The 10000 g pellet's lipolytic activity
The investigation of the lipolytic activity in the 10000 g pellet fraction is shown in Figure 2. A linear relationship was obtained between the amount of pellet extract added and enzymic response.

The conjugated lipolytic activity of lipid body plus pellet
We mixed the pellet extract with the oil-body fraction in the presence of free Ca²⁺ (200 μM). We observed (Figure 3) that the resulting activity was 4-fold higher with the addition of 130 μg of protein pellet. This corresponds to a ratio of oil body to pellet of 1:3.

Conclusion
The subcellular distribution of lipase could be regulated. ATP and calcium seemed to be required for lipolytic activity in germinating sunflower seedlings. Is the studied lipolytic system so far from that of animals?

References
2 Mukherjee, K. D. (1994) Prog. Lipid Res. 33, 165-174

Assaying Arabidopsis lipase activity
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Abstract
A low lipase activity from a crude extract of Arabidopsis seedlings was assayed using three sensitive methods (radiolabelled triacylglycerols, commercial resorufin ester and triacylglycerols containing the naturally fluorescent parinaric acid as substrates). The specific activity of the extract was found to be similar using the three methods. However, the plant lipase activity measured using the radioactivity and the fluorescence assays could be abolished by heating the extract, contrary to the apparent activity measured using the commercial colorimetric assay. Unlike the radioactivity assay, the fluorescence assay can be monitored continuously. The parinaric acid-based method is therefore the only one to provide a sensitive, specific and continuous assay.

Introduction
True lipases (EC 3.1.1.3) are enzymes that catalyse the hydrolysis of the carboxyl ester bonds of long-chain acylglycerols at an oil/water interface [1]. These enzymes have been well characterized in mammals and micro-organisms [2]. By contrast, very little is known about plant lipases. This is mainly due to the very low abundance of these proteins, which makes them difficult to purify in amounts sufficient to get access to amino acid sequence information. Therefore, no plant true lipase has ever been cloned.

During post-germination of an oilseed plant such as Arabidopsis thaliana, the growth of the

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Comparison of three sensitive lipase assays

Conditions are as in [9]. IU, International Units (μmol of non-esterified fatty acids released per min); HPL, human pancreatic lipase.

<table>
<thead>
<tr>
<th>Type of assay</th>
<th>Substrate</th>
<th>Lipase source…</th>
<th>Continuous assay</th>
<th>Sensitivity (ng of HPL)</th>
<th>Specific activity (mIU·mg of protein⁻¹)</th>
<th>Activity remaining after heating (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactivity</td>
<td>Triolein</td>
<td>Pure HPL</td>
<td>No</td>
<td>0.1</td>
<td>15</td>
<td>9.1</td>
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<tr>
<td>Fluorescence</td>
<td>Parinari TAGs</td>
<td>Arabidopsis seedling homogenate</td>
<td>Yes</td>
<td>0.1</td>
<td>1</td>
<td>17.5</td>
</tr>
<tr>
<td>Colorimetry</td>
<td>Resorufin ester</td>
<td></td>
<td>Yes</td>
<td>0.1</td>
<td>36</td>
<td>112</td>
</tr>
</tbody>
</table>

Seedsling is supported mainly by hydrolysis of the oil reserve [3]. Since lipases are involved in the first steps of this series of reactions, they might control a crucial step in post-germination of seeds.

In addition to their importance for proper growth of seedlings, plant lipases are enzymes that play important roles in various agroindustrial processes. Their activity is important for production of good-quality bread and beer, while they appear to be detrimental to the long-term storage of plant-derived foods, because they participate in the process that turns food products rancid [4].

Finally, lipases from various origins have been found to have numerous applications in the food, cosmetics, detergent and pharmaceutical industries. One of their more exciting uses concerns organic chemistry. Almost a third of the reactions carried out for biotransformation in organic chemistry utilize lipases as a biocatalyst. The uncovering of a new group of lipases (such as oilseed lipases) with different catalytic properties is therefore of increasing potential value in chemistry [5].

Since lipases are present in very low amounts in homogenates from germinating oilseedlings that contain many other carboxylesterases, it is critical that purification procedures use specific and sensitive lipase assays. A recent review [6] of the main lipase detection and assay methods is available on our laboratory’s web site (http://lle-sg.cnrs-mrs.fr).

We have recently set up a lipase assay using parinaric acid [9(cis),11(trans),13(trans),15(cis)-octadecatetraenoic acid], a naturally fluorescent fatty acid that contains four conjugated double bonds [9]. The TAGs purified from Parinari glaberrimum seed oil are fluorescent and more than half of the fatty acids from Parinari oil are known to contain parinaric acid in its esterified form. Under the assay conditions used, the excitation and emission wavelengths of Parinari oil were 324 and 420 nm, respectively.

The presence of detergents (sodium taurodeoxycholate, CHAPS, Sulfobetaine SB12, Tween® 20, Brij® 35, Dobanol® and n-dodecylglucoside) above their critical micellar concentrations dramatically increases the fluorescence of the free parinaric acid released by various lipases. This increase in the fluorescence intensity is linear with time and proportional to the amount of lipase added. This new method, performed under non-oxidative conditions, was applied successfully to detecting low lipase levels in crude protein extracts from plant seedlings and could be scaled down to microtitre-plate measurements. Lipase activity can also be assayed in acidic media (pH 5). One drawback of this method, however, is the sus-
Lipid Catabolism: Lipid Degradation

The susceptibility of parinaric acid to oxidation by atmospheric oxygen. This can be overcome by adding antioxidant agents to the buffers and by performing incubation steps under an argon or a nitrogen atmosphere. In addition, this method requires the presence of a selected detergent in order to solubilize the released parinaric acid into mixed micelles; and of course, this detergent must not inhibit the lipase activity [9].

We have compared this parinaric acid-based fluorescent assay with a classical assay based on a radioactive TAG (triolein) and a fast commercial colorimetric assay based on the use of a resorufin ester as substrate (La Roche-Boehringer). The sensitivity, expressed as the minimal amount of pure human pancreatic lipase that can be detected under standard conditions (pH 8), was found to be similar in the three assays (Table 1).

We used a crude extract of germinated A. thaliana seeds expressing a very low level of lipase activity to further compare the three above-described methods. The specific activity of the homogenate, as well as the remaining activity after heating the extract (5 min at 95 °C), were evaluated using the three methods (Table 1). The plant lipase activity measured using the radioactivity and fluorescence assays can be abolished by heating the extract. However, with the resorufin assay, the change in optical absorbance detected at 550 nm using the same crude extract was not abolished when the lipase sample was heated. This indicates that the apparent activity measured on resorufin ester is likely to be due to non-enzymic hydrolysis and/or hydrolysis by other thermo-stable carboxylesterases.

To conclude, the parinaric acid-based assay exhibits a good sensitivity. In addition, it can be used to process numerous samples in a microtitre-plate assay. Contrary to the resorufin ester, parinaric acid-containing TAGs are specific substrates for lipases. The parinaric acid-based method provides a sensitive, specific and continuous assay. It is therefore a particularly promising method for measuring low lipase activities in crude biological media such as seedling homogenates.

References

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Altered membrane lipase expression delays leaf senescence
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Abstract
A cDNA clone encoding a lipase that is up-regulated in senescing leaves and flower petals has been isolated by screening an expression library. The abundance of the lipase mRNA increases as flowers and leaves begin to senesce, and expression of the gene is also induced by treatment with ethylene. Transgenic Arabidopsis plants in which levels of the senescence-induced lipase protein have been reduced show delayed leaf senescence.

Introduction
Membrane deterioration leading to leakiness and loss of selective permeability is an early and ubiquitous feature of senescence whether it occurs naturally or whether it is induced prematurely in response to episodes of environmental stress [1]. It affects both the plasma membrane and intracellular membranes, and results in loss of ionic and

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