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DIFFUSION MEDIATED VOLATILE ALDEHYDE ASSAY

Dale O. Wilson, Jr. and M. B. McDonald, Jr.¹

Introduction

Problems with vigor and storability of soybean [*Glycine max* (L.) Merr.] seeds have stimulated the adoption of vigor tests to supplement the standard germination test as a basic quality control tool in soybean seed production. Some the more accepted vigor tests suffer from excessive subjectivity (tetrazolium test) or require more than one week for completion (cold test). The attempt to combine predictive value and practical utility has resulted in a search for new biochemical vigor tests which would eliminate subjectivity and require only a day or less for completion. Many of these tests, such as the quantification of respiration rate (Woodstock 1968) or ATP (Ching 1973), have proven difficult, inconvenient and expensive to conduct (AOSA 1983).

Recent work (Woodstock and Taylorson 1981; Harman, Nedrow, Clark and Mattick 1982) has demonstrated an association between volatile aldehyde production during early germination and low soybean and pea seed vigor. The source of these aldehydes in the seed is not known although volatile aldehydes are clearly products of lipid peroxidation (Frankel, Neff and Selke 1981; Dillard and Tappel 1979) and can be produced by the action of lipoxygenase found in a wide variety of seeds (Grosch 1976). Aldehydes are formed during germination in many plant species (Stotzky and Schenk 1976) and may result from the action of hydroperoxide lyase on fatty acid hydroperoxides (Vick and Zimmerman 1967; Sekiya, Kajiwara and Hatanaka 1979). If lipid peroxidation is a primary cause of seed deterioration (Stewart and Bewley 1980; Wilson and McDonald 1985), the accumulation of hydroperoxide, which is the primary product of lipid peroxidation, may be a basic index of the physiological status of the seed and might serve as a useful index of seed vigor.

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Supplies (Figure 1)

3-Methyl-2benzothiazolione hydrazone (MBTH)	FeCl ₃ -6H ₂ O
Tetamethyl thiuram disulfide (Thiram)	Acetone
Benomyl (Benlate fungicide)	Formaldehyde
Filter paper, 9 cm Whatman #1	Neoprene stoppers #7
Erlenmeyer flasks, 500 ml	Test tubes, 16 x 150 mm
Distilled or deionized water	Parafilm
Spectrophotometer or colorimeter	

Assay Procedure

Equilibrate all seed samples to same moisture level. We used soybeans with 8% water (fresh wt. basis). This can be done by placing small paper bags of seed together in an airtight container for a week. Just before the test, dry treat the seed with a finely ground mixture of benomyl and thiram at a rate of 0.75 g of each active ingredient per one kg seed. Place nine disks of filter paper in the bottom of each flask and add 15 ml distilled water. Place 100 soybeans in each flask on top of the filter paper. Prepare a control flask by adding only the fungicide. Into each flask place a test tube containing 10 ml 0.2% MBTH solution (0.2g MBTH and distilled water to 100 ml). Seal each flask with a stopper or parafilm and incubate in the dark at 25⁰C for 24 hours (Figure 2A). Prepare a second labeled set of test tubes, each containing 2.5 ml of 0.23% ferric chloride solution (0.38 g FeCl₃-6H₂O and distilled water to 100 ml). Remove test tubes from the germination flasks and cover with parafilm. Mix the contents of each tube by inverting three times. Remove parafilm one tube at a time and transfer 1 ml of the solution into the corresponding labeled tube of FeCl₃ solution. Prepare a reagent blank by adding 1 ml of fresh MBTH to an FeCl₃ tube, cap all the tubes with parafilm and invert three times to mix. Let react at room temperature for five minutes then add 6.5 ml acetone to each. Cap with parafilm and mix by inverting several times. With the colorimeter adjusted to 635 nm, set the absorbance to zero using the blank sample and measure the absorbance of the contents of each tube within a few minutes. Preferably, run the standard curve at the same time (Figure 2B).

Standard Curve

Make up 0.002% formaldehyde as follows: Mix 2.70 ml of 37% formaldehyde solution with distilled water to make 100 ml of 1% solution. Take 2.0 ml of this solution and bring volume to 1000 ml with distilled water to make a 0.002% formaldehyde solution. Add 5 ml 0.4% MBTH solution to each of 8 test tubes. Add varying amounts of water and 0.002% formaldehyde according to Table 1. Cap with parafilm and mix by inverting 3 times. Let react at room temperature for 20

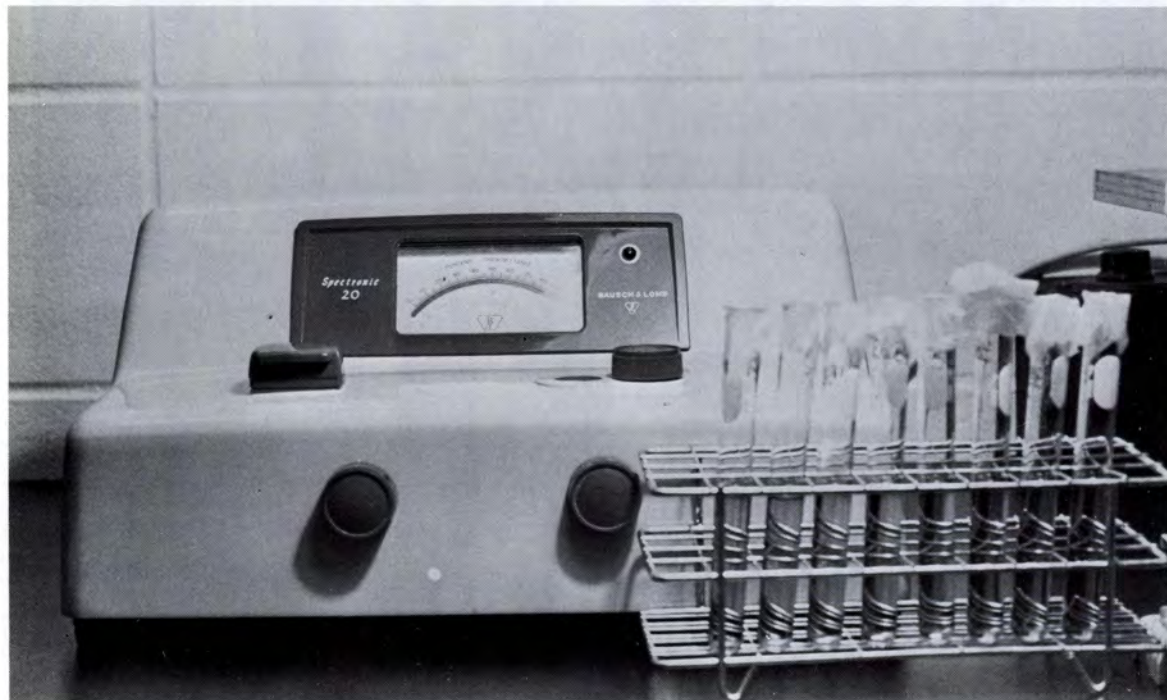
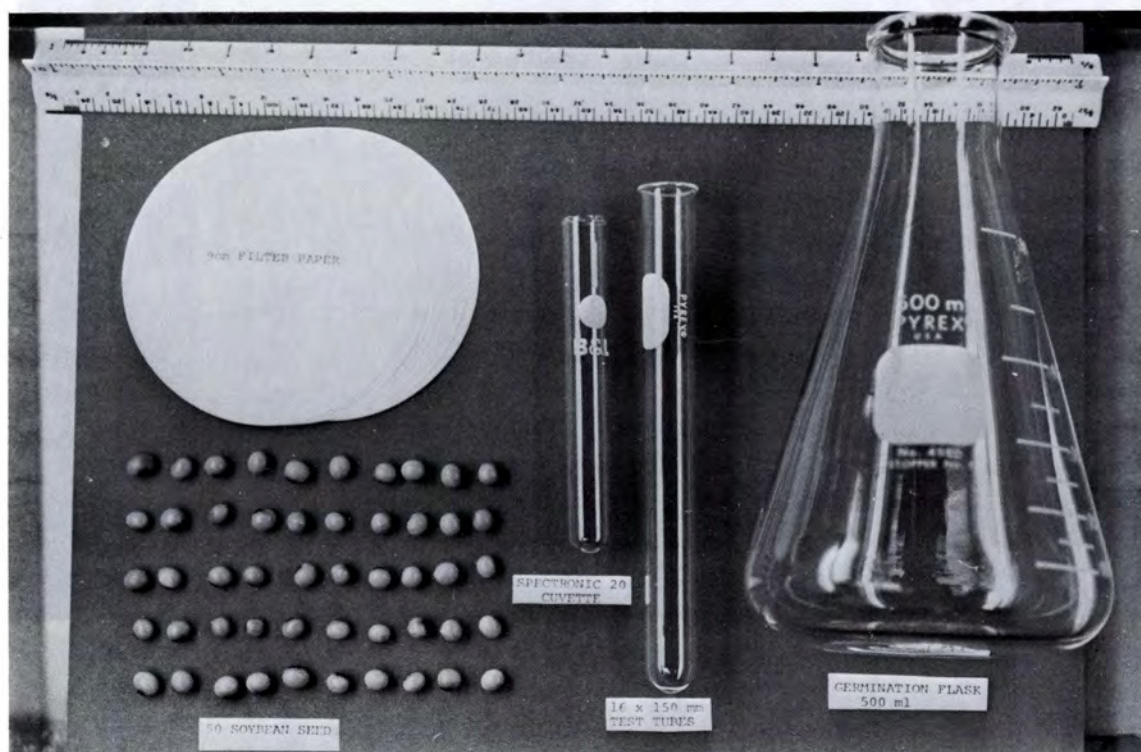


Figure 1. The basic equipment needs used for conducting the volatile aldehyde assay.

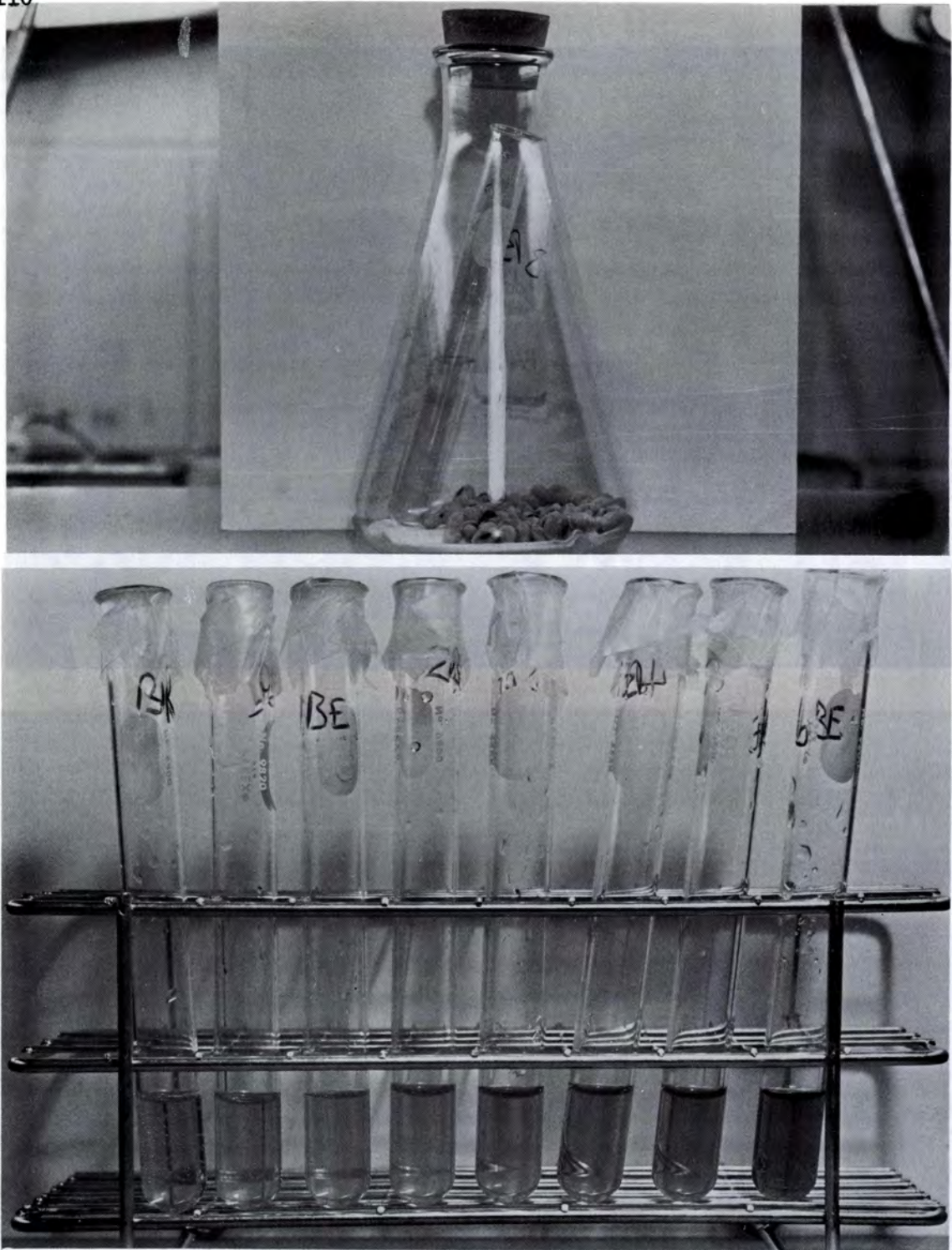


Figure 2. (above) Passive trapping apparatus used to capture aldehydes by diffusion from germinating soybean. (below) An array of solutions at the completion of the chemical test; the darker the solution color the lower the seed quality.

Table 1. Composition of the reaction tubes used to construct the standard curve during the course of the aldehyde assays.

μg Formaldehyde	Working Standard	ml	
		H_2O	0.4% MBTH
0	0	5	5
1	0.05	4.95	5
2	0.1	4.9	5
5	0.25	4.75	5
10	0.50	4.5	5
20	1.0	4.0	5
50	2.5	2.5	5
100	5.0	0	5

minutes. Transfer 1 ml from each tube into corresponding tubes containing 2.5 ml FeCl₃ solution. Mix by inverting and let sit 5 min. Add 6.5 ml acetone to each tube and measure absorbance at 635 nm using the 0 μg tube as a blank. Construct the standard curve by plotting absorbance versus μg formaldehyde. Absorbance values from seed samples can be converted to "μg aldehyde as formaldehyde" using the curve.

Preliminary results indicate that for soybeans, a capture of about 2 μg aldehyde as formaldehyde per 100 seeds is normal for high quality seed. From 4 to 6 μg indicates soybeans which, though possibly able to germinate well in the laboratory, have suffered a loss in vigor detectable by field planting or vigor tests such as accelerated aging. Samples yielding more than 8 μg aldehyde will probably exhibit a decline in laboratory germination as well as very poor field emergence.

Modifications

To increase sensitivity, decrease the amount of MBTH solution placed in the test tube. Adjust the standard curve accordingly. The whole reaction sequence could be done in a single tube, perhaps in a spectronic 20 cuvette by using smaller quantities of reagents. In the absence of a colorimeter, a color chart might be built by comparison with the standard curve using layers of colored plastic film.

References

- Assoc. Off. Seed Anal. (1983). Seed Vigor Testing Handbook. Contr. No. 32. To the handbook on seed testing.
- Ching, T. M. (1973). Adenosine triphosphate content and seed vigor. Plant Physiology 51, 400-402.
- Dillard, C. J. and Tappel, A. L. (1979). A comparison of pentane, ethane, hexanal and acetone as in vivo indices of lipid peroxidation. Lipids 14, 980-993.
- Frankel, E. N., Neff, W. E., and Selke, E. (1981). Analysis of autoxidized fats by gas chromatography-mass spectrometry: VII. Volatile thermal decomposition products of pure hydroperoxides.
- Grosch, W., Laskany G., and Weber, F. 1976. Formation of volatile carbonyl compounds and cooxidation of β-carotene by lipoxxygenase from wheat, potato, flax, and beans. Journal of Agricultural and Food Chemistry 24, 456-459.

- Harman, G. E., Nedrow, B. L., Clark, B. E. and Mattick, L. R. (1982). Association of volatile aldehyde production during germination with poor soybean and pea seed quality. Crop Science 22, 712-716.
- Sekiya, J., Kajiwara, T. and Hatanaka, A. (1979). Volatile C₆-aldehyde formation via hydroperoxides from C₁₈-unsaturated fatty acids in etiolated alfalfa and cucumber seedlings. Agricultural and Biological Chemistry 43, 969-980.
- Stewart, R. R. C. and Bewley, J. D. (1980). Lipid peroxidation associated with accelerated aging of soybean axes. Plant Physiology 65, 245-248.
- Vick, B. A. and Zimmerman, D. C. (1967). Lipoxygenase and hydroperoxide lyase in germinating watermelon seedlings. Plant Physiology 57, 780-788.
- Wilson, D. O. and McDonald, M. R., Jr. (1985). The lipid peroxidation model of seed aging. Seed Science and Technology (Submitted).
- Woodstock, L. W. (1968). Relationship between respiration during imbibition and subsequent growth rates in germinating seeds. p. 136-146. In: E. A. Locker (ed.) 3rd International Symposium on Quantitative Biology of Metabolism.
- Woodstock, L. W. and Taylorson, R. B. (1981). Ethanol and acetaldehyde in imbibing soybean seeds in relation to deterioration. Plant Physiology 67, 424-428.

PROCEDURES FOR DETECTING ENDOPHYTES
IN PLANT AND SEED TISSUE¹

Stain Test for Plant Tissue

- a. Tillers must be randomly collected; one tiller each, from a minimum of thirty plants. The more tillers taken per sample, the more accurate the test.
- b. Samples arriving in the mail should be free of contaminating fungi and other grasses such as annual ryegrass, orchardgrass and crabgrass.
- c. Freezing upon arrival will preserve samples and make subsequent peeling of tissue easier.
- d. Remove the outermost sheath from the tiller. Tissue should have no obvious discoloration from saprophytes and should have as little chlorophyll as possible.
- e. Isolate a longitudinal section of sheath approximately 3-5 mm in width.
- f. Place the section on a microscope slide and scrape gently with a scalpel. Separating the upper and lower epidermis and exposing the mesophyll tissue. Place the epidermis side down in both halves.
- g. Stain immediately with aniline blue-lactic acid stain². Allow dye to remain at least 15 seconds but no more than one minute.

¹Information compiled from various references and actual experience by the authors; Charles Sciple, Don Blasingame and M. V. Patel.

²Method of Preparation of Aniline Blue Stain:

1. Prepare a 1% w/v aqueous aniline blue solution in water.
2. Prepare a solution of 1 part lactic acid (85%) to 15 parts water.
3. Mix one part of solution 1 with 2 parts of solution 2.
4. Use stain as is or dilute with water if sections are too dark.

- h. Blot off excess dye with a tissue. Sections should remain on the slide, but may adhere to the tissue; if so, remove the sections and place them in their original position on the slide.
- i. Place a coverglass on the sections and flood with water.
- j. Examine each half of each section at 200x magnification. Score a section as positive if any identifiable hyphae are present (Figure 1).

Stain Test for Seed

- a. Seed sample should be properly collected. (Sample all of 1-5 bags, 10% of all remaining bags). Seed in bulk should be probed.
- b. Take a subsample of the seed sample (2g is sufficient).
- c. Digest seed overnight (8 hr. minimum) in a 5% solution of sodium - hydroxide.
- d. Rinse the digested seed thoroughly in running tap water.
- e. De-glume seed with forceps and place on microscope slide in a drop of aniline blue stain. Crush seed with scalpel. Wipe the scapel blade between seeds to prevent carryover of hyphae.
- f. Place coverglass on seed and squash with gentle pressure.
- g. Examine at 200x magnification, scoring a seed as positive if any identifiable hyphae are present.

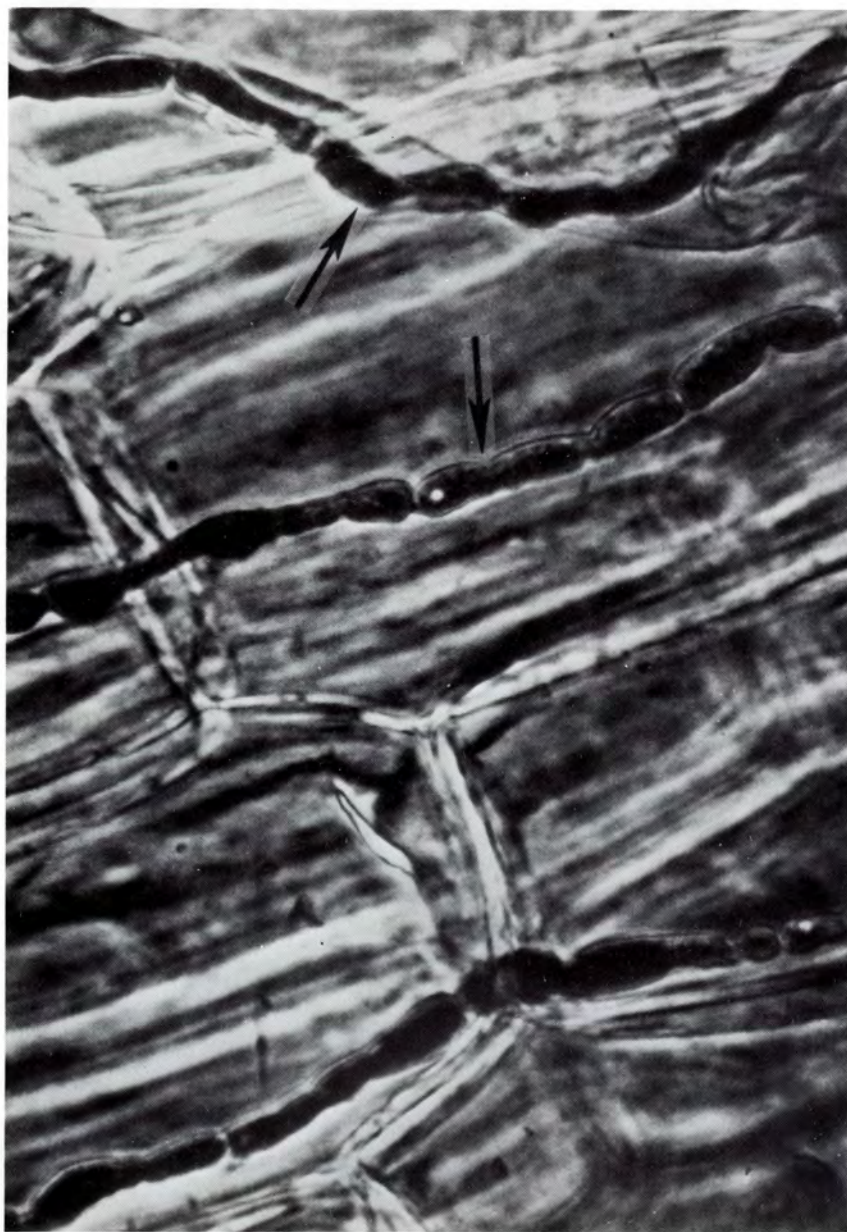


Figure 1. Hyphae of an endophytic fungus (arrows) shown in the cells of a leaf.