Vital stains for microscopy

Vital dyes are used to distinguish living from dead cells. In general, a combination of two dyes is used. One dye is excluded from living cells – it cannot cross the cell membrane because it is too large or too highly charged to penetrate. However, this dye usually can stain dead plant tissues and other material. The other dye, of contrasting colour or fluorescence, is taken up by the cell and accumulates in the cytoplasm or vacuole. Thus the cells are one colour if alive and a contrasting colour if dead.

A common combination used for light microscopy is Evans blue and Neutral red. Evans blue does not penetrate living cells, whereas neutral red accumulates in the cytoplasm and vacuole and stains living cells a pink-red colour.

Example protocol:
Stain cells or tissues with 0.1-0.5% Evans blue in aqueous solution. For delicate tissues, buffer to pH 6.5 and add appropriate osmoticum, e.g. 0.3 M mannitol.
Generally stain between 10 and 30 min, or longer for tissues with less permeable cell walls.
Rinse in water or buffer.
Stain with 0.01-0.1% Neutral red in aqueous solution. As with Evans blue staining, for delicate tissues, buffer to pH 6.5 and add appropriate osmoticum, e.g. 0.3 M mannitol.
As with Evans blue, generally stain between 10 and 30 min, or longer for tissues with less permeable cell walls.
Rinse and observe.

A common combination used for fluorescence microscopy is propidium iodide with fluorescein diacetate (FDA) or carboxyfluorescein diacetate (CFDA). Propidium iodide is non-fluorescent in aqueous solution and is charged, so cannot enter living cells with intact cell membranes. PI stains plant cell walls and cell nuclei as it is only fluorescent when it binds into certain polymer configurations, e.g between the base-pairs of DNA or between parallel strands of cellulose in plant cell walls. FDA and CFDA are also non-fluorescent in aqueous solution, and have neutral charge so they can cross the cell membrane and enter the cell. In the cytoplasm, the acetate groups are cleaved off the molecule by cytoplasmic esterases and the fluorescent molecules fluorescein or carboxyfluorescein accumulate within the living cell, often ending up in the vacuole. Neither of these fluorescent molecules are very membrane permeable, so they do not escape from the cell, at least in the short term.

Example protocol:
Stain tissues with 0.001% (10μg/ml) propidium iodide and 0.0005% (0.5μg/ml) FDA or 0.001% (1μg/ml) CFDA. (If these concentrations do not produce significant fluorescence, increase the concentrations.) Under blue illumination, all cell walls, plus nuclei in dead cells, will fluoresce red, and cell contents in living cells will fluoresce bright yellow-green after 5-10 min. To stop uptake of the dyes, rinse tissues after 15 min. Fluorescein and carboxyfluorescein become toxic if too much of the dye accumulates in the cells.
Stock solution of PI – 1 mg/ml in water stored in the fridge – lasts for a long time.
Stock solution of CFDA/FDA – 100 μg/ml in DMSO stored in fridge or freezer. Make up by adding correct amount to aqueous solution and vortexing immediately to get into solution.

CROP and SOIL NEWS/NOTES
OSU Extension Service
February, 1999
Vol. 13, No. 2

Seed Laboratory
Sabry Elias and Adriel Garay
http://cropandsoil.oregonstate.edu/newsnotes/9902/seed-lab.html

Tetrazolium Test (TZ) - A Fast, Reliable Test for Seed Viability Determination

The Need for A Fast Viability Test

The need for a quick, dependable seed viability evaluation test has become urgent to meet today's competitive seed market and the continuous dynamic changes in the seed industry. The development of the TZ test as a quick method of determining seed viability is probably the most significant advance in seed testing technology of this century. Today, the TZ test is widely recognized as an accurate, fast method of determining the viability of field crop, grass, horticultural, flower, and tree seeds throughout the world.

The TZ test results can be particularly valuable for providing fast information on the viability level of any dormant or non-dormant seeds. This information can be useful in helping growers, buyers, seed dealers and cleaners to make fast decisions.

The Principle of the Test

TZ is a biochemical test, which distinguishes between viable and dead tissues of seed embryos on the basis of dehydrogenase enzyme activity (respiration enzymes). Upon seed hydration, the activity of de-hydrogenase enzymes increases, resulting in the release of hydrogen ions, which reduce the colorless tetrazolium salt solution (2,3,5-triphenyl tetrazolium chloride) into a chemical red compound called formazan. Formazan stains living cells with a red color, while dead cells remain colorless. The viability of seeds is interpreted according to the staining pattern of the embryo and the intensity of the coloration.

Seed viability is an indication of the capability of seeds to germinate and produce a normal plant under suitable germination conditions.

The Tetrazolium Testing Handbook, No. 29, by the Association of Official Seed Analysts has detailed techniques of how-to conduct TZ test for a wide range of species, as well as guidelines for interpretation of the results.

Objectives and Applications

1. A rapid determination of viability/quality level of a seed lot.
2. A prompt evaluation of dormant seeds.
3. Timely guidance in quality control program.
4. A useful research and teaching aid in seed studies.
5. The detection of seed weaknesses before they become evident in germination tests.

Procedures

1. Hydration: seeds must be completely imbibed in order to initiate the dehydrogenase enzyme activity. This process is needed to release hydrogen ions, which reduces the colorless TZ solution to red formazan.

2. Cutting or puncturing: This process permits the access of the TZ solution to the embryos of seeds. For some species, e.g., clover, cutting is not necessary, where the TZ solution can be added to the intact seeds with a clearing agent (e.g., lactic/phenol solution).

3. Staining: After hydration, cutting and piercing, seeds are placed in a TZ solution for a certain period of time, as indicated in the TZ Handbook of AOSA, for complete coloration.

4. Evaluation: Critical evaluation of the TZ staining pattern and intensity is needed for accurate interpretation. Seed analyst should be familiar with the structure and the anatomy of the seeds in question to determine the staining pattern of the embryo (the most critical organ during germination). This will enable the analyst to determine if seeds have the capacity to produce normal seedling.

Advantages of the TZ Test

1. Provides rapid evaluation of seed viability. It is particularly useful when the time factor is critical such as for immediate shipment of seed lots without waiting for completion of standard germination tests.

2. It takes between 24-48 hours to perform a TZ test compared to 2-4 weeks, depending on the species, to break dormancy and conduct a standard germination test. So it is useful in providing a prompt viability determination of seed lots, especially those with a high level of dormancy.

In order to achieve accurate TZ results, the seeds should be evaluated by a qualified, experienced seed analyst. This will reduce the level of subjectivity in interpreting the staining pattern of seeds and increase the precision of the test results.

The tetrazolium test is expected to gain more acceptance as the testing procedures become standardized and more analysts are trained to use them. It is the advanced seed testing technology gift of the 20th century to the seed industry of the 21st century.

The determination of seed viability by TZ method is available at OSU Seed Laboratory for a wide range of field crop, grass, vegetable, flower, and tree species. The test is conducted by skilled experienced seed analysts.

For more information contact Adriel Garay or Sabry Elias at (541) 737-4464. Fax (541) 737-2126. E-mail garaya@css.orst.edu or Sabry.Elias@orst.edu

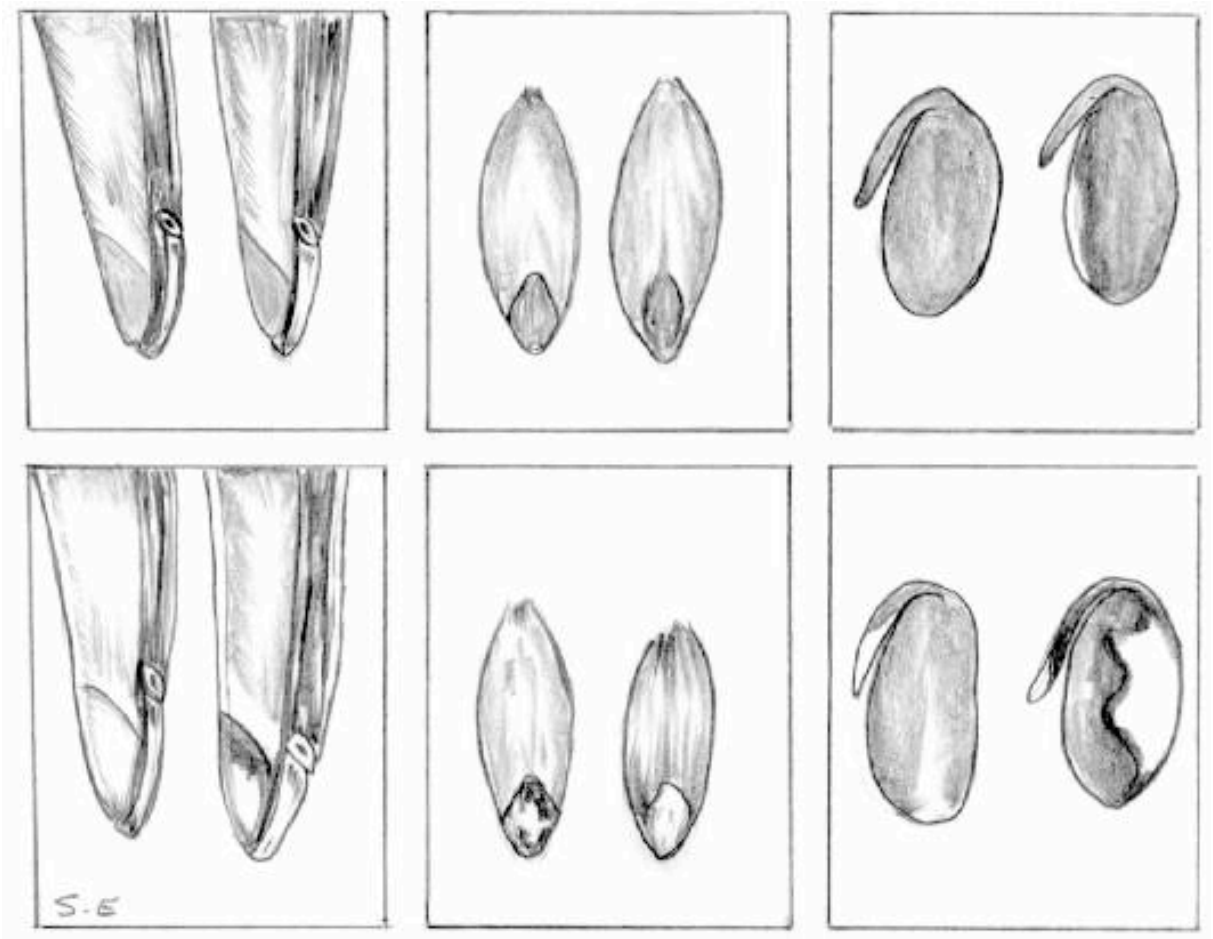

Figure 1. A. Tall Fescue, B. Bluegrass, and C. Soybean. The upper seeds are viable (germinable), embryos are entirely stained. The lower seeds are non-viable (non-germinable), Embryos are poorly developed and stained.

Jones KH, Senft JA (1985) An improved method to determine cell viability by simultaneous staining with fluorescein diacetate-propidium iodide. J Histochem Cytochem 33:77-79
A rapid, simultaneous double-staining procedure using fluorescein diacetate (FDA) and propidium iodide (PI) is described for use in the determination of cell viability in cell suspension. Air-dried slide preparations can be made from the cell suspensions so that an accurate estimate of the viability of the cells in the original suspension can be made up to 1 week later. Viable cells fluoresce bright green, while nonviable cells are bright red. Furthermore, when FDA-PI staining is compared to trypan blue dye exclusion as a method to determine cell viability, FDA-PI is found to be more consistent over prolonged periods of exposure to the dyes. Therefore, double staining with FDA-PI is a rapid, convenient, and reliable method to determine cell viability.

Class S, Braun P, Siegert A (1989) Fluorochromatic determination of pollen grain viability of Triticum aestivum L. for the evaluation of chemical hybridizing agents. [Fluoreszenzoptische Vitalitatsprufung an Pollenkornern von Triticum aestivum L. zur Bewertung pollensterilisierender Agenzien.] Angewandte Botanik 63:1-6
In a study of 7 stains, the most suitable technique was a double-staining procedure using fluorescein diacetate (FDA) and propidium iodide (PI). Viable pollen fluoresced bright green whereas non-viable pollen fluoresced bright red. Fluorescence of the cytoplasm is considerably stronger than when uranin (fluorescein sodium) is used. Staining with PI takes place over a period of 1-2 minutes in isolated pollen grains but may take up to 30 minutes in young pollen at the tetrad stage. In fully viable cells the cytoplasm continues to fluoresce for several hours under light and dark storage. Gametocidal effects were detected fluorochromatically as early as 3-5 days after application.

Zhang G, Campenot MK, McGann LE, Cass DD (1992) Flow cytometric characteristics of sperm cells isolated from pollen of Zea mays L. Plant Physiology 99:54-59
Sperm cells were isolated from maize cv. 129 Crusader pollen and purified with Percoll density centrifugation. Their flow cytometric characteristics were determined on a FACScan flow cytometer with the fluorescent dyes, fluorescein diacetate and propidium iodide. Freshly isolated sperm cells appeared as a dot cluster on the forward scatter and side scatter dot plot. This dot cluster contained 85-95% of the 10 thousand counts collected. More than 98% of cells from the cluster were fluorescein diacetate positive, with no propidium iodide positivity, indicating high cell viability. After 5 h in 15% (w/v) sucrose at room temperature (23 degrees C), scattering properties, cell number and percentage of fluorescein diacetate-positive cells remained the same. In contrast, Brewbaker and Kwack salts in 15% sucrose resulted in the emergence of a new cell population, as well as a decrease in cell number at 5 h. Further investigations with individual components of the Brewbaker and Kwack salts showed that calcium was mainly responsible for the deleterious effects. It was concluded that these results demonstrate the utility of flow cytometry as a tool to determine viability and to monitor morphological changes of plant sperm cells and to challenge current views on the ability of Brewbaker and Kwack salts to maintain viability of isolated sperm cells.

Vital staining of root hairs in 12 warm-season perennial grasses.
Oprisko MJ; Green RL; Beard JB; Gates CE
Crop Science. 1990, 30: 947-950
To distinguish between live and dead root hairs, thought useful when studying components of drought resistance, sprigs of 12 genotypes representing 6 species were grown in sand culture in the greenhouse. Vital stain effectiveness in 0.05 M PO_4 buffer (pH 7.0) was established by the percentage of genotypes in which vitality could be determined using living/dead colour differences. The effectiveness of the 5 vital stains varied widely among the 12 genotypes. The 5 vital stains (given per litre), with their percentage effectiveness over the 12 genotypes and their average absolute colour difference values (none = 1, greatest = 4), were: 0.5 g Evan's blue, 100%, 3.3; 0.1 g methylene blue, 67%, 1.9; 1 g Congo red, 58%, 1.8; 0.1 g phenosafranin, 50%, 1.7; and 0.1 g neutral red, 25%, 1.4. Thus, Evan's blue was the best stain, in terms of greatest precision and least variability.

Isolation of protoplasts from tomato fruit (Lycopersicon esculentum): first uptake studies.

Fieuw S; Willenbrink J
Plant Science (Limerick). 1991, 76: 9-17
In order to study the uptake of sugars, protoplasts were isolated from pericarp and placental tissue of immature green fruits by treatment with macerozyme, pectolyase and several cellulases. Using a flotation technique with percoll, clean protoplasts were collected which were stable for at least 24 h. Viability was determined by cytoplasmic streaming and by accumulation of fluorescein in the cytoplasm. Exclusion of Evan's blue and uptake of neutral red in the vacuole indicated the retention of selection membrane permeability during the isolation procedure. At the plasma membrane, glucose was transported preferentially and the affinity for glucose was higher in pericarp (K_m = 0.5 mM) than in placental (K_m = 1.3 mM) protoplasts, whilst V_{max} amounts were 37 and 48 nmol/mg protein per h, respectively. Investigation of the specificity of glucose uptake revealed that 2-deoxyglucose and to a lesser extent fructose were competing with glucose at the carrier binding site. Fructose and sucrose permeated into pericarp protoplasts with a lower affinity than glucose (K_m = 12 mM and 7 mM, respectively). After 15 min (^{14}C) glucose uptake, 65 and 35% of radioactivity was found in sucrose for pericarp and placental protoplasts, respectively.

Viability and guggul steroid production in immobilized tissue cultured cells of Commiphora wightii.
Prashant Phale; Subramani J; Bhatt PN; Mehta AR; Phale P
Indian Journal of Experimental Biology. 1989, 27: 4, p338-340
A cell suspension culture of C. wightii was generated from leaf-derived callus, which was developed on MS medium containing 0.2 mg/litre each of 2,4-D and kinetin. The cell suspension medium contained 0.15 mg/litre each of 2,4-D and kinetin. Cells were immobilized, using the calcium entrapment method, in early (5-day-old), mid (12-day-old) and late (16-day-old) log phase. Viability of immobilized cells was measured every 2 days using Evan's blue and fluorescein diacetate stains. Immobilized early and mid log phase suspension cultures were active in steroid biosynthesis and remained viable till day 60 (64-70% viability). The immobilized stationary phase cells were less viable showing only 40% viability within 10-12 days of immobilization but they were very active in steroid synthesis. Guggul sterone production in cell suspension culture was 4.5- to 5-times lower than in leaves of the intact plant, whereas in the immobilized cells it was 2- to 2.5-times lower than in the intact plant.

Recognition responses in pathogen/non-host and race/cultivar interactions involving soybean (Glycine max) and Pseudomonas syringae pathovars.
Baker CJ; Mock N; Glazener J; Orlandi E
Physiological and Molecular Plant Pathology. 1993, 43: 2, p81-94
Early responses leading to a hypersensitive response (HR), including the K^+/H^+ response (increase in extracellular pH and K^+) and active oxygen production, were observed in soyabean following recognition of pathogens of other host species (including P. syringae pvs tomato, pisi and syringae) and incompatible races of P. syringae pv. glycinea. Pathogens of other host species stimulated the K^+/H^+ response in leaf disc and cell suspension assays in all soyabean cultivars tested, and also stimulated the active oxygen response in cell suspension assays (observed using luminol-dependent chemiluminescence). Races of P. s. pv. glycinea stimulated these responses in incompatible interactions which lead to early cell death (monitored by Evans Blue). However, the race/cultivar compatibility under the leaf disc or cell suspension assay conditions differed in some cases from that observed in intact leaves. In all cases, the K^+/H^+ and active oxygen responses preceded hypersensitive cell death by several hours. It is concluded that the K^+/H^+ and active oxygen responses appear to occur in response to recognition by the host of pathogen stimuli in pathogen/non-host and race/cultivar interactions.

Leafy spurge (Euphorbia esula) cell cultures for screening deleterious rhizobacteria.
Souissi T; Kremer RJ
Weed Science. 1994, 42: 2, p310-315
Bioassays using cell cultures and callus tissues of E. esula were devised to evaluate the potential of rhizobacteria as biological control agents. Rhizobacteria isolated from the roots

of E. esula seedlings were screened in suspension-cultured E. esula cells. Cell viability was assessed using the Evan's blue bioassay 48 h after bacterial inoculation. Among the 30 isolates tested, LS102 and LS105 consistently caused intensive cell death, determined by measuring the A sub(630) of the inoculated cell cultures. Cell death was 2.5- and 3-fold greater in cultures inoculated with LS105 and LS102, resp., than in the control. Population levels of the 2 isolates within cell cultures and callus tissues of E. esula increased during the 1st 48 h. E. esula callus tissues were inoculated with rhizobacteria either directly or by using the Host Pathogen Interaction System (HPIS). The latter exposes calli to bacteria without any physical contact. LS102 caused cellular leakage and eventually death of the callus tissue. Callus growth was reduced by approx. 30 and 70% when exposed to LS102 and LS105, resp. It was suggested that these 2 isolates may affect E. esula at the cellular level by different mechanisms. It was concluded that a screening method based on cell cultures and callus tissues offers a good and rapid technique for detecting deleterious rhizobacteria with potential as biological control agents for E. esula.

Protocol for assessing the viability of Sphaerotheca fuliginea conidia by tetrazolium chloride (INT) reduction.
Weiersbye-Witkowski IM; Straker CJ
South African Journal of Botany. 1997, 63: 6, p498-506
The use of tetrazolium salt reduction for assessing the viability of fungal spores is reviewed, and a protocol utilizing 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) salts is described for the vital staining of spores of S. fuliginea. This protocol was compared with other methods used to assess spore viability (germination assays and Evans Blue exclusion). It is suggested that Evans Blue staining is an unreliable indicator of viability as conidia rapidly become permeable to Evans Blue during immersion. Sensitivity of conidia to immersion appears to be related to spore maturation stage, as Evans Blue inclusion by conidia still attached to the conidiophore was greater than in free conidia. In contrast, INT was taken up by viable and non-viable fungal spores. Oxidised INT is colourless and non-fluorescent, but respiratory reduction of INT within viable spores resulted in the intracellular deposition of formazan crystals. INT-formazan is bright red and has detectable fluorescence between 515 and 565 nm when illuminated with near-UV light. Variability in INT-formazan staining was related to spore developmental stage, with free conidia staining more rapidly and intensely than those still attached to the conidiophore. Fluorescing INT-formazan in viable spores incubated at 20 deg C was detected as early as 1 hour after incubation providing background autofluorescence was low. Fluorescent detection was optimal by 6 hours, and detectable increases in fluorescence stopped after 12 hours. Detection of INT-formazan under transmitted light was optimal by 12 hours, with detectable increases for up to 20 hours. It is suggested that as a time-course analysis of Evans Blue exclusion by immersed spores found that membrane integrity was compromised by 12 hours, INT viability staining is suitable for S. fuliginea spores that are intolerant of longer periods of liquid immersion.

Axillary bud banks of two semiarid perennial grasses: occurrence, longevity, and contribution to population persistence.
Hendrickson JR; Briske DD
Oecologia. 1997, 110: 4, p584-591
The occurrence, longevity, and contribution of axillary bud banks to population maintenance were investigated in a late-seral perennial grass, Bouteloua curtipendula, and a mid-seral perennial grass, Hilaria belangeri, in a semiarid oak-juniper savanna near Sonora, Texas. Axillary buds of both species were evaluated over a 2-year period in communities with contrasting histories of grazing by domestic herbivores. A double staining procedure utilizing triphenyl tetrazolium chloride and Evan's blue indicated that both viable and dormant axillary buds remained attached to the base of reproductive parental tillers for 18-24 months which exceeded parental tiller longevity by approximately 12 months. Bud longevity of the late-seral species, B. curtipendula, exceeded bud longevity of the mid-seral species, H. belangeri, by approximately 6 months. Younger buds located on the distal portion of the tiller base were 3.2 and 1.4 times more likely to grow out than older proximal buds of B. curtipendula and H. belangeri, respectively. The percentage of older proximal buds, which included comparable portions of viable and dormant buds, that grew out to produce tillers following mortality of

parental tillers was 6.0% for B. curtipendula and 8.4% for H. belangeri. In spite of the occurrence of relative large axillary bud banks for both species, the magnitude of proximal bud growth did not appear sufficient to maintain viable tiller populations. No evidence was found to support the hypothesis of compensatory bud growth on an individual tiller basis for either species. Grazing history of the communities from which the buds were collected did not substantially affect the number, status, longevity, or outgrowth of axillary buds on an individual tiller basis for either species. However, long-term grazing by domestic herbivores influenced axillary bud availability by modifying population structure of these two species. Bud number per square meter for B. curtipendula was 25% lower in the long-term grazed compared with the long-term ungrazed community based on a reduction in both tiller number per plant and plant number per square meter. In contrast, bud number per square meter for H. belangeri was 190% greater in the long-term grazed than in the long-term ungrazed community based on a large increase in plant density per square meter. Minimal contributions of axillary bud banks to annual maintenance of tiller populations in this mid- and late-seral species underscores the ecological importance of consistent tiller recruitment from recently developed axillary buds. Consistent tiller recruitment in grasslands and savannas characterized by intensive grazing and periodic drought implies that (1) bud differentiation and maturation must be remarkably tolerant of adverse environmental conditions and/or (2) tiller recruitment may resume from buds that mature following the cessation of severe drought and/or grazing, rather than from mature buds that survive these disturbances.

The use of Evan's blue stain to test the survival of plant cells after exposure to high salt and high osmotic pressure.
Taylor JA; West DW
Journal of Experimental Botany. 1980, 31: 121, p571-576
The survival of plant cells was tested rapidly and conveniently by staining tissue sections in a sol. of Evan's blue (0.5% w/v) after exposure to sol. of high salt conc. or low osmotic potential. Living cells retained the ability to exclude Evan's blue at the plasma membrane and remain their natural colour. Cells damaged by salt or osmotic stress were unable to exclude Evan's blue, stained deep blue, and were readily distinguished upon microscopic examination. The technique was tested on cells of Phaseolus vulgaris.

Protoplast isolation from Ulmus americana L. pollen mother cells, tetrads, and microspores.
Redenbaugh MK; Westfall RD; Karnosky DF
Canadian Journal of Forest Research. 1980, 10: 3, p284-289
Meiotic protoplasts of U. americana are potentially valuable for producing interspecific elm hybrids through protoplast fusion. Meiotic cells (pollen mother cells, tetrads, and microspores) were incubated in either a cellulase, hemicellulase and pectinase enzyme solution or a beta -1,3-glucanase (laminarinase) solution. Respective protoplast isolation frequencies for the three meiotic cell types were 100, 50, and 10%. Exclusion staining with 0.2% Evans blue and 0.1% methyl blue suggested protoplast viability. Some of the microspore protoplasts were vacuolated, which is an important condition for cell division. Although attempts to regenerate cell walls and induce cell division were unsuccessful, these two problems may be superceded by protoplast fusion with more regenerative protoplasts.

Relationship between vital staining and subculture growth during the senescence of plant tissue cultures.
Smith BA; Reider ML; Fletcher JS
Plant Physiology. 1982, 70: 4, p1228-1230
At 4-day intervals beginning on day 14, after cell division and expansion had stopped, cells of rose cultures, cv. Paul's Scarlet were stained separately with Evans Blue, fluorescein diacetate and phenosafranine. The degree to which parent cultures stained was compared to the dry weight of their subcultures harvested after 9 and 21 days' growth. Evans Blue and fluorescein diacetate accurately established when senescing cells died. However, the staining properties of aging cultures did not correlate well with their ability to be subcultured, because an increasing proportion of the living cells appeared to lose their ability to divide as senescence progressed.

The in vitro testing of grapevine cultivars for Oidium and Plasmopara resistance.
Stein U; Heintz C; Blaich R
Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz. 1985, 92: 4, p355-369
Spores from pure cultures of O. tuckeri (Uncinula necator) and P. viticola were used in lab. tests on 18 grapevine cultivars. Proportions of viable and damaged spores were indicated by staining with fluorescein diacetate and Evans Blue. This also allowed observation of fungus growth on the leaf surface. U. necator conidia germinated on glass slides and on vine leaves to the same extent. Germ tube growth on glass, however, stopped after 48 h and the spores died. In aqueous solution germination was depressed by c. 50%. Inoculation of whole grapevine plants in the field and in the greenhouse and of leaf discs cut from these plants in vitro led to comparable results. Most susceptible to both pathogens were the Vitis vinifera cultivars Kerner, Riesling and V 3125. Some interspecific cultivars were susceptible to U. necator, but most were resistant (Vidal, Ga 49-22, Ga 50-34). They were all moderately resistant to P. viticola, this being ascribed to the unnaturally high inoculum density used in these tests, as field resistance in this group is high.

Separation of intact and damaged plant -protoplasts by using a cell-sorter equipped with a two-channel Piezo valve chamber.
Bromova M; Knopf UC
Plant Science, Irish Republic. 1987, 52: 1-2, p91-97
Protoplasts were prepared from a suspension culture of Solanum tuberosum cv. Bintje, and either stained with fluorescein diacetate (FDA) or killed by heat treatment. Live and damaged protoplasts were mixed in equal proportions and treated with Evans Blue which stained the damaged protoplasts. Flow cytometry, using a cell-sorter equipped with a 2-channel Piezo valve chamber, separated the FDA-stained and the blue protoplasts. Further analysis showed that 75% of protoplasts remained intact after sorting.

Ethylene action inhibitors suppressed clomeprop metabolite [2-(2,4-dichloro-m-tolyloxy) propionic acid]-induced electrolyte leakage from radish roots.
Sunohara Y; Matsumoto H; Usui K; Ishizuka K
Weed Research, Japan. 1996, 41: 3, p234-240
The involvement of ethylene was investigated in the physiological mechanism of electrolyte leakage from radish cv. Shogoin roots induced by DMPA (2-(2, 4-dichloro-m-tolyloxy) propionic acid), the hydrolytic metabolite of clomeprop. Treatment of radish roots with DMPA resulted in increased electrolyte leakage from the roots, suppression of root growth and stimulation of ethylene production. Although the ethylene inhibitors, 2,5-norbornadiene (NBD) and cis-propenylphosphonic acid (PPOH), did not suppress ethylene evolution from the DMPA-treated radish roots for the first 24 h, these compounds suppressed electrolyte leakage and, to some extent, restored the growth of lateral roots. Evan's blue dye tests revealed that death of the root cells occurred 12 h after the DMPA treatment. This indicated that increase in electrolyte leakage preceded the death of root cells. These results suggest that the ethylene induced by DMPA is attributable to the leakage of electrolyte from the radish roots.

A rapid viability assay for plant shoot apical meristems.
Popov AS; Vysotskaya ON
Russian Journal of Plant Physiology. 1996, 43: 2, p263-269 (translated from Fiziologiya Rastenii (1996) 43 (2) 303-309)
The tetrazolium chloride procedure and staining with various dyes (phenosafranine, Evans blue and fluorescein diacetate) were compared to assess the viability of shoot apical meristems of potatoes cv. Simvol and strawberries cv. Gora Everest in vivo and in vitro. In some cases, tetrazolium chloride did not stain living apices, because its reduction depended on the conditions of plant culture and on meristem pretreatments with some chemicals. Phenosafranine and Evans blue stained only damaged tissues of viable apices. The most successful procedure was apex staining with 0.005% fluorescein diacetate. When treated apices were irradiated with blue-violet light, viable, but not dead, apices fluoresced. Greenish fluorescence appeared within 10 min after apex incubation in fluorescein diacetate and was noticeable for at least an hour.

An improved method for monitoring cell death in cell suspension and leaf disc assays using Evans blue.
Baker CJ; Mock NM
Plant Cell, Tissue and Organ Culture. 1994, 39: 1, p7-12
Cell viability or cell death is an important variable to monitor in many studies of host/pathogen interactions. However, for studies that focus on events within the first few hours of the interaction, many of the viability assays currently being used are either too laborious and time consuming or measure the cell's temporary metabolic state rather than irreversible cell death. Evans blue has proven over the years to be a dependable stain for microscopic determination of cell death. The stain was used to develop a spectrophotometric procedure that allows rapid, reproducible quantification of the stain retained by dead cells. This spectrophotometric procedure was used to compare plant/bacteria interactions involving either soyabean/Pseudomonas syringae pv. glycinea or tobacco/P. syringae pv. syringae. Relative increases in cell death during these interactions in suspension cell systems were measured by both the spectrophotometric and microscopic technique and found to be similar. The spectrophotometric procedure was also adapted for leaf disc assays.

Tracer dyes for following water uptake into plants

As noted above, propidium iodide does not penetrate living cells. It is also unable to permeate highly suberised walls, and can be used as a tracer dye for localised uptake across roots. Dye uptake will be blocked at the first impermeable barrier in the root. This method can be used to detect where the root endodermis first becomes impermeable to water, or where roots develop other impermeable layers in the subepidermis or cortex. Other dyes that can be used in this way include sulforhodamine G, HPTS and basic fuchsin.
Long-distance transport through the xylem can be followed with a number of dyes, including those listed above. Here, the stem is cut under water or in the dye solution, and appearance of the dye observed up the stem.