the harvest value for grain, in relative units (HTO concentration in leaves at end exposure)



Rel.OBT leaf 1,2,4h,1d,harv.

%

#### Diurnal trend of DLI, 1 hour exposure, linear grain filling period Strack&Diabate, unpublished



## COMMENTS

- Immediately after the end of exposure, the highest relative OBT concentrations were observed in leaves under day-time conditions (1.25±0.34%), about 3 times higher than under night conditions (0.38±0.05%).
- In day time there is a clear reduction in the first day, due to assimilate export, which seems to start immediately after end exposure. In night condition assimilate export is slower and perhaps more active in the next morning.
- Despite the large difference in leaf OBT at end exposure, in all experiments the OBT in grain at harvest shows similar relative values (mean = 0,25±0,07%).
- This can be partly explained by the longer residence of leaf HTO in night time (experiment F6 F12) allowing a larger contribution of metabolic processes to OBT formation.

Courses of relative OBT concentrations in leaves and grains from exposure to HTO to harvest. The data represent means  $\pm$  1SD of 7 exposures under day-time conditions and

#### of 2 exposures under night-time conditions



It seems that translocation in the night experiments is delayed until next morning and take longer. The total OBT per plant increases in the first 2 days and can decrease until harvest at 80 % from maximum value.

## Empirical correlation; OBT in grain at harvest and integrated TWT concentration (day Leaf+0.5\*day Ear)+0.2(night leaf+night Ear)) STRACK UNPUBLISHED



 $OBT_{grain}$  at harvest related to  $TWT_{integrated}$  in leaves and ears

Diabaté, S., Strack, S. and Paunescu, N. (1998). Tritium uptake in green bean and potato plants after short-term exposure to atmospheric tritium Preprint IFIN-HH/ RB-53 (2001)

Plant part	exposure at 900 μmol m <sup>-2</sup> s <sup>-1</sup>	exposure at 120 μmol m <sup>-2</sup> s <sup>-1</sup>	exposure at night
Time after flowering	4	1	12
Bean leaves	0,7	0,3	0,5
Bean stem	0,4	0,2	0,3
Bean pods	0,1	0,03	0,4
Time after flowering	<u>20</u>	<u>13-25</u>	<u>15-23</u>
Potato leaves	0,2	0,2	0,2
Potato steam	0,2	0,1	0,2
Potato tubercle	0,3	0,2	0,2

At a first impression, it seems that night translocation in bean is close with wheat but lower for potato. More experimental data are needed

### rice

- Ichimasa, linear period, hulled, 25 day before harvest; TLI
- 0.73% for day 0.54% night
- Atarashi-Andoh, 5 days after flowering
- 0.36 % for the day case and only 0.03 % for the night case.
- Korea to find

#### Soybean, Ichimasa



At final harvest TLI is near 0.7 % in day time and 0.5 in night.

From the paper details on pods development stage at exposure is difficult to asses. More information is needed but the researcher retired.

Note that these results are for an exposure of 8 hours, when air concentration gradually increased in the glasshouse. A crude translation for one hour exposure will be to divide the TLI by 4-6

# Uptake of HTO in night and leaf resistance

Aqueous pores enhance cuticular conductance (Scho<sup>¨</sup>nherr J. 2006.). Data on water permeance in leaves (Riederer M, Schreiber L. 2001) suggest a large variability of night uptake rate of water. From the tritium experiments low uptake have been seen for tomato leaves and lettuce (Boyer thesis) and high ones for sunflower. Wheat, bean and potato are at intermediate range. It will be useful to have direct experimental data for each major crop of interest.

		rate h <sup>-1</sup>	resistance porometer s/cm	rate using porometer h
Night 95	Komatsuna	$0.65 ~\pm 0.19$	5.7–40	0.06–0.44
	Orange	$0.06\ \pm\ 0.29$	49–55	0.04–0.05
Night 96	Komatsuna	$0.20\pm0.04$	2.7–3.2	0.82–0.97
	Radish	$0.31\pm0.05$	2.6–3.4	0.72–0.95
	Tomato	$0.12\pm0.02$	6.9–15	0.16–0.36

#### TLI %, cherry tomato, Canada

	DAA	TLI %	OBS
n3	15	0.0146	
n4	17	0.0075	growth dilution added
n5	48	0.5	
n6	49	0.346	
d7	41	0.044	day suny
d8	42	0.12	day cloudy

Relative OBT concentration in tomato fruits



# Provisional conclusion from experimental data

- NOT mandatory that night TLI are less than day TLI
- Dependence on plant development stage possible
- Dependence on plant type must be investigated
- For night release, next morning processes very important
- Not enough experimental data

## Briefing of process analysis

- A large portion of assimilated C is used in maintenance respiration for upkeep of existing structures and in growth respiration to produce new components.
- A substantial part of growth respiration involves oxidation of photosynthate necessary to produce the organic acid C skeletons required for assimilation of N (See figure from Lewis et all 2000). Many enzymes are contributing to all processes (see figure).
- Assimilate is a soluble sugar with structure of glucose. *Glucose*, fructose, and galactose are monosaccharides; their structural formula is C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>. Part of assimilate is converted to sucrose, stored in the leaf but also exported.
- A chain of enzymatic reactions in mitochondrion and chloroplast produce amino acids and lipids, mostly exported (see figure from Lewis).
- Part of assimilate is stored as un-soluble polysaccharide, starch, that function to store energy. Plants produce starch to store carbohydrates. In the night starch is hydrolyzed and soluble sugars are exported.
- The <u>cell walls</u> of <u>plants</u> are composed of cellulose. Cellulose is composed of beta-glucose monomers; starch and glycogen are composed of alpha-glucose.

#### Processes analysis



#### Assimilate export



FIG. 1. Diurnal changes in net CER (O) and assimilate export rate ( $\Delta$ ) of a maize leaf during a normal day/night cycle. The solid bar at the bottom of the figure indicates darkenss and the open bar, light.

soybean night export maximize after 6 hours of dark period

Maize Export in night is lower than in the day and decrease after dusk



FIG. 4. Rate of starch mobilization from leaves of Amsoy 71 and Wells II soybean during a 10-h dark period. Rates of starch mobilization were determined from the first derivative of equations used in Figure 3 and presented as positive values.

Assimilate export



FIG. 7. Relationship between rates of daytime and nighttime assimilate export and PPFD in Amsoy 71 and Wells II leaves. Rates of export were estimated from the linear rate of change in SLW between 2200 and 0800 h and rates of CO<sub>2</sub> exchange. Inset: relationship between PPFD and rates of daytime and nighttime export as a percent of carbohydrate production rates.

#### Night/day ratio depends on cultivar and previous irradiance





FIG. 5. Contents of and transfers among source leaf pools during phases of the diurnal cycle in sugar beet. Underlined numbers are amounts expressed as  $\mu g$  C cm<sup>-2</sup>. Other numbers are rates expressed as  $\mu g$  C cm<sup>-2</sup> min<sup>-1</sup>. Numbers associated with sucrose pools refer to total without distinction between cytoplasmic and vacuolar compartments. Amount of structural material was calculated from average dry weight values less starch and ethanol soluble materials. Unaccounted for material was computed from rates of input to intermediates minus rates of output.

In specific cases it is possible to detect more details and assess transfers between pools in day and night conditions

675

### Some conclusion

- The goal of the coordinated diurnal regulation of sucrose and starch metabolism is to maintain a balanced carbon supply for export during the day and night. This regulation requires that the degradation of starch and the allocation of carbon between starch and sucrose synthesis be controlled in accord with the integrated daily rate of carbon assimilation and photosynthetic duration. By its nature, diurnal regulation
- maintains this goal throughout a day and therefore is not readily nor rapidly changed during the span of a single photoperiod. As most of the newly fixed carbon is
- allocated to either starch or sucrose, any increase in daytime sucrose synthesis and export would come at the expense of carbon available for export at night. Consequently, a diurnal change in carbon allocation would not result in greater total export over the 24 h period but would only upset the balance between daytime and nighttime carbon supply(Fondy).

### Models etmod AECL

- **2.5 Dry Matter Production in Plants:** Gross photosynthesis rates are calculated using the CO2 consumption model [Weir et al. 1984, Sellers 1985, Mitchell et al. 1991, Pinder et al. 1988] and depend on air temperature, the resistance to CO2 uptake by the plant and the photosynthetically active radiation reaching the plant, which in turn depends on leaf area index. The production rate of dry matter is based on net photosynthesis (the difference between gross photosynthesis and respiration), taking into account both growth and maintenance respiration. Plant dry mass is updated using the dry matter produced in the time step. The wet vegetation mass is then calculated from the dry mass and the fractional water content, which is assumed to remain constant as the plant grows. The calculation stops when a pre-specified plant mass or harvest time is reached.
- **2.6 OBT Formation in Plants:** The dry matter produced at a given time is assumed to have a T/H ratio equal to 0.6 times the T/H ratio in the plant water that takes part in the photosynthesis at that time. All dry matter production and OBT formation is assumed to take place in the above-ground part of the plant, even for root crops. ETMOD assumes that dry matter production and OBT formation do not occur at night in the absence of photosynthesis. OBT concentrations following exposure decrease due to dilution with new uncontaminated dry matter. ETMOD does not account for the slow conversion of OBT to HTO in plants due to metabolic processes.
- COBT(Bq/kgdm)/0.08(kgH/kgdm)=0.6\*Chto(Bq/L)/0.111kgH/L
- Cobt(Bq/kgdm)=(0.6\*0.08/0.111)\*Chto
- Cobt(Bq/kgdm)=0.43Chto
- OBTprod=DMPROD\*0.43\*Chtomed
- **2.7 Translocation:** ETMOD can handle five types of crops (pasture, leafy vegetables, non-leafy vegetables, root vegetables and grain). In each case, the plant is treated as a single compartment with uniform concentrations throughout. This means that translocation between different parts of the plant must be addressed outside ETMOD.
- NO NIGHT until now

## Models MyFDMH IFIN

- The production of OBT in plants is assumed to be proportional to the assimilation (net photosynthesis) rate during daytime and to the basic metabolic rate at night. This leads to the following equations:
- POBT = fac1\*fac2\*CO2as\_rate\*tim\*chtomean (daytime) (4)
- POBT = fac1\*fac2\*ratenight\*(LAI/maxlai)\*tim\*chtomean (night-time)
- where:
- POBT = OBT produced per m2 in time period tim
- fac1 = correction for fractionation and non-exchangeable tritium = 0.6
- fac2 = conversion from CO2 to H2O assimilation rate = 0.41
- CO2as\_rate = net CO2 assimilation rate = gross assimilation rate respiration rate kg CO2 per unit time and unit surface of crop;
- chtomean = mean concentration of HTO in plant water during time period tim
- ratenight = maximum night production rate (= 1.2x10-3 kg CO2 m-2 h-1 for a fully developed plant) under metabolic processes
- maxlai = maximum value of the leaf area index
- In the same conditions of time and space, the net dry matter production is
- PD= 30/44 Pc
- Pobt=0.6\*0.6\*Pd\* CHTO
- The newly formed OBT is stored in the edible part of the crop using the partition fraction derived for the deposition day.
- Plant growth is modeled using elements of the WOFOST model; plant parameters are adapted to the region of interest [Melintescu et al., 2002].

## Models GAZAXI CEA

- The dry matter produced is assumed to have a T/H ratio of 0.95. To calculate the ۲ exchangeable fraction, the dry matter is weighted by a factor 0.53, which corresponds to the T/H ratio multiplied by 90 (the molecular weight of five water molecules, the number of water molecules in one cellulose molecule) and divided by 162 (the molecular weight of cellulose (C6H10O5)n).
- Dans le cas d'un végétal à récolte unique, l'activité du végétal au moment de sa • récolte est donc :

$$C_{v\acute{e}g}^{dir}(t_{i}) = C_{HTO}^{dir}(t_{i}) (1 - \tau_{ms}^{v\acute{e}g}) + 0.53 \frac{CI_{HTO}^{dir}}{86400 \,\Delta t_{i}} \tau_{ms}^{v\acute{e}g}$$

- $CI_{HTO}^{dir}$ : activité intégrée en eau tritiée libre (Bq.s.kgeau-1)
- 86400 : facteur de conversion du temps (s.j-1)
- ∆ti : durée de croissance du végétal
  - (i) : taux de matière sèche dans le végétal (kgvégétal sec.kgvégétal
- $\tau_{\rm ms}^{\rm vég}$ frais-1)
  - : coefficient de pondération 0,53