

THE TETRAZOLIUM TEST FOR SOYBEAN SEEDS





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Jose B. França Neto, Francisco Carlos Krzyzanowski,
Nilton Pereira da Costa



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Soybean seeds with different lesions caused by stink bug
(Photo: J. B. França Neto)

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FOREWORD

The evaluation of soybean seed physiological quality has been a major task for seed technologists. This is due to the influence of several factors which can affect seed quality in each phase of the soybean production system. The tetrazolium test has contributed to screening these factors and pointed out the most important ones.

Embrapa, through its National Center for Soybean Research, at Londrina, Parana, has been contributing to improve the methodology of the tetrazolium test for over 20 years, through several studies developed by the Seed Technology research team. Their objective was to provide a powerful tool to the seed industry and to seed research programs to obtain more reliable information related to the seed quality evaluation.

The result of this work along those years culminated with this publication, which is released to share the knowledge with all Seed Science community.

*PAULO ROBERTO GALERANI
Head of Research and Development
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Jose B. França Neto¹
Francisco Carlos Krzyzanowski¹
Nilton Pereira da Costa¹

1

INTRODUCTION

The production and utilization of high quality seeds of soybean [*Glycine max* (L.) Merrill] are important and basic keys for the success of the crop. To achieve these requisites, the quality control program of the soybean seed industry must be versatile and dynamic, thus promptly providing accurate results. Several determinations, such as varietal and physical purity, moisture content and level of mechanical damage, can be evaluated within minutes, thereby partially fulfilling these requirements.

The time-consuming determination of viability by the germination test presents a serious limitation to the entire process of decision making within the seed industry. In addition to this limitation, the germination test provides very restricted information that is most reliable when ideal conditions are provided for the seed. For example, the results provided by the test are frequently affected by imbibitional damage (França Neto *et al*, 1997) and can be severely influenced by seed infection by different pathogens, such as *Phomopsis* spp., *Fusarium semitectum*, and *Colletotrichum truncatum* (Henning and Franca Neto, 1980; Franca Neto and West, 1989a, 1989b). Such limitations of the test may result in serious losses to the seed producers, since they negatively affect

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the decision making with regards to several seed production practices, such as harvesting, processing, storage and commercialization.

Faster and more comprehensive results than those provided by the germination test are provided by the tetrazolium (tz) test. In addition to germination potential, the test also provides a vigor index, and reveals the causes of seed weakness, such as mechanical damage, field and storage deterioration, stink bug damage, and damage to heat and frost. The identification of the causes of seed weakness and its feedback to seed producers will enable them to make corrections to promote improved soybean seed quality in future crops.

This diagnostic feature, provided by the tz-test, is responsible for the high level of its adoption by soybean seed analysts in Brazil. Additionally, due to the publication of the first versions of the present manual (França Neto *et al.*, 1985; França Neto *et al.*, 1988), and due to the intensive training offered by seed specialists of Embrapa and other institutions, the test is performed in all seed laboratories in Brazil that deal with soybean seeds. As a consequence, Brazil is today the world leader with regards to the utilization of this test. Some numbers illustrate this leadership: one million tons of soybean seed were used in sowing 12.5 million hectares of the crop in 1997. This volume represented approximately 100,000 seed lots. Considering that 80% of these seed lots were evaluated at least twice by the tz-test, during the quality control process, close to 160,000 tz-analyses were performed in 1997, with soybean seed only.

The use of the tetrazolium test in Brazil stands not only for its quantitative aspects, as illustrated by the numbers presented above, but mainly for its qualitative features. The performance of the tz-test, in conjunction with other tests, assures that only the seed lots that effectively have good quality will be placed in the market. This fact has resulted in a more reliable seed quality control system, warranting good profits to the seed producer, by the production of high quality seed at a low cost. According to frequent feedback from several seed producers, the use of the tz-test has decreased to levels of seed lot replacement and replanting to indexes close to zero.

Several aspects about the tz-testing of soybean seed will be considered in this manual: a) the major events and accomplishments which contributed to the development and perfecting of the test; b) the basic principles of the test; c) necessary equipment and supplies; d) procedures for seed preparation and

evaluation; e) basis for the correct interpretation of the results; f) advantages and limitations of the test; and g) accuracy of the results.

2

HISTORY

“The successful development of the tz-test represents the accomplishment of many milestones in the history of seed research and in the attainment of new insights into seed life” (Moore, 1985, p. 2). More detailed reviews about the history of the tz-test were published by Cottrell (1948), Delouche *et al.* (1962), Gadd (1950), Isely (1952), Lakon (1953), Lindenbein (1965) and Moore (1962a, 1966, 1969, 1976). A brief summary of its history and major accomplishments follow.

The development of quick and accurate methods for estimating the physiological quality of seeds has been investigated by seed technologists for several years, mainly after the end of the 19th century, when the early stages of an organized seed production system were established in some European countries.

Many early testing methods used certain seed characteristics, such as color, appearance, volumetric weight, rate of imbibition, electrical conductivity, density and heat of respiration, to estimate seed viability. Nevertheless, the results obtained by these methods were not accurate. During the early 1920s the activity of certain enzymes, such as peroxidase, catalase, oxidase, reductase and phenolase received special attention, but the lack of success of enzyme detection tests was likely due to the fact that individual seeds were not evaluated. Several stains, such as indigo carmine, sulfuric acid, methylene blue, neutral red, and malachite green were also used. Here too, the lack of precision of the tests was the major problem.

As reported by Moore (1969), the first successful attempts to evaluate seed viability by vital stains were accomplished by Turina of Yugoslavia in 1922, and by Neljubow of Russia in 1925. Turina worked with the reduction of tellurium and selenium salts in seed cells, and Neljubow reported some

success with indigo carmine. Hasegawa of Japan, working with tree seed in the early 1930s, improved the application of selenium and tellurium salts in the staining of seed embryos. Most of his work was published in Japanese, thus making his achievements inaccessible to most of the scientific community. Part of his studies was widely publicized after he released some of his findings in English (Hasegawa, 1935), and in German, during a meeting of the International Seed Testing Association in Europe. During this trip, Hasegawa revealed certain details of his testing procedures to the German scientist, F.E. Eidmann, who also improved the selenium method (Moore, 1969).

Lakon, from Hohenhein, Germany, who had shown a great interest in seed physiology since the early 1920s, perfected the selenium method developed by Hasegawa and Eidmann, culminating with the development of the topographical selenium method (Lakon, 1940). After he realized the poisonous characteristics of selenium for laboratory use, Lakon searched for a similar but non-toxic salt that could be used for the same purpose. After Kühn and Jerchel (1941), as pointed out by Cottrell (1948) and Isely (1952), first called attention to the reduction of tetrazolium compounds in living tissues, Lakon tested several of these salts and concluded that 2,3,5, triphenyl tetrazolium chloride was the most appropriate for the topographical test. Lakon developed his test on several cereal crops and corn.

As reported by Moore (1976), knowledge of the existence and merits of the tz-testing was first received in America in 1945 from U.S. Military personnel, who investigated research activities in Germany after World War II. The first research work with the tz-test conducted in the U.S. was published by Porter *et al.* (1947), from Iowa State University. Other pioneer studies in America, as pointed out by Moore (1976), were published in 1948 by Flemion and Poole, from the Boyce Thompson Institute of Yonkers, New York; by Goodsell, from Pioneer Hi-Bred Corn Company of Johnston, Iowa; and by Bennett, from Iowa State University.

Substantial achievements and improvements on the tz-test were achieved during the 1950s. Several researchers from different U.S. universities made outstanding contributions. They include Isely, Bass, Smith and Throneberry, from Iowa State University, and Parker, from the University of Idaho.

In the 1960s important developments concerning the practical application of the tz-test were obtained by Delouche, Still, Raspet and Leinhard, from Mississippi State University, who published the first tz-test handbook for a large number of seed species (Delouche *et al.*, 1962). Jensen, Pierpoint, Hayes and Grabe, from Oregon State Seed Laboratory, and Copeland, Bruce and Midyette, from Virginia, also provided important improvements to the test.

In 1970 another milestone was achieved. The use of the tz-test was accepted by the Association of Official Seed Analysts (AOSA), with the release of the “Tetrazolium Testing Handbook” (Grabe, 1970). In 1983 the AOSA published the “Seed Vigor Testing Handbook” (AOSA, 1983), which compiled important information about the methodology of the tz-test for soybean, cotton, corn, and wheat.

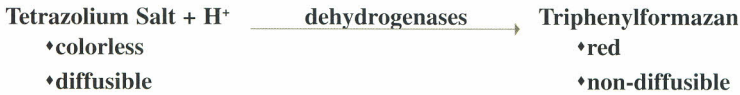
Special recognition and tribute are due to Dr. Robert P. Moore from the North Carolina State Seed Laboratory. Between 1955 and 1985 he released more than 230 publications about the tz-test and edited the outstanding “Handbook on Tetrazolium Testing” (Moore, 1985), published by the International Seed Testing Association. This publication contains details and procedures for the application of the test to more than 650 species.

The tz-test was also successfully accepted and used in several other countries. The test was introduced in Brazil by several seed technologists that were trained at Mississippi State University. The test was perfected for soybean seed, a very important crop to Brazil, by Seed Technology specialists at Embrapa Soja, National Center for Soybean Research, who published three manuals containing specific procedures for the test with seeds of this species (França Neto, 1981; França Neto *et al.*, 1985; 1988). Today, the tz-test is routinely and successfully employed in all seed laboratories that works with soybean in Brazil.

3

PRINCIPLES OF THE TEST

The tz-test relies on the activity of dehydrogenase enzymes (AOSA, 1983; Bulat, 1961; Copeland *et al.*, 1959; Moore, 1973; Smith, 1952; Smith and Throneberry, 1951), which catalyze the reactions in glycolysis and the citric acid cycle. These enzymes, particularly malic acid dehydrogenase, carry out the reduction of the tz-salt (2, 3, 5-triphenyl tetrazolium chloride - TTC) in living tissues. When a soybean seed is immersed in the colorless TTC-solution, the TTC penetrates into the seed tissues, where it interferes with the reduction processes of the living cells by accepting a hydrogen ion. In the reduced form, the TTC-salt is a red-colored, stable, non-diffusible substance, called triphenylformazan.



When triphenylformazan is formed in the seed tissue, it means that there is respiratory activity in the mitochondria in the cells of the seed tissue, which is concluded to be alive. Therefore, the resulting red color in the seed tissue is a positive indicator of its viability, by indirectly detecting respiratory activity at the cellular level. Non-viable seed tissues do not react with TTC, and consequently do not stain.

If the tissue is vigorous, a normal faint red color will result; if it is weak, an intensive red will develop, due to an intensive diffusion rate of the TTC-solution through the damaged cell membranes of the seed tissue; if it is dead, no reduction will occur, and the dead tissue will contrast as white (non-colored) with the stained living tissue. These color differences, together with the knowledge of several seed features, permit an assessment of the presence, location, and nature of disturbances within embryo tissues (Moore, 1973).

4

EQUIPMENT AND SUPPLIES

The following equipment and supplies are needed to perform the test:

a) reagent:

- ♦ tetrazolium salt: 2, 3, 5-triphenyl tetrazolium chloride, normally available in 10-g-flasks;

b) plastic and glassware:

- ♦ Petri dishes;
- ♦ 50-ml beakers or plastic cups;
- ♦ dark plastic or glass bottles, to store the TTC-solution, which is reduced under light.

Important: the use of metallic flasks must be avoided, since the TTC-salt might be reduced when in contact with certain metals (Bulat, 1961).

c) single edge razor blades;

d) staining oven or germinator, with temperature capability of 35°C to 40°C;

e) magnifying lens (6X) with fluorescent lamps, preferably circular;

f) a refrigerator for storage of the TTC-solutions and stained samples;

g) germination paper.

TTC solutions

The use of a 0.075% (v/v) solution of the tz-salt is recommended, since the solution at this concentration results in an adequate staining of the seeds and permits the seed analyst to visualize recently occurred mechanical damage, in the form of bruises, that normally would not be detected if less dilute solutions (0.5 to 1.0%) were used, as recommended in the international literature (AOSA, 1983; Delouche *et al.*, 1962; Grabe, 1970; Moore, 1985). In addition, the use

of a 0.075% solution is cost saving: 200 seed samples of 100 seeds each can be analyzed with only 10 g of the salt, as compared to merely 15 samples if a 1.0% solution were used.

A 1.0% (w/v) stock solution is initially prepared by diluting 10.0 g of the tetrazolium salt in 1.0 liter of distilled water. This solution must be stored in dark bottles, in a dark and cool environment, preferably in a refrigerator.

As needed, the 0.075% working solution is prepared. This solution must be stored with the same care as for the working solution.

1.0 liter of the 0.075% solution = 75 ml stock solution (1.0%) + 925 ml of H₂O.

The water used for the working solution may be either distilled or normal tap water, if its pH is between 6 and 7.

Seed Preparation

♦ Seed Sampling

The Rules for Testing Seeds prescribe for the germination test (in sand or in rolled paper towel), the use of 400 seeds per seed sample (8 subsamples of 50 seeds each). However, the use of 100 seeds (2 subsamples of 50 seeds each) is suggested for the tetrazolium test in soybean (AOSA, 1983; Moore, 1973; França Neto, 1981; França Neto *et al.*, 1985; 1988). The need for the use of a reduced number of seeds for the tetrazolium test is due to the homogeneous conditions that the seeds are subjected to during preparation and conditioning. This is not always the case in the germination test, which may be influenced by several sources of variation, such as: moisture and temperature gradients in the germinator; pH and texture of the substrate (sand or paper); differences in the amount of water added to the substrate; and the presence of certain seedborne pathogens (*Phomopsis* spp. and *Fusarium* spp.). Additionally, in the tz test, each seed is analyzed individually and this is not the case with the germination test.

♦ **Seed Preconditioning**

Seeds are kept overnight in a moist germination paper towel at 25°C for 16 h. This operation permits the seed to imbibe water slowly, thus activating the germination process. During preconditioning, wrapped seeds in moist paper must remain in a saturated environment, such as in a germinator a plastic bag or desiccator, with a layer of water instead of silica gel.

The seed coats of dark coated varieties of soybean are impermeable to the tz-solution. Therefore, seed coats must be removed from these seeds after preconditioning and before staining begins.

To speed up this process, an alternative methodology may be used, as suggested by Costa *et al.* (1998): precondition the seeds for 6 hours at 41°C. This method reduces seed preparation in 10 hours, without sacrificing the precision of the obtained results.

♦ **Staining**

After conditioning, the seeds are placed in plastic cups or beakers, and covered with the 0.075% tz-solution. These cups are then placed in an oven or germinator at 35 to 40°C for 150 to 180 minutes. This temperature is achieved with the use of an oven or a germinator. Special care must be exercised to store the tz-solutions and to stain the seeds in darkness, since the reagent is light-sensitive (Lakon, 1949).

♦ **Sample Rinsing**

After staining, the seeds are rinsed with tap water several times to stop the staining reaction. It is important to keep the seeds submerged in water to avoid dehydration. Stained seeds can be kept in a refrigerator for a period of up to 12 h before they are evaluated.

5

SEED EVALUATION

Unlike the requirement of very simple and inexpensive equipment and supplies, the tz-test demands the expertise of a well-trained seed analyst. Knowledge of seed and seedling structures is a major element required of the analyst. Experience, judgment and perhaps imagination are also necessary for the analyst to visualize the kinds of seedling abnormalities revealed by the tz-test. Accuracy and reliability are dependent upon the knowledge of all test techniques.

As pointed out by Moore (1985), there are three basic objectives of seed evaluation: a) to determine the germination potential of a seed lot under the most ideal conditions; b) to stratify the seed into different categories of viability, in order to report a vigor test rating; and c) to diagnose the possible causes of seed weaknesses that resulted in the loss of viability. The first two objectives can be achieved by the interpretation of four basic characteristics: condition and color of the tissue after staining, and position and extent of damage. The ability of the seed analyst to recognize the symptoms of different kinds of damage is imperative for the correct diagnosis of the causes of loss of viability.

Individual seeds are examined through a magnifying lens (6X to 10X) under fluorescent light. A single edge razor blade is used to cut the seed through the seed coat and longitudinally through the midsection of the embryonic axis (Fig. 1). Care must be exercised to section the embryonic axis exactly at its midsection. If the cut is off-center, the evaluation of the condition of the embryonic axis should be made on the seed half which contains the larger portion of the axis, exposing its midsection after additional slicing.

After the seed is sectioned, the seed halves are separated and the seed coat is removed to expose the outer surface of the cotyledons. The analyst should observe the inner and outer surfaces of the cotyledons, searching for all types of defects.

Special care must be applied during the evaluation of the radicle-hypocotyl axis. This axis is composed of two kinds of tissues: the **cortex**, and the **stele, or vascular cylinder** (Fig. 2). The stele is the most critical structure

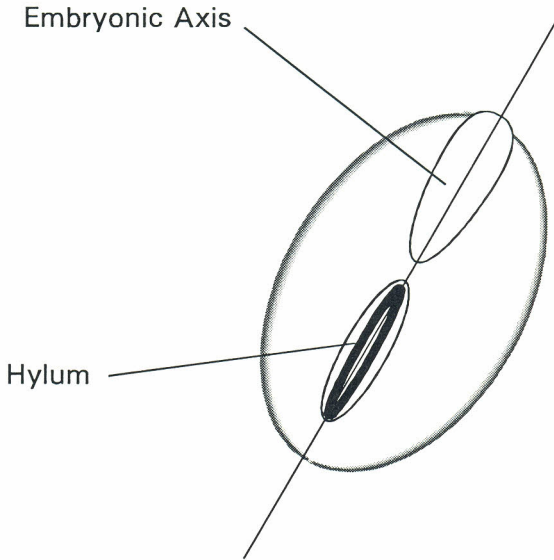


FIG. 1. Illustration of cutting site, through the midsection of a soybean seed.

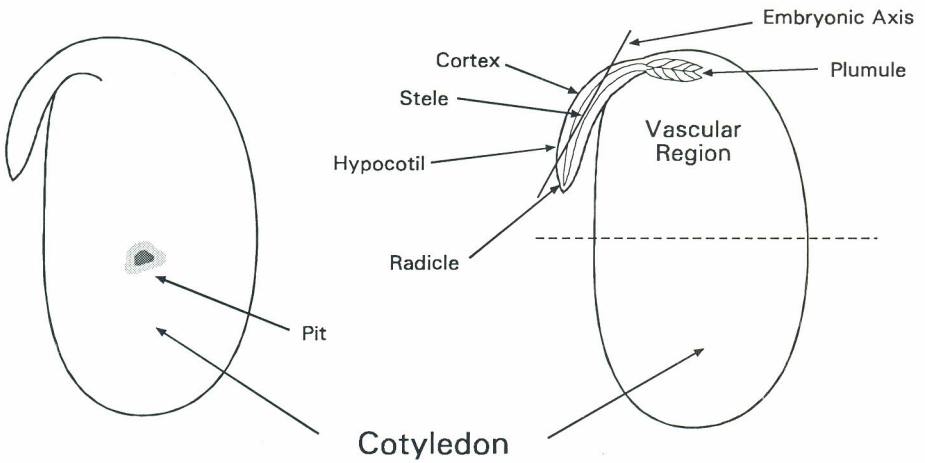


FIG. 2. Structures of an imbibed soybean seed with the seed coat removed.

of the radicle-hypocotyl axis. As a general rule, if damage occurs on this axis, but is not deep enough to injure the stele, the seed may be considered viable. However, if the damage harms the stele, then the seed is rated as non-viable (Fig. 3).

In addition to the stele, another critical region of the seed must be observed with extra care: the **vascular region** (Figs. 3 and 4). Through this region there are minute vessels that connect the embryonic axis to the cotyledons, which transport reserve material from the cotyledons to the developing seedling, in the initial stages of germination and emergence. If this region is injured, seed vigor and/or viability may be compromised.

Other factors that must be observed are the differential colors of the seed tissues:

- ♦ faint red carmine: vigorous and healthy tissue;
- ♦ dark red carmine: deteriorating tissue;
- ♦ white: dead tissue.

According to Moore (1985), sound tissues tend to stain gradually and uniformly from the exposed surface inward (Figs. 5 and 6). A high level of turgidity is present in moist tissues. Dark red carmine is typical of weak tissues,

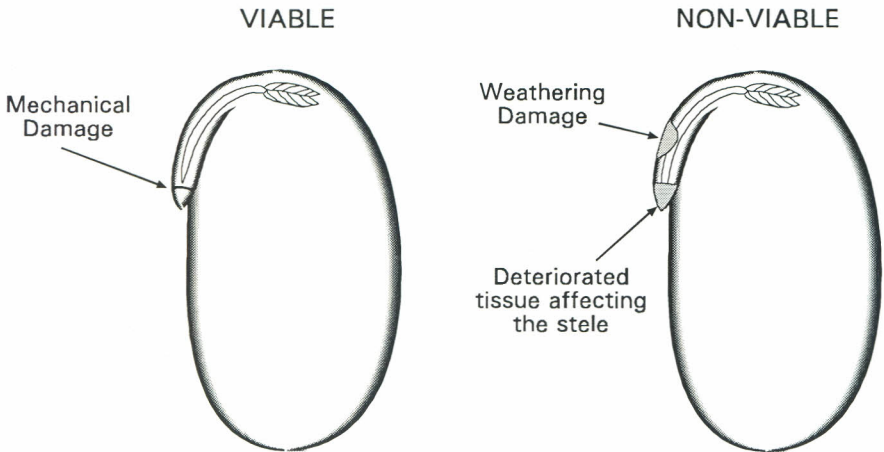


FIG. 3. Illustration of different severities of damage to the radicle-hypocotyl axis.

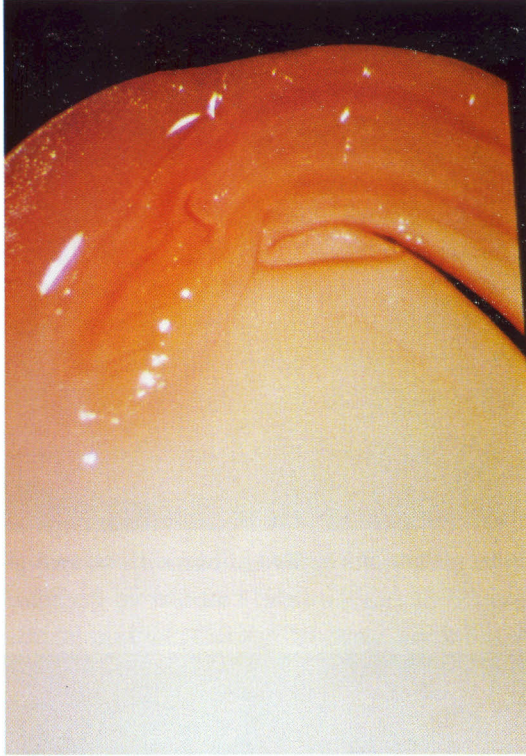


FIG. 4. Close-up of the vascular region and plumule of a vigorous soybean seed. Note the meristematic region and the cortex and stele of the hypocotyl. (Photo: J. B. França Neto).

which are in the process of deterioration. Upon exposure to dry air, these tissues will lose turgidity more rapidly than vigorous tissues. White identifies dead tissue, which lacks the enzymatic activity necessary for the production of triphenylformazan. Dead tissues are usually flaccid and commonly chalky-white, but may show yellowish, greenish or grayish tones, especially when the tissue has suffered stink bug damage. On some rare occasions, some dead tissues may show a mottled red intensity, due to high microbial activity. However, these tissues are easily differentiated from weak tissues due to their extreme flaccidity and friability.

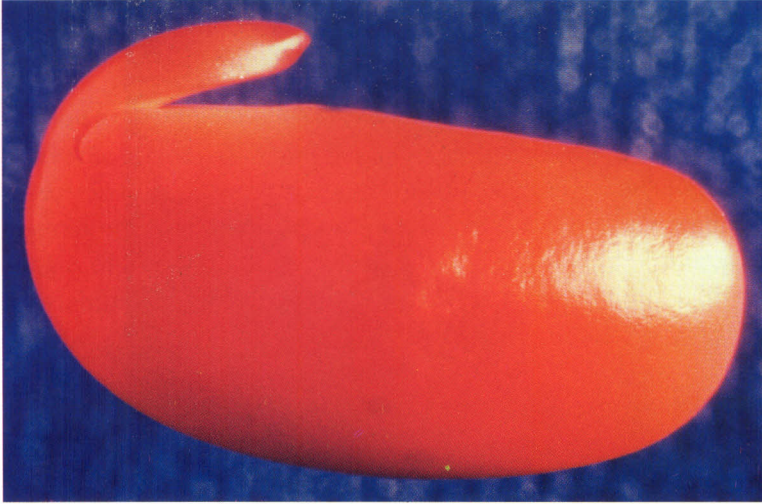


FIG. 5. Typical staining pattern of a highly vigorous soybean seed. (Photo: J. B. França Neto).

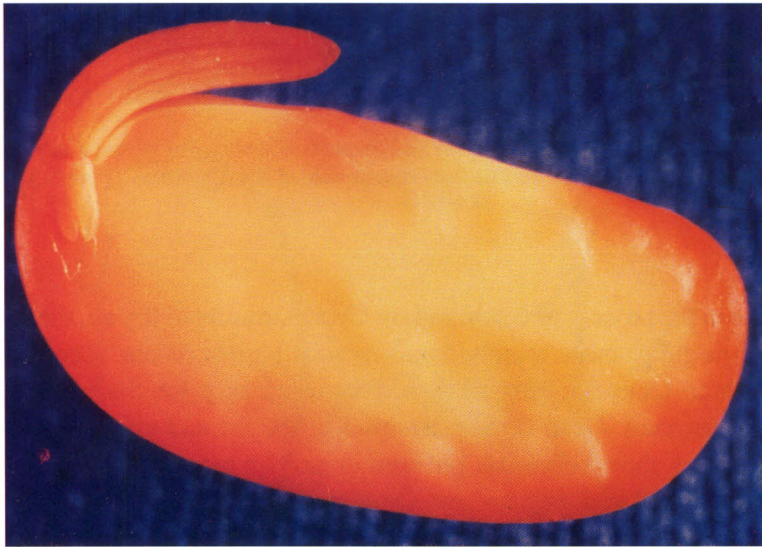


FIG. 6. Staining pattern of the internal surface of a highly vigorous soybean seed. (Photo: J. B. França Neto).

It should be emphasized that after the seed is sectioned, the internal surfaces of live cotyledons are normally white, due to the lack of diffusion of the tz-solution to the inner tissues of the cotyledons (Fig. 6). Moore (1985) reported that viable non-stained tissues are usually turgid, lustrous and pinkish-or yellowish-white.

The position and extent of a certain damage are characteristics of crucial importance for the correct evaluation of the seed and must be considered in combination. For example, in soybeans, a small area of damage caused by the feeding of a stink bug on the hypocotyl, which damages the stele, will result in more serious consequences than a large area of damage by the stink bug feeding on the lower half of a cotyledon, far away from the embryonic axis.

5.1. Diagnosis of the Causes of Soybean Seed Deterioration

Several factors contribute to lowering the quality of soybean seed. The major ones, as described by Moore (1960, 1962a, 1973) and by Franca Neto (1984) are mechanical and weathering damages, stink bug damage, heat and drought damage, drying damage and freeze injury. Each kind of injury is associated with very typical staining patterns and tissue characteristics, that are briefly illustrated below.

♦ **Mechanical damages** (Figs. 7 to 12) result from physical impacts, especially during harvesting, threshing, processing, drying and sowing of soybean seed. There are three types of mechanical injury which are easily identified by the tz-test: cracks, splits and bruises. The latter is typically identified by the presence of dark-red speckles, if of recent occurrence, or by white and flaccid tissues, if not recent. It is very common for an inexperienced analyst to mistake the pit (Fig. 2) for a mechanical injury. The pit is composed of a group of specialized cells on the abaxial surface of the cotyledons in direct opposition to the seed coat antipit, an enlarged layer of cells on the ventral surface of the seed coat (Yaklich *et al.*, 1984, 1986).

♦ **Weathering damage** (Figs. 12 to 19), as described by Moore (1973), Franca Neto (1984) and Pereira and Andrews (1976), is the result of the exposure of

soybean seeds to alternate wetting-drying cycles before harvest. Damages will be of greater magnitude if these conditions occur in warm environments. Weathered seeds often show characteristic wrinkles on the cotyledonary region opposite the hilum, or on the hypocotyl axis. If stained, seeds will reveal the presence of dark-red and white patches on embryonic tissues just beneath these wrinkles. Frequently these lesions are associated with infection by certain fungi. The lesions can be deep, and if the stele is damaged, or if more than 50% of the cotyledonary tissue is destroyed, the seed is considered non-viable. A very typical characteristic of most weathered seeds is the symmetry of the lesions on both seed halves (Figs. 13 and 14).

♦ **Stink bug damage** (Figs. 20 to 22) can seriously affect the soybean seed quality. Among the species of stink bugs that attack soybean, the southern green stink bug, *Nezara viridula* (L.), is generally found in most soybean producing fields (Turnipseed and Kogan, 1976). Other species, such as *Piezodorus guildini* (Westwood) and *Euschistus heros* (Fabricius), are also reported to cause severe damages to soybean seeds. When stink bugs feed on soybean seeds they also inoculate them with the yeast fungus *Nematospora coryli* Peglion (Sinclair, 1982). Colonization of this fungus in seed tissue often causes severe losses of seed viability and vigor (Bowling, 1980; Villas Boas *et al.*, 1982). This infection results in characteristic circular, sometimes shrunken and deep lesions. Lesioned tissues are dead and flaccid and appear typically white, or sometimes greenish, yellowish, or grayish white. A distinct dark-red boundary commonly exists between damaged and sound tissues (Fig. 22). Multiple lesions on a single seed might occur, and if they overlap, the typical circular wound will not be distinguishable. Frequently, a minor puncture caused by the insect can be noticed in the center of the circular lesion. Deep punctures by the insect might result in inoculation of central seed tissues by *N. coryli*. Therefore, colonization of the tissues by the fungus will cause internal damage that is not always revealed on the outside of the seed.

♦ **Heat and drought damages** (Fig. 23) are found in seeds of certain cultivars, when high temperatures (above 30°C) and drought occur during the seed-filling period. Visible symptoms on dry seeds are variable. Typical lesions may range from as little as a dimple on the cotyledonary area opposite the hilum, to completely shriveled and distorted seeds. Some seeds produced under these

stressful conditions may become impermeable to water. When dimpled seeds are stained, dark-red or white patches occur in the lesioned tissues, and can be mistaken for weathering damage. The presence of dimples on dry seeds will help the analyst to avoid misinterpretations. Shriveled seeds, after staining, might be deformed and reveal white and dark-red patches scattered over the cotyledons, with a higher concentration on the upper half of the seed, close to the embryonic axis (Fig. 23). Dead tissues are flaccid and friable. Severely shriveled seeds will not germinate due to a complete crushing of the embryonic axis tissues and upper cotyledons.

♦ **Excessive drying damages** result from drying the seeds to moisture contents below 10.0%. They are characterized by the presence of high levels of soybean seeds with transversal cracks in the cotyledons, always in the same position (Fig. 24). Excessively dried soybean seeds are susceptible to break in this location, when subjected to a mechanical impact.

♦ **Freeze injury** in soybean seeds, as described by Moore (1973), will vary with the stage of development of the seed when exposed, temperature and duration of frost period. Immature seeds are generally killed and remain green; dry and mature seeds tend to resist damage. Damaged tissues are identified with the tz-test by embryos being darker red than normal and by a tendency to release leachate that produces a red precipitate, which accumulates within seed coats and in the testing solution. Freeze injured tissues, as revealed by Osorio (1987), tend to develop a greenish- or brownish-red appearance.

The intensity of the above described lesions may intensify during storage. The rate of this intensification is dependent on the conditions of temperature and relative humidity during storage. Mechanical and weathering damages are the types of injuries that most commonly progress during storage, resulting in severe reductions in vigor and viability, markedly during the final weeks of storage.

More than one of the above described defects can be observed on a single seed (Fig. 12). Other damages are associated with ageing and poor storage conditions. Warm and humid conditions during storage will result in severely damaged seeds. With the tz-test the symptoms of improper storage are similar to the symptoms for weathering. The correct diagnosis of the cause of seed

deterioration can be achieved by conducting a bio-assay, such as the blotter test. High levels of infection by storage fungi, such as *Aspergillus* spp. and *Penicillium* spp., will be found on seeds with storage problems, while *Phomopsis* spp., *Fusarium* spp., *Cercospora kikuchii*, or other field pathogens will normally be found on seeds with weathering damage.

The identification of these causes for seed weaknesses might seem complex with first reading, but with good training and experience the seed analyst can readily recognize and differentiate these symptoms.

5.2. Identification of Levels of Viability

The tz-test is based upon the analysis of the condition of individual seeds. Each seed is rated as viable or non-viable and the causes for seed weaknesses are recorded. Moore and Smith (1956), as cited by Copeland *et al.* (1959), and Moore (1961, 1962b, 1967), have worked out a relative classification for corn and soybean seed. Each seed was assigned a soundness rating of 1 to 5, if viable, and of 6 to 8, if non-viable. The presence, location and nature of staining and the physical condition of embryo structures are used in this classification. This methodology was modified and described in detail for soybean seed by França Neto *et al.* (1985, 1988).

A description of several situations for each category follows. The illustrations were prepared from seeds stained in a 0.075% (v/v) tz-solution. Each diagram presents a seed that was sectioned longitudinally. The exterior of the seed is exemplified on the left and the interior on the right.

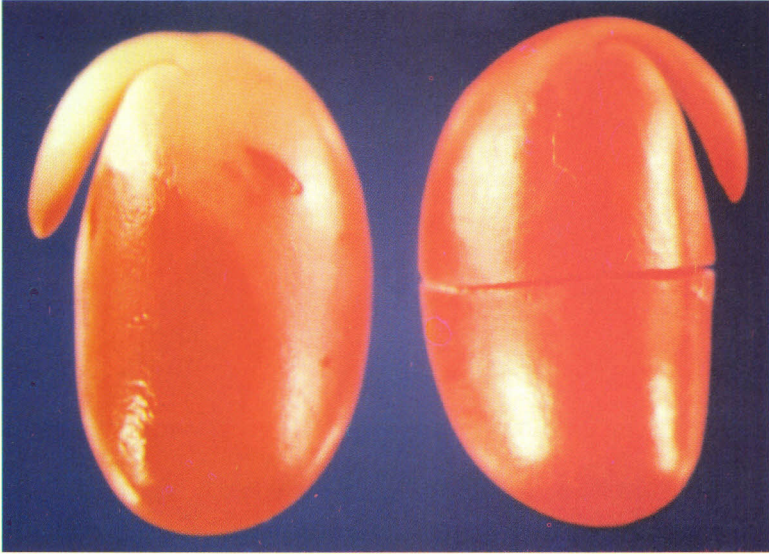


FIG. 7. Soybean seeds with typical lesions of mechanical damage. Left: seed with latent damage (bruised seed). Right: immediate damage (cracked seed). (Photo: J. B. França Neto).

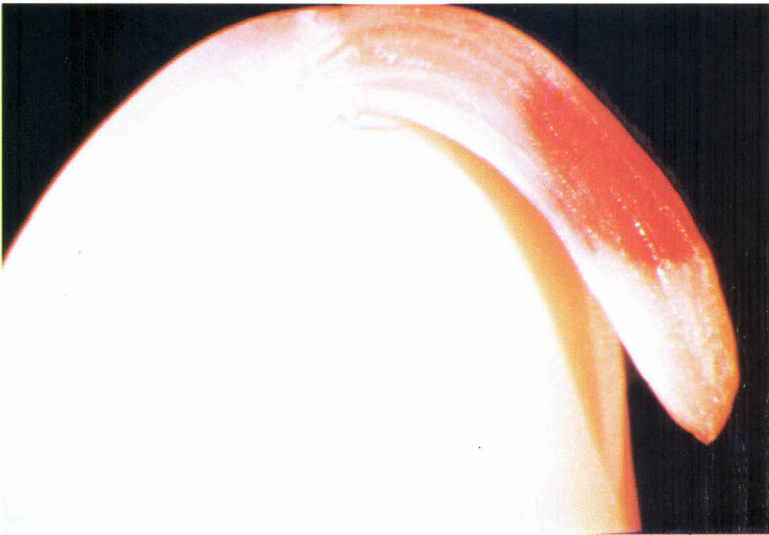


FIG. 8. Critical mechanical damage on the radicle-hypocotyl axis, reaching the stele. (Photo: J. B. França Neto).

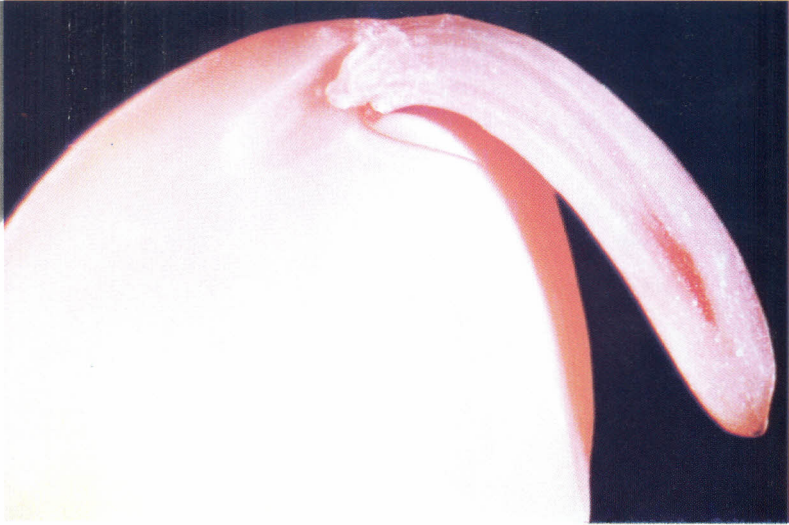


FIG. 9. Bruised hypocotyl due to mechanical impact, reaching the stele. (Photo: J. B. França Neto).



FIG. 10. Mechanical damage affecting the meristematic region of the plumule. (Photo: J. B. França Neto).

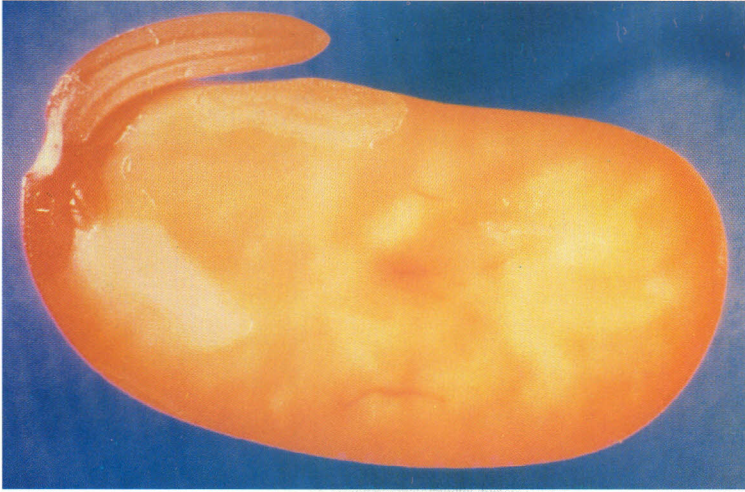


FIG. 11. Minor mechanical damage affecting a vital region of the stele and plumule. (Photo: J. B. França Neto).



FIG. 12. Soybean seed with typical lesions of mechanical damage on the radicle and cotyledon and superficial weathering damage on the cortex of the hypocotyl. (Photo: J. B. França Neto).



FIG. 13. Soybean seed with typical lesion of weathering damage. Note the symmetry of the lesions and the symptoms on the embryonic axis. (Photo: J. B. França Neto).

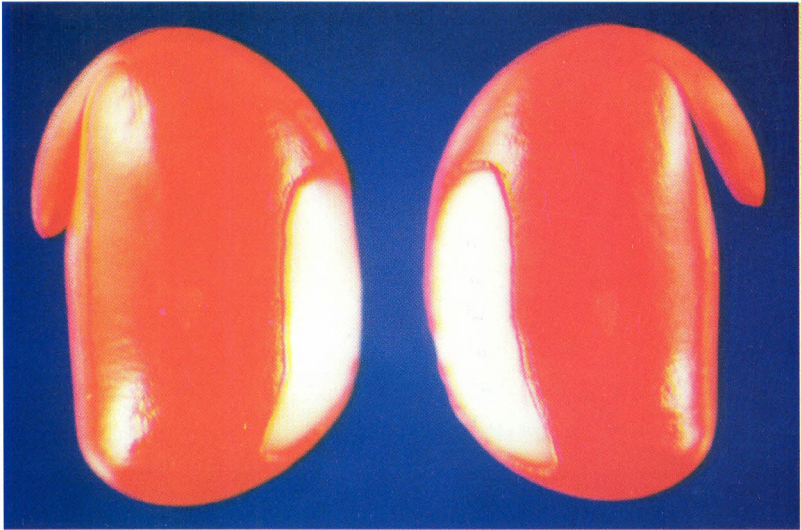


FIG. 14. Soybean seed with typical lesion of weathering damage. Note the symmetry of the lesions on the cotyledons. (Photo: J. B. França Neto).

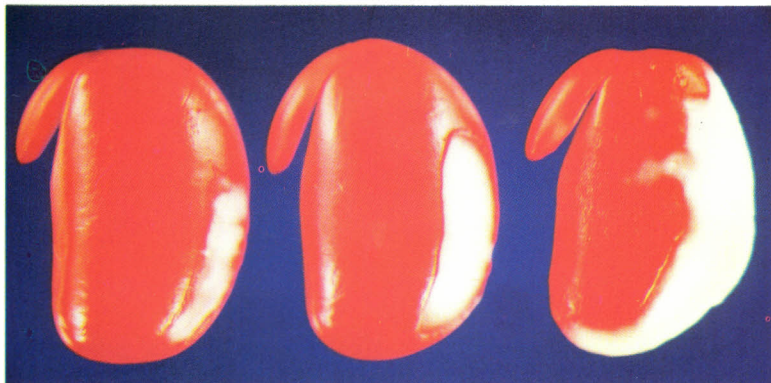


FIG. 15. Soybean seeds with three levels of weathering damage. (Photo: J. B. França Neto).

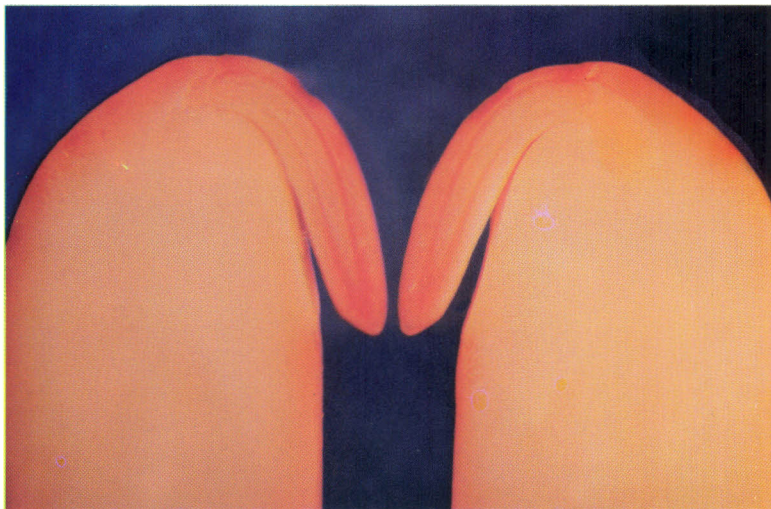


FIG. 16. Soybean seed with superficial lesions of weathering damage on the cortex of the hypocotyl. (Photo: J. B. França Neto).

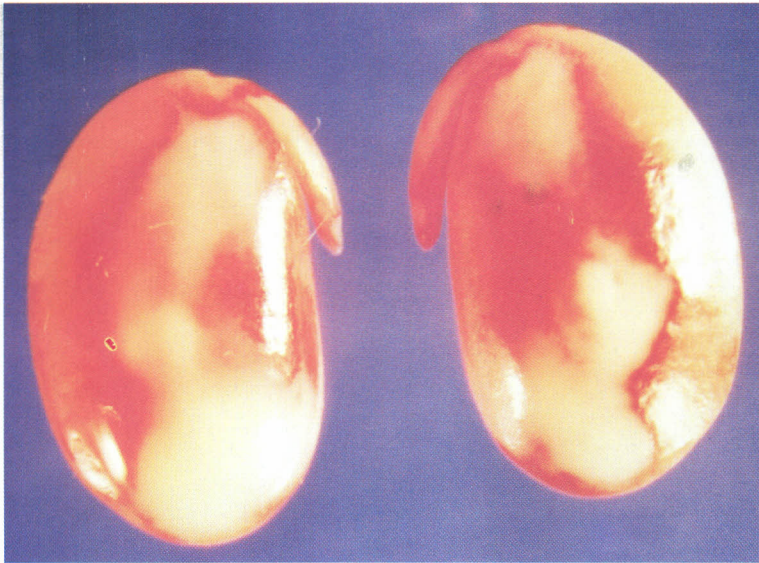


FIG. 17. Severe lesions of field weathering on soybean seeds. (Photo: J. B. França Neto).

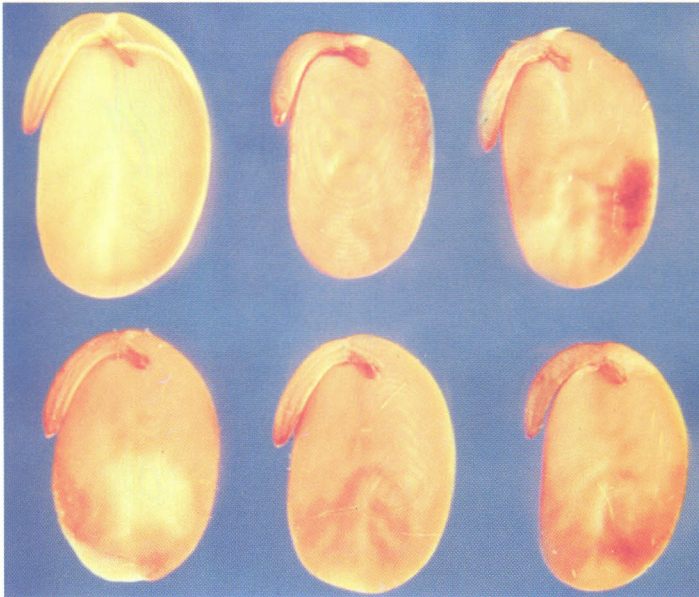


FIG. 18. Stained plumules of soybean seeds due to intense field weathering. (Photo: J. B. França Neto).

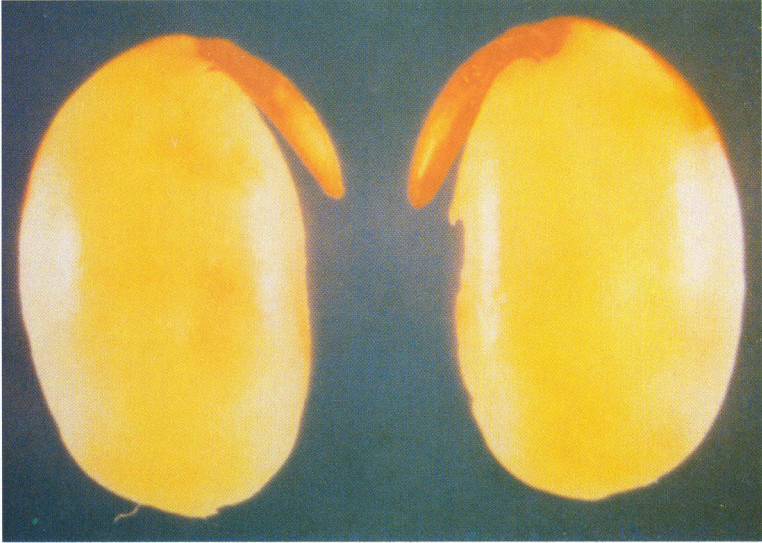


FIG. 19. Class 8 soybean seeds, due to intense field weathering. (Photo: J. B. França Neto).

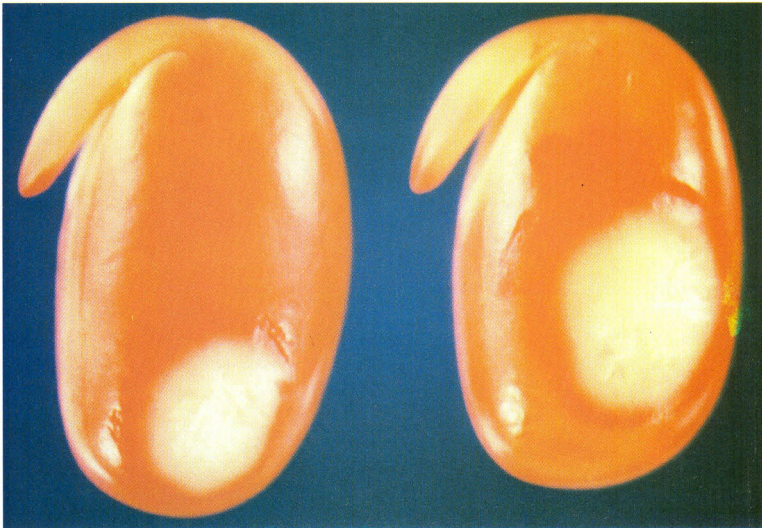


FIG. 20. Typical damages caused by stink bug on the cotyledons. (Photo: J. B. França Neto).

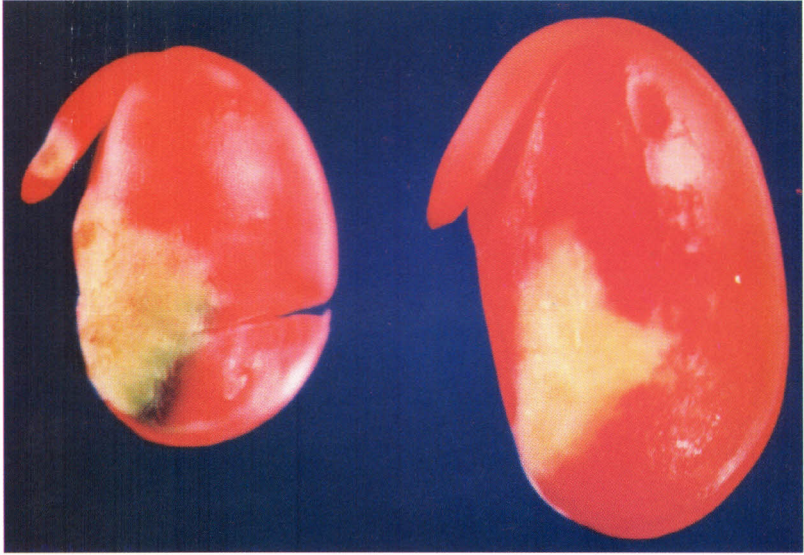


FIG. 21. Soybean seeds with stink bug damages on the cotyledons and embryonic axis. (Photo: J. B. França Neto).

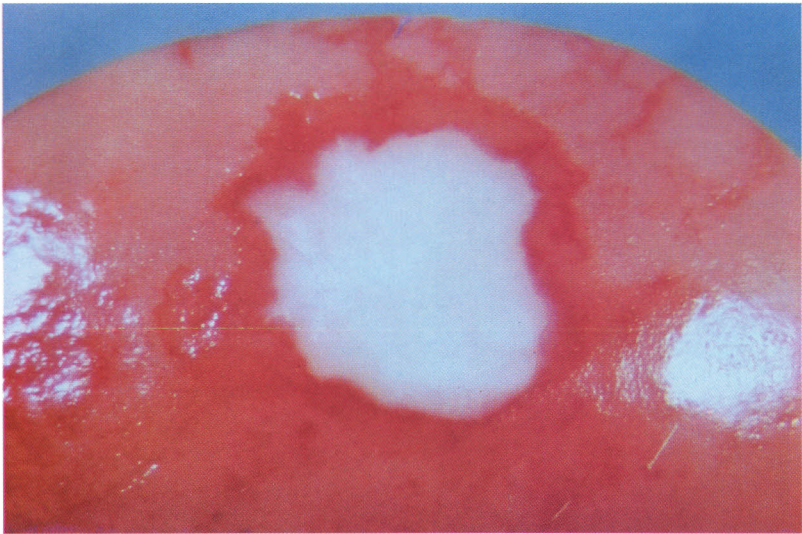


FIG. 22. Typical stink bug damage on the cotyledon. Note the circular configuration of the lesion and the intensive red ring (deteriorating tissue) surrounding the dead tissue. (Photo: J. B. França Neto).

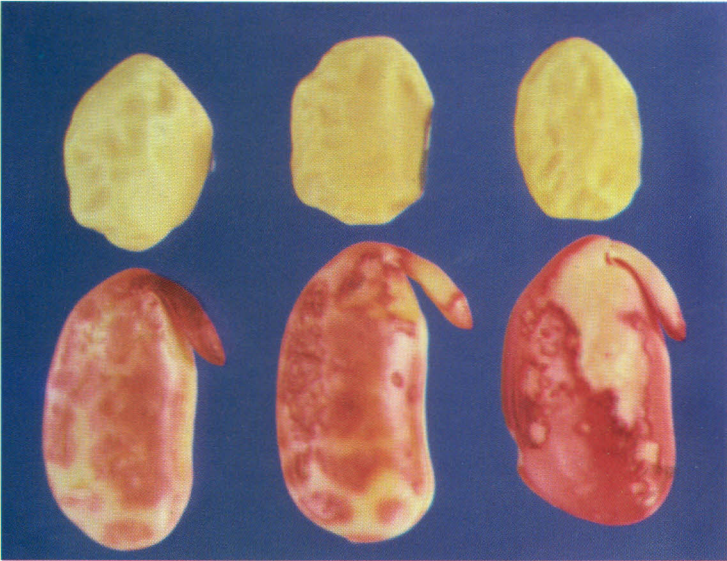


FIG. 23. Soybean seeds with lesions caused by the occurrence of high temperature and water deficit during seed filling. Top: dry seeds; bottom: stained seeds. (Photo: J. B. França Neto).

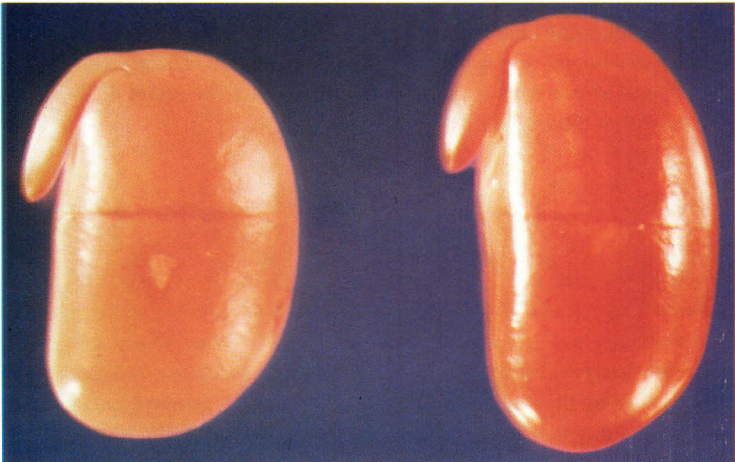
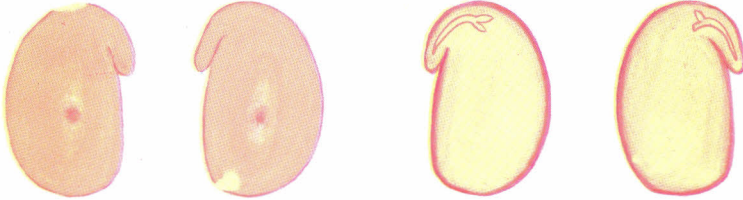
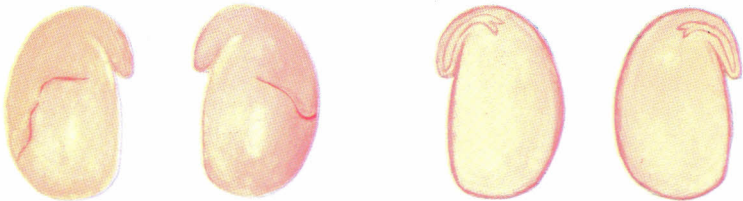


FIG. 24. Soybean seeds with characteristic damage caused by excessive drying: lots with this problem present high levels of seeds showing transversal cracks on the cotyledons. (Photo: J. B. França Neto).

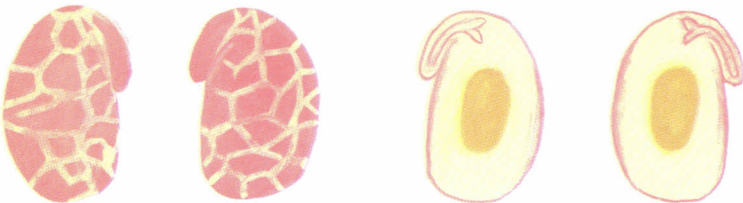
CLASS 1 (very high vigor)



- 1a. Seeds are characterized by uniform and superficial staining, due to a slow diffusion of the tz-salt; internal surfaces of the cotyledons are not stained except for their borders; all seed tissues are normal and firm.



- 1b. Seeds present the same characteristics as illustrated in 1a, except for the presence of one or two more intensively stained stripes per cotyledon. These stripes are superficial (depth of one or two tenths of a millimeter) and are the result of the first stages of water penetration during the weathering process.

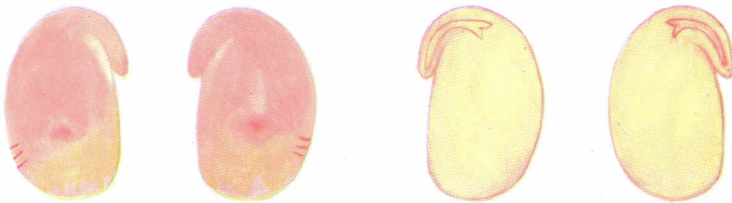


- 1c. Seeds show staining with a mosaic pattern (Fig. 25), due to slow imbibition; seed tissues are firm; the inner surface of the cotyledons are curved, resulting in a hollow space in between them. This is due to insufficient water absorption by the seeds and usually occurs on semi-permeable seeds. It may also be related to inadequate imbibition during preconditioning, or to the use of extremely dry seeds.

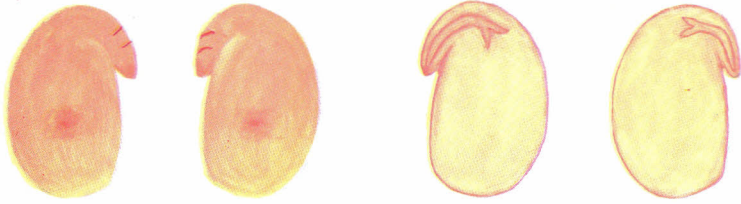


FIG. 25. Soybean seed with mosaic staining caused by improper preconditioning (imbibition) process. (Photo: F.C. Krzyzanowski).

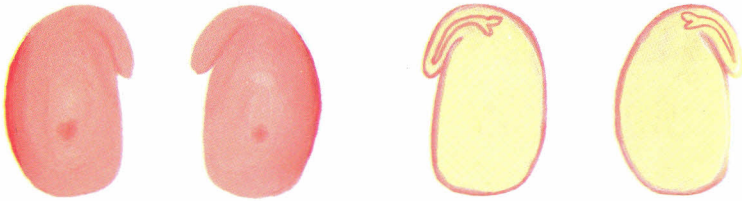
CLASS 2 (high vigor)



- 2a. Small stripes are present on the outer surface of the “back” of the cotyledons, i.e., opposite the embryonic axis. These stripes result from successive hydrations (expansions) and dehydrations (contractions) of the seed coat and cotyledons, during the weathering process. A pressure exerted by the seed coat on the cotyledons, due to an unequal expanding and contracting capability of these structures, will superficially harm the cotyledons, causing the stripes. These stripes are not deeper than 0.5 mm. The internal surfaces of the cotyledons are as described for 1a. Damages on the cotyledons are generally symmetrical.



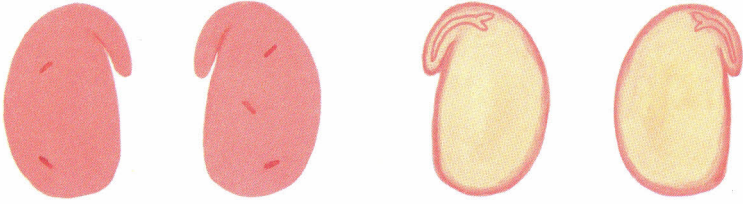
- 2b. Small stripes are present on the embryonic axis. These lines are due to the reasons described for 2a. These lesions are superficial and cannot be observed on the internal tissues of the embryonic axis after sectioning, as illustrated in 1a. Damages to cotyledons are generally symmetrical.



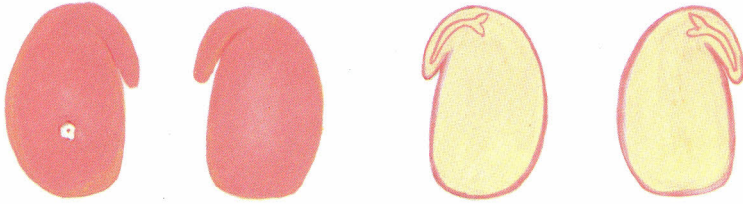
- 2c. Intensive red bands are present on the "back" of the cotyledons. These bands result from the grouping of several small stripes (as described in 2a). The internal surfaces of the cotyledons present no defects, as illustrated in 1a. Damages to cotyledons are generally symmetrical.



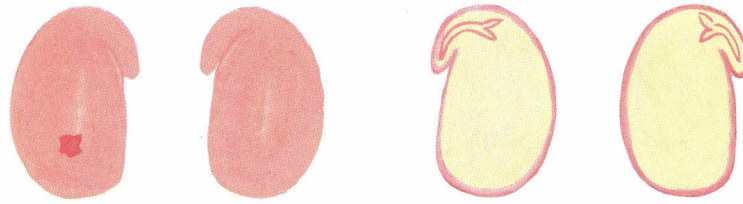
- 2d. Small bands of non-stained (white and dead) tissue are present on the "back" of the cotyledons. These bands should not be larger than that illustrated on the figure. The internal surfaces of the cotyledons are as described in 1a. All lesions, illustrated from Figs. 2a to 2d, in general, are symmetrical on both cotyledons. This characteristic is used to differentiate weathering damage from other types of injuries.



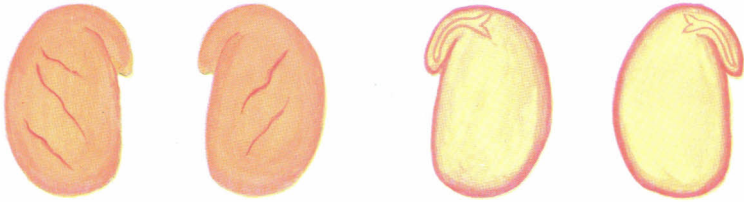
2e. Minor bruises, due to mechanical impact, are observed on the external surfaces of the cotyledons. Lesions should not be deeper than 0.5 mm. The internal surfaces of the cotyledons are as described in 1a.



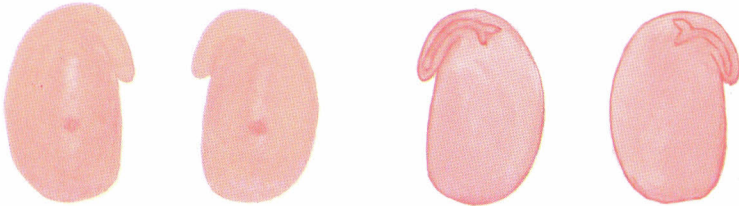
2f. Minor stink bug damage(s) is (are) observed on the seed. Damages are superficial (no more than 0.5 mm deep) and comprise an area not larger than the one illustrated in the figure. Damages are located away from the vascular region, i.e., on the seed half which does not contain the embryonic axis. Lesioned tissues are dead and friable and appear typically white, or sometimes greenish-, yellowish-, or grayish-white. The puncture site left by the insect may be often observed. The internal surfaces of the cotyledons are as described in 1a.



2g. Seeds reveal similar damage(s) as described in 2f, but the lesioned tissues are not dead (white). Affected tissue is wrinkled with an intensive red coloration. These lesions result from damages due to stink bugs that occur during the late stages of seed maturation, while the seeds are dehydrating.



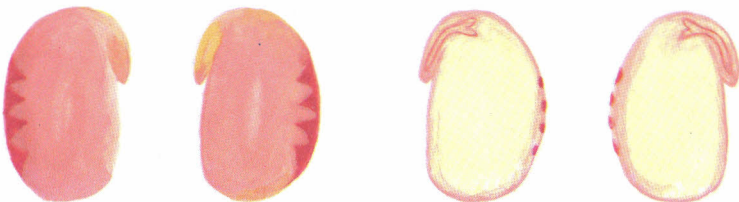
2h. Three or more stripes, as described in 1b, are present per cotyledon. Internal surface of the cotyledons are as described in 1a.



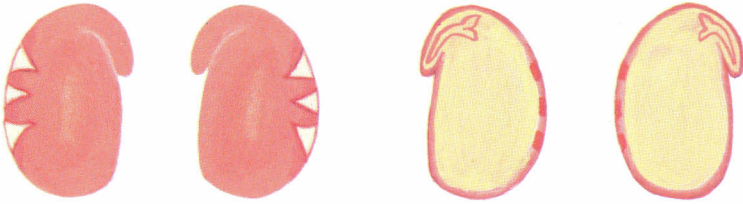
2i. External surfaces of the cotyledons display normal coloration, as described in 1a. Internal surfaces of the cotyledons are uniformly stained due to the penetration of the tz-salt through a rupture on the seed coat.

Note: seeds classified in Classes 1 or 2 do not present any damage on the internal surfaces of the cotyledons.

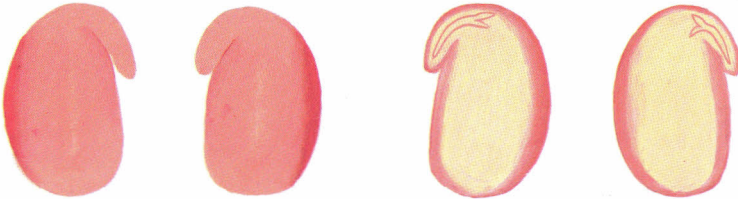
CLASS 3 (medium vigor)



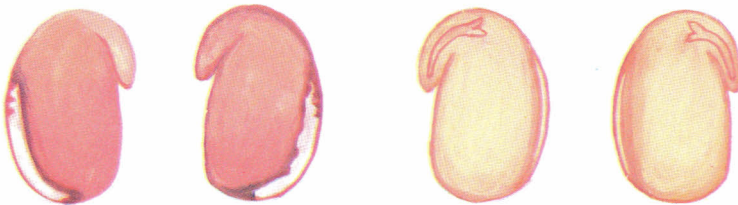
3a. Intensive red stripes are observed on the “back” of the cotyledons, with a total area not larger than the one illustrated on the figure. The internal surface of the cotyledons may display small dark red patches, with a maximum depth of 0.5 mm.



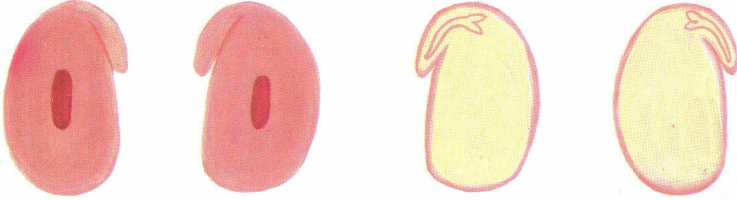
3b. Seed with similar lesions as described in 3a, but the stripes are milky-white. The internal surface of the cotyledons is similar to the one illustrated in 3a.



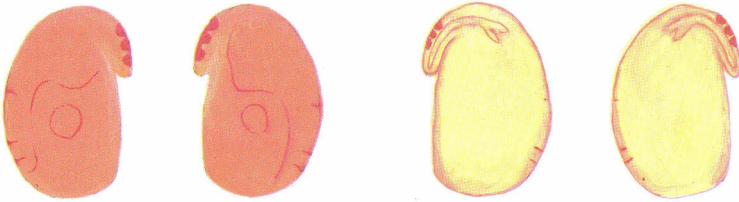
3c. Intensive red bands on the “back” of the cotyledons, as illustrated in the figure. These bands probably result from the junction of several small stripes during the process of seed weathering. Correspondingly, a dark band may also be observed on the internal surface of the cotyledons, with a maximum thickness of 0.5 mm.



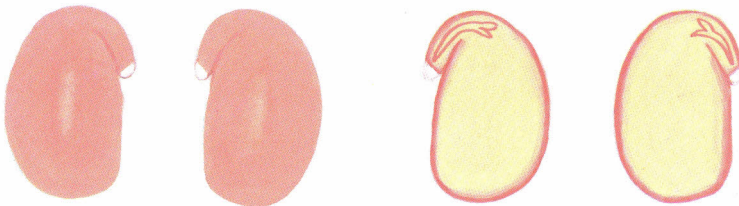
3d. The seeds have similar lesions as described before, but the band is milky-white (dead tissues). The area of the damaged tissue is not larger than the one illustrated in the figure, with a maximum thickness of 0.5 mm.



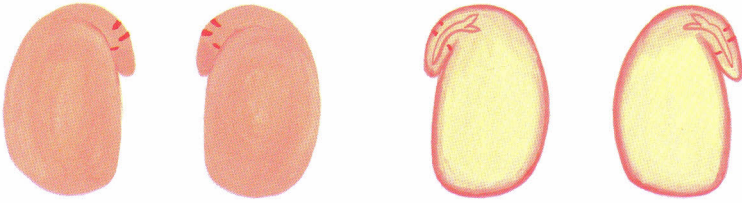
- 3e. An intensive red bruise, due to mechanical damage, is present on one or both cotyledons. Damages are superficial and not deeper than 0.5 mm. The internal surface of the cotyledons are as described in 1a.



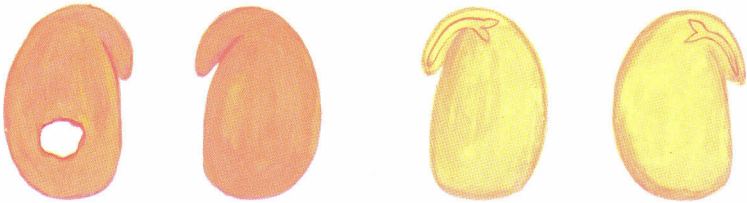
- 3f. Dark red patches are present on the cortex of the embryonic axis, but do not affect the stele.



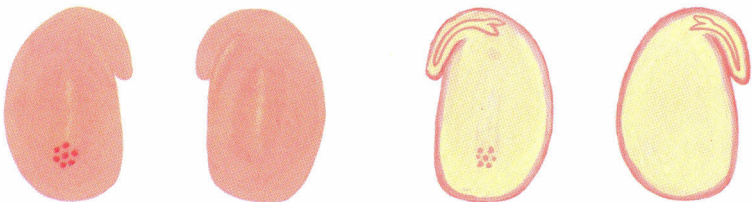
- 3g. The cortex tissue of the radicle tip is intensive red or milky-white, but the stele is not affected.



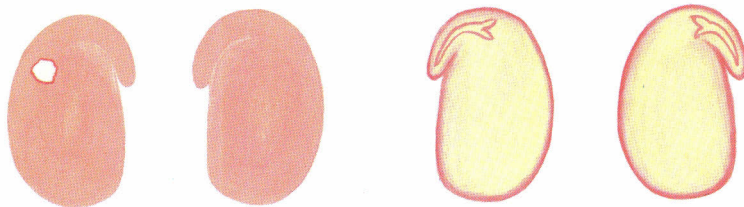
- 3h. Small fractures are observed on the radicle-hypocotyl axis, but does not affect the stele.



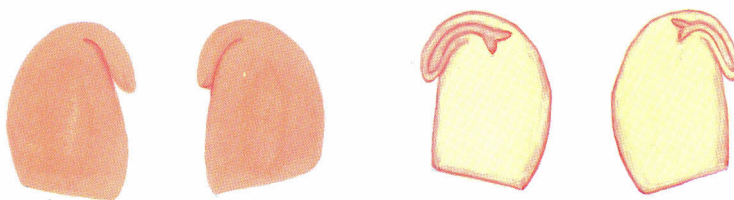
- 3i. Stink bug damage(s) as illustrated in the figure, is(are) observed on the cotyledon. This damage is a little larger than the one illustrated in 2f and is located on the seed a distance half-way from the embryonic axis. Affected tissues have a friable consistency and may be either intensive red or white (dead). Damage is superficial, and not deeper than 0.5 mm. The internal surface of the cotyledons are as described in 1a.



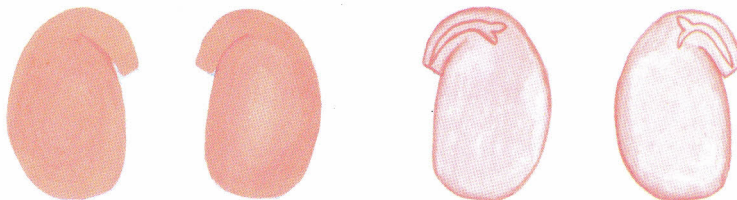
- 3j. Stink bug damage(s) not larger than the one illustrated in the figure, is(are) observed on the cotyledon. Damaged tissue is not white (dead) and is located on the seed a distance half-way from the embryonic axis. Damage(s) are on the internal surface of one cotyledon, which displays wrinkled patches with little red specks. The internal surface of the cotyledons may be uniformly stained as described in 2i.



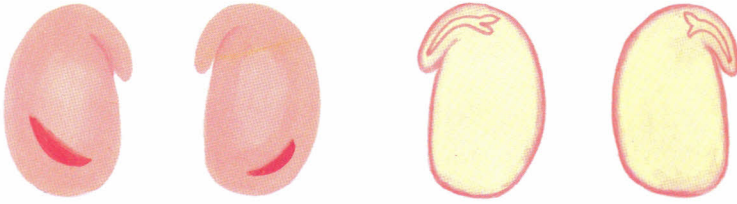
- 3k. Minor stink bug damage(s) not larger than the one illustrated on the figure. Damage is located on the seed half-way near to the embryonic axis. Damage is superficial (not deeper than 0.5 mm) and does not affect the vascular connections between the cotyledon and embryonic axis.



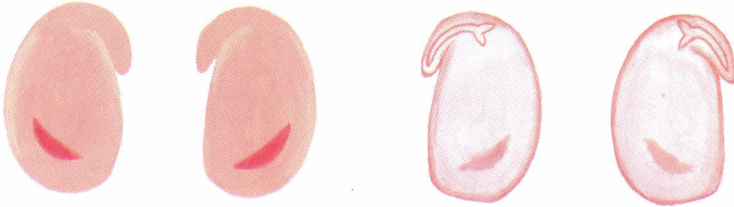
- 3l. Fractured cotyledons are observed, with an extension of damage not larger than the one illustrated in the figure. The internal surface of the cotyledons may be as described in 1a or 2i.



- 3m. A fractured radicle tip is present, but the damage does not affect the stele. The internal surface of the cotyledons may be as described in 1a or 2i.

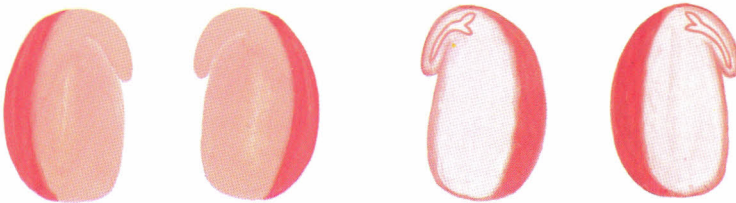


- 3n. Mechanical damage(s) is(are) present on the seed half-way from the embryonic axis. Affected tissues may be dark red or white (dead), but may not be deeper than 0.5 mm. The internal surface of the cotyledons may be as described in 1a or 2i.

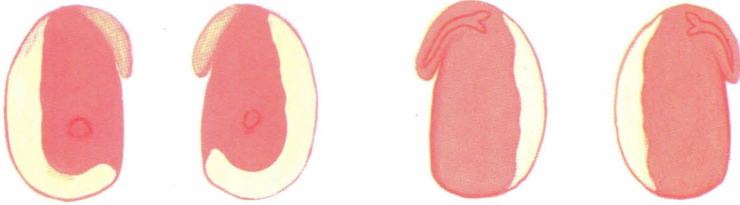


- 3o. Mechanical damage(s) is(are) present on the seed half-way from the embryonic axis. Damages are deep and can be observed on the internal surface of the cotyledons, as illustrated.

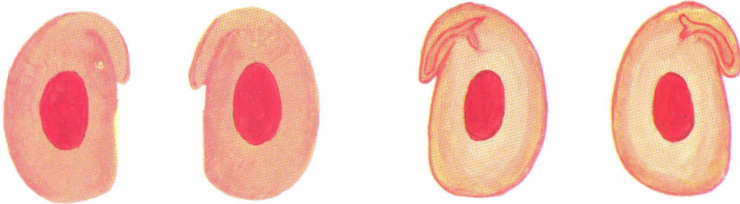
CLASS 4 (low vigor)



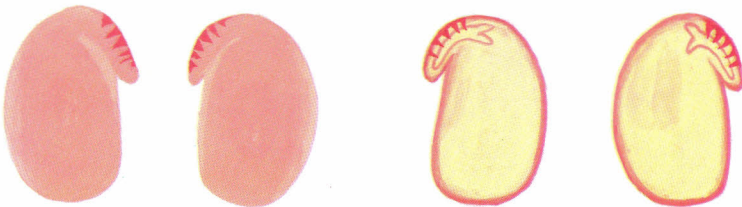
- 4a. An intensive red area is present on both cotyledons. The affected tissues comprise less than half of the cotyledonary tissues. The internal surface of the cotyledons is also affected and dark red. The vascular region between the embryonic axis and cotyledons is not distressed.



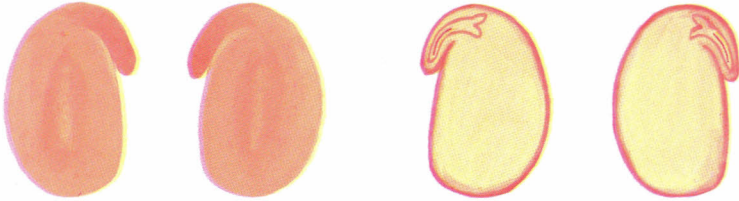
- 4b. Damages are similar to the ones described before, but affected tissues are dead (milky-white).



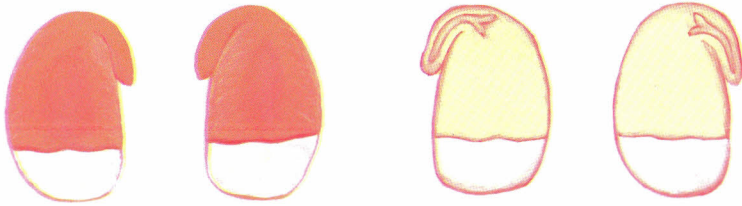
- 4c. External and internal central areas of the cotyledons are dark red. Part of the vascular region, between the embryonic axis and cotyledons, may be affected. Nevertheless, most of the vascular connections are still functional, allowing the transport of the cotyledonary reserves to the embryonic axis.



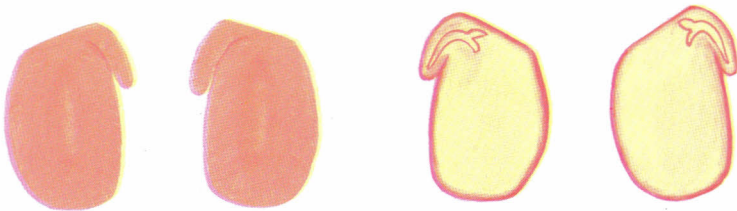
- 4d. Stripes are present on the embryonic axis. Lesions are deep and affect the stele, but no more than half of its thickness.



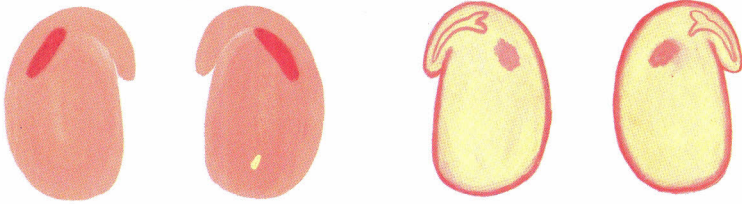
- 4e. An intensive red area is observed on the embryonic axis, but the structures of the cortex and stele are well defined.



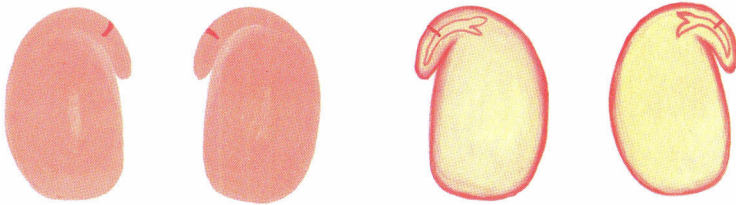
- 4f. Dead tissue area is less than half the area of the cotyledons.



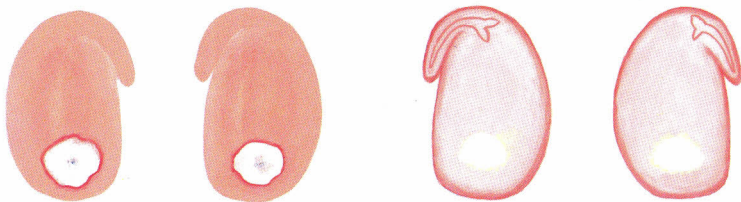
- 4g. One or both cotyledons may be fractured on the vascular region between the embryonic axis and cotyledons. Nevertheless, most of the vascular connections are still functional, allowing the transport of the cotyledonary reserves to the embryonic axis.



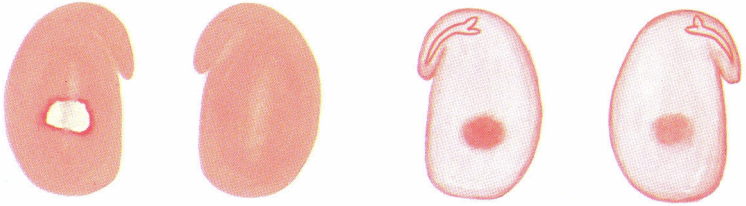
- 4h. Mechanical damage(s) is(are) present on the vascular region of the cotyledons and may be noticed on the inner surface of the cotyledons. Nevertheless, most of the vascular connections are still functional, allowing the transport of the cotyledonary reserves to the embryonic axis.



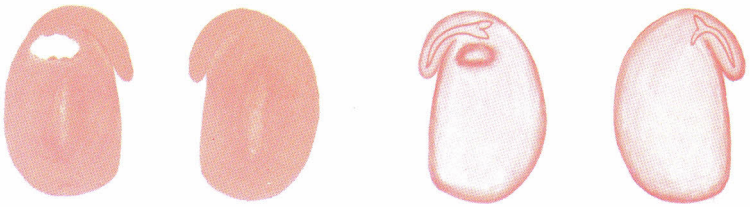
- 4i. Fractures are present on the embryonic axis. Fractures are deep and affect the stele, but no more than half of its thickness.



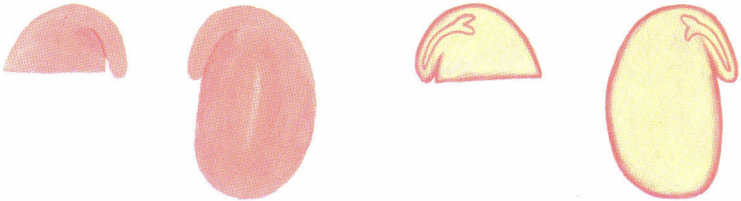
- 4j. Stink bug damage(s), with necrotic (white) tissue, is(are) present on the seed half-way from the embryonic axis. Lesions are deep and can be noticed on the inner surface of the cotyledons.



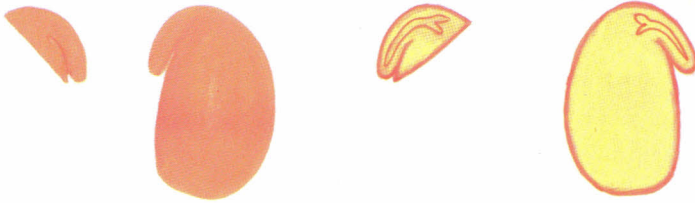
- 4k. Stink bug damage(s) is(are) present on one cotyledon, which becomes practically non-functional. The internal surface of the second cotyledon is also affected, but only superficially.



- 4l. Stink bug damage(s) on the vascular region of one cotyledon completely block(s) the vascular region, thus making this cotyledon non-functional. The germinating seedling will rely on the reserves from only one cotyledon.



- 4m. More than half of one cotyledon is fractured. The other cotyledon is in good condition.



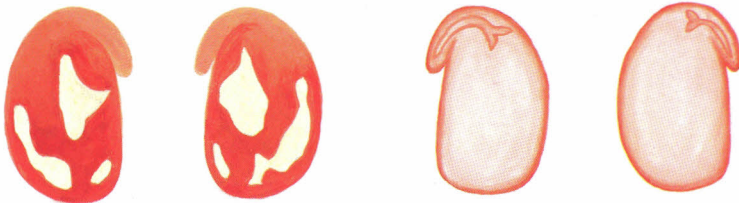
- 4n. One cotyledon is severely fractured, but the embryonic axis (including the plumule) remains sound.



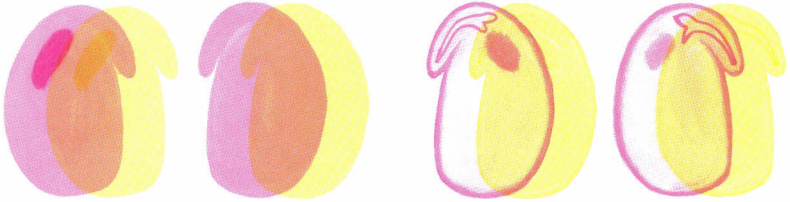
- 4o. Both cotyledons are fractured severely but the remaining part encompasses more than 50% of viable and functional cotyledonary tissues.

CLASS 5 (very low vigor)

The critical area of the tz-evaluation is between categories 5 and 6, the latter being considered non-viable. If a sample contains many seeds rated as 5 or 6, there could be discrepancies between the results of the standard germination test and the tz test.



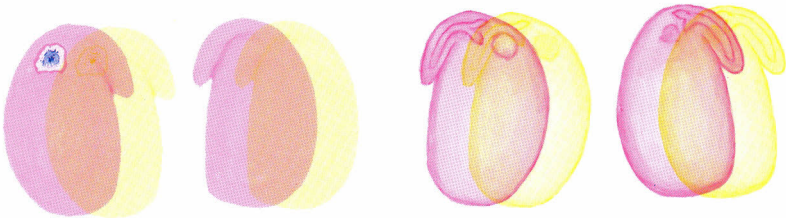
- 5a. Both cotyledons present lesions on a mosaic pattern. Dark red patches are associated with faint-red or white (dead) tissues. Damaged areas are extended to more than 1.0 mm deep, but do not exceed half the thickness of the cotyledon. Some areas on the internal surface of the cotyledons are stained more intensively. The embryonic axis is well defined and the cortex and stele are well delineated.



- 5b. Mechanical damage(s) on one cotyledon impair(s) the vascular region between this cotyledon and the embryonic axis. The corresponding internal surface of the second cotyledon is affected partially.



- 5c. Cotyledons are fractured severely, in such a way that it is difficult to visualize if the remaining parts comprise more than 50% of the total seed.

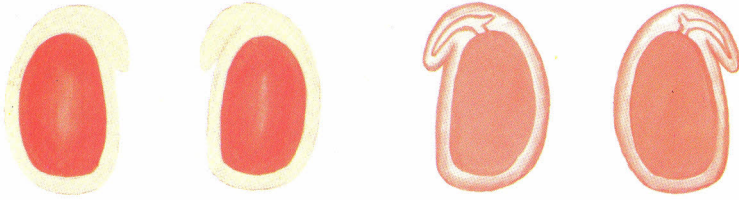


- 5d. Stink bug damage(s) affect(s) the vascular region as described in 5b.

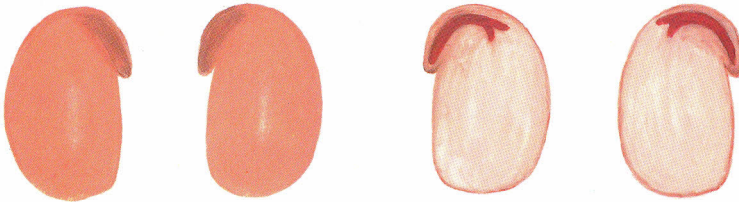
CLASS 6 (non-viable)

Seeds, rated as class 6, exhibit lesions similar to the ones described in category 5 (please refer to illustrations). Nevertheless, the extent of the damaged areas is larger, resulting in non-viable seeds.

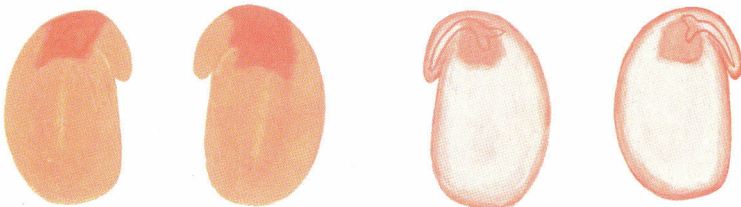
CLASS 7 (non-viable)



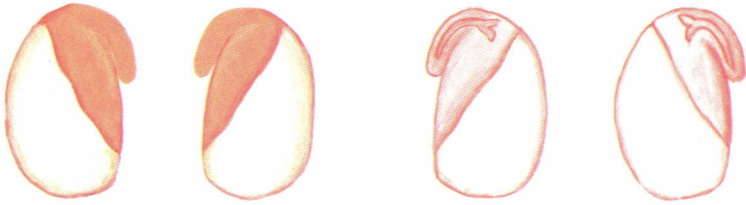
- 7a. Both cotyledons show lesions in a mosaic pattern. Dark red patches are associated with faint-red or white (dead) tissues. Lesioned tissues exceed half of the thickness of the cotyledons, thus blocking completely the vascular region.



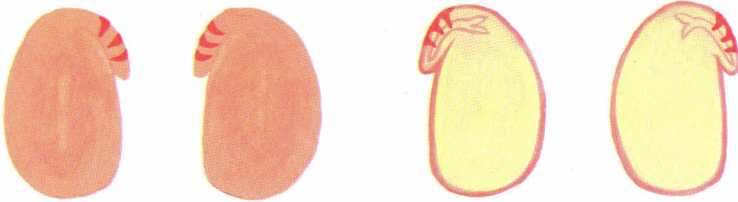
- 7b. Dark red tissues extend all over the embryonic axis.



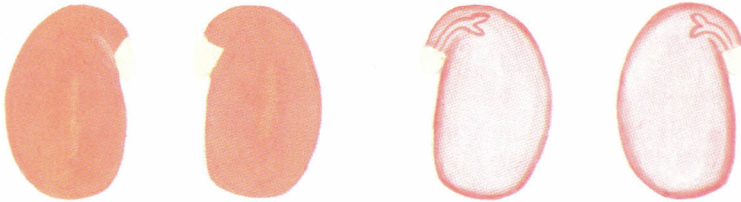
- 7c. Dark red (or milky-white) tissues stretch over the vascular region on both cotyledons.



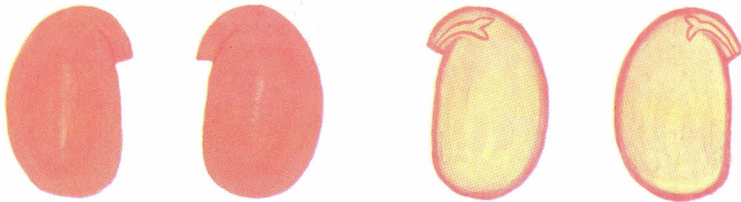
7d. More than 50% of the seed tissues are milky-white (dead).



7e. Dark red stripes are present on the embryonic axis. Lesions are deep and they stretch over more than half the thickness of the stele.



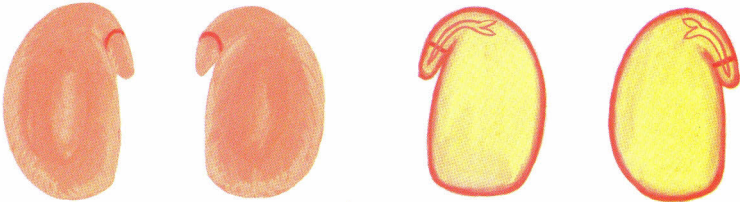
7f. Cortex and stele areas at the radicle tip are dead (milky-white).



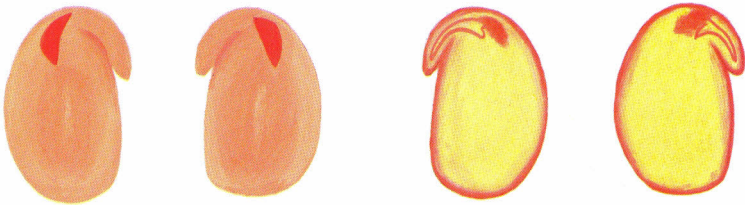
7g. Severe fracture on the radicle tip, affecting the cortex and stele.



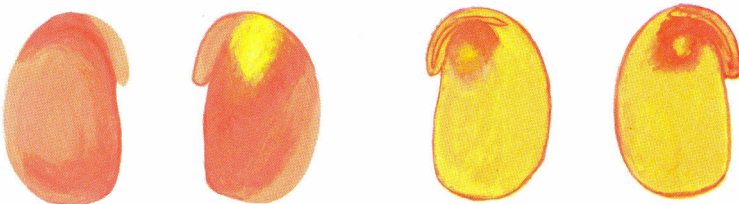
7h. Embryonic axis attached to fractured cotyledons. Remnants of cotyledons are less than 50% of the entire seed size.



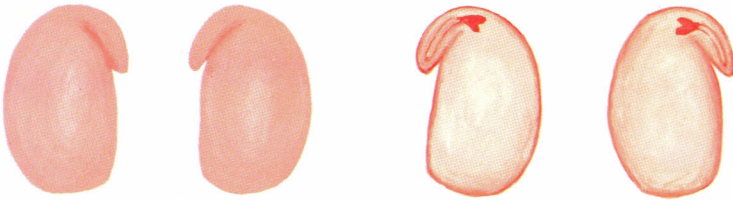
7i. The embryonic axis shows a fracture which affects the stele.



7j. Mechanical damage(s) severely affects the vascular region between both cotyledons and embryonic axis.



7k. Stink bug damage(s) severely affects the vascular region between both cotyledons and embryonic axis.



71. Plumule is deteriorated (intensive red).

CLASS 8 (dead seed)

Seeds are totally dead, being usually white and sporadically pinkish-white. Seed tissues are friable and flaccid.

Hard Seeds: special procedures are required, when hard seeds are observed in the seed sample. If a low level of hardseededness (2% to 5%) is noticed, these seeds may be considered as viable and vigorous, being recorded as Class 1. However, if its level is above 5%, such seeds must be scarified with a fine sandpaper, preconditioned in wet paper, stained, and interpreted accordingly.

Abnormal embryos: certain types of embryo abnormalities can be noticed when running the tz-test (Figs. 26 and 27). It is common to observe the presence of three or more cotyledons, folded cotyledons and misplaced embryonic axis. Most of these seeds will not produce a normal seedling, as the one pictured in Fig. 27. On the other hand, seeds with the abnormality illustrated in Fig. 26 may result in normal seedlings, if serious damages are not observed on critical seed components.

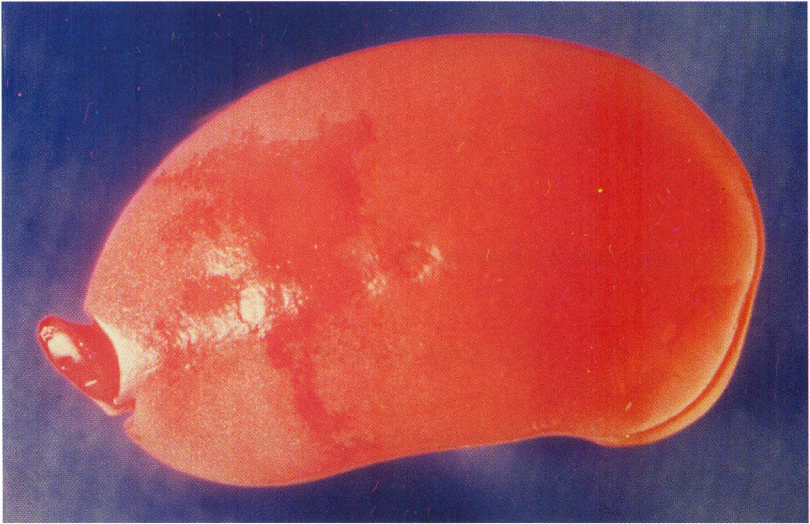


FIG. 26. Abnormal embryo of a soybean seed, detected by the tetrazolium test. (Photo: J.B. França Neto).

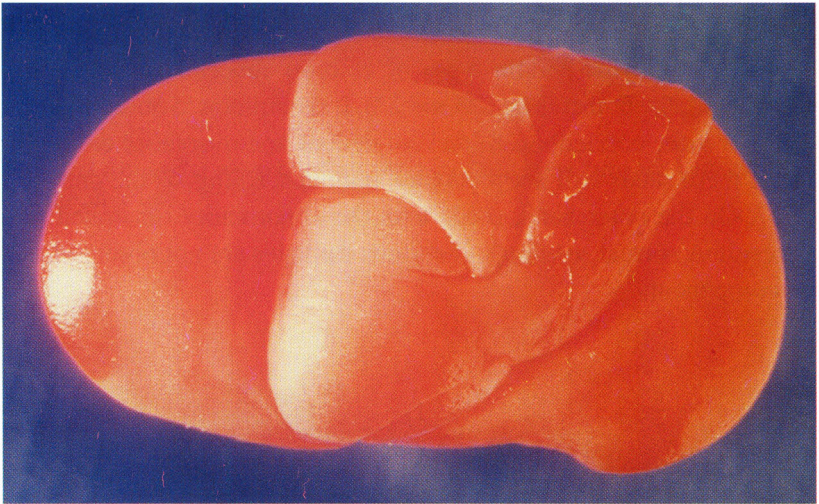


FIG. 27. Soybean seed with embryonic abnormality. Note the improper location of the embryonic axis. (Photo: J.B. França Neto).

5.3. Recording and Interpreting the Results

Each seed from a seed sample is graded into one class of viability, and the kind(s) of weakness on each seed is recorded, using a report form, as illustrated in the appendix. The following symbols may be used:

- χ = no damage (used for category 1);
- L = mechanical damage (MD);
- f = stink bug damage (SB);
- P = weathering damage (WD);
- \angle = MD + SB;
- \underline{L} = MD + WD;
- \overline{P} = WD + SB;
- \overline{E} = MD + WD + SB.

Important Observation: a non-viable seed (classes 6 and 7) may show signs of two or more types of damage (Fig. 12). If this is the case, and only one type of injury is responsible for the loss of viability, this particular kind of weakness must be annotated on the report form with its symbol in bold. For example: a seed displays a severe mechanical damage that totally fractured the embryonic axis, resulting in loss of viability; this seed also shows superficial signs of weathering on the cotyledons, away from its critical areas (Fig. 28). This seed will be graded as class 7, with signs of mechanical and

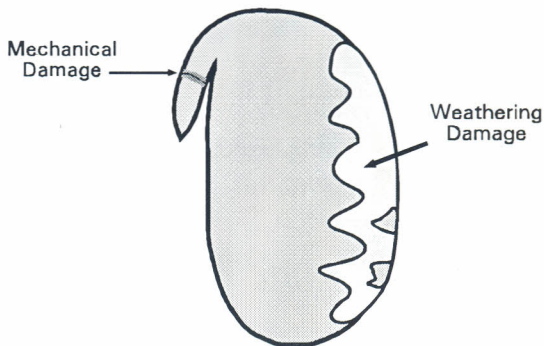


FIG. 28. Soybean seed with weathering damage on the cotyledons and a severe mechanical damage that fractured the embryonic axis.

weathering damages. However, this weathering damage was not intense enough to affect viability. In this case, mechanical damage, which is the only damage truly responsible for the loss of viability, must be marked down on the report form with a line in bold. This procedure must be followed when similar situations occur, annotating in bold the types of damage that effectively result in loss of viability and in a normal intensity those damages that are not severe enough to affect viability. Please refer to the report forms in the Appendix.

After 100 seeds (2 X 50 seeds) from a seed sample are evaluated, the percentages of seed rated within each category (1 to 8) are determined. The germination potential is calculated by the summation of the percentages of seeds in categories one through five. The vigor rating is determined by the seed total within categories one through three.

The results of viability and vigor for the two sub-samples of a seed lot are annotated on the bottom part of the report form. The percentage of seeds injured by all types of damages determined by the test (mechanical, weathering and stink bug damages) for classes 1 through 8 and 6 through 8 are calculated and also recorded. For the level (1-8), the number of seeds in each sub-sample with signs of mechanical damage are counted, for example; the number obtained is then multiplied by "2" to obtain the percentage value. The same procedure is done for the second sub-sample and the average of these two values is calculated and recorded on the form. This same procedure is followed for the other kinds of injuries.

For the level (6-8), the same actions are carried out. However, for those seeds with more than one type of damage, only the damage(s) that effectively contributed to the loss of viability (marked in bold) should be computed. Please refer to the report forms in the Appendix for a better understanding.

Need for Reanalysis: the seed sample should be reanalyzed when the viability values for the two sub-samples differ from each other by 10% or more.

5.3.1. Interpreting the results

The vigor level can be interpreted according to this classification:

- ♦ very high: 85% and above;
- ♦ high: 75% to 84%;

- ♦ medium: 60% to 74%;
- ♦ low: 50% to 59%;
- ♦ very low: below 49%.

The interpretation of the viability results should be the same as for the germination values.

The diagnosis for the cause(s) of low seed quality is achieved by the determination of the percentages of seeds affected by each kind of weakness, such as mechanical damage, weathering and stink bug injury, from classes 6 through 8. These numbers mean the percent reduction in viability caused by each kind of damage. These levels of damage may be interpreted according to the following classification:

- ♦ no serious problem (acceptable): up to 6%;
- ♦ serious problem: 7% to 10%;
- ♦ very serious problem: more than 10%.

If a serious, or very serious problem, is diagnosed by the seed analyst using the tz-test, e.g., a high level of mechanical damage, or stink bug damage, a corrective action can be taken, to improve seed quality. The seed producer can be advised, for example, to adjust the threshing system of his combine harvester, or to improve his methods for the control of stink bugs.

Examples of results provided by the tz-test are provided in Table 1. Seed lot no. 1 shows good viability and vigor and no serious problems with mechanical damage, weathering, or stink bug damage. Seed lot no. 2 has 82% viability and the vigor level (65%) was rated as medium, mainly due to serious problems (10%) with mechanical damage. Lot no. 3 had 75% viability and very low (49%) vigor, due to both weathering (12%) and stink bug damage (9%). The report form used to record the results for these three seed lots is illustrated in the Appendix.

An example of the tremendous value of feed-back to producers of information provided by the tz-test is illustrated by Costa *et al.* (1987). High levels of mechanical damage were determined for soybean seeds produced in the state of Parana, Brazil. After the problem was identified, farmers were instructed on how to improve the adjustments of the combine, through wide-

scale extension training programs. In two years, levels of mechanical damage on soybean seed were drastically reduced to the acceptable levels of 6%.

TABLE 1. Illustration of the results provided by the tetrazolium test for three soybean seed lots.

Parameter	Lot No. 1	Lot No. 2	Lot No. 3
Viability	93	82	75
Vigor ¹	86	65	49
Mechanical Damage	2 ²	10	5
Weathering Damage	4 ²	5	12
Stink Bug Damage	1 ²	4	9

¹ Vigor level: very high: $\geq 85\%$; high: 75 to 84%; medium: 60 to 74%; low: 50 to 59%; very low: $\leq 49\%$.

² Percentage loss of viability caused by the respective type of damage.

6

PRECISION AND ACCURACY OF RESULTS

A good level of reliability and precision of the tz-test was demonstrated by Franca Neto *et al.* (1986). Several samples of soybean seed were sent to 41 seed laboratories, with specific instructions for the performance of the following tests: a) standard germination, as prescribed by the Rules for Testing Seeds (Brasil, 1976); b) accelerated aging by the tray and chamber methods, according to the procedures described in the Seed Vigor Testing Handbook (AOSA, 1983); c) tz-test, as described by Franca Neto *et al.* (1985b); and d) emergence in sand, according to specific procedures sent with the samples. The tz-test was ranked as the second most precise test with regards to repeatability, after the standard germination test.

In most situations, the percentage of germinable seed in the tz-test and the standard germination test are similar. Up to a 5.0% difference between

these tests is considered acceptable. However, discrepancies might exist and might be due to one of the following reasons: a) sampling differences; b) improper tz-testing techniques; c) improper techniques used in the standard germination test; d) presence of hard seeds in the sample; e) use of seed lots with low or medium vigor; f) presence of high levels of seeds with mechanical or stink bug damages; and g) presence of high levels of seeds infected by *Phomopsis* spp., *Fusarium* spp. or *Colletotrichum truncatum*.

Lakon, as pointed out in a report by Gadd (1950, p. 253), stated that his “long experience in comparing the tetrazolium method and the ordinary germination methods has shown that where there are differences it was the germination test which failed.”

7

ADVANTAGES AND LIMITATIONS OF THE TEST

7.1. Advantages

Listed below are the major advantages of the tz-test:

- a) it by-passes major environmental disturbances that might affect the performance of growth tests;
- b) focuses attention on the physical and physiological conditions of the embryo structures of each individual seed;
- c) provides quick evaluation: 8 h for soybean;
- d) allows identification of the level of seed vigor;
- e) diagnoses the causes of seed deterioration;
- f) requires only simple and inexpensive equipment.
- g) an experienced analyst may analyze between four to five seed samples (2 X 50 seeds) per hour.

7.2. Limitations

The major disadvantages of the tz-test are listed:

- a) it requires knowledge of the seed structures and interpretation techniques;
- b) is relatively tedious, because examination of individual seeds requires patience and experience;
- c) consumes more time per sample than the standard germination test in spite of being a quick test; however, the tz-test provides more information than the standard germination test;
- d) does not show the efficacy of chemical seed treatments nor the damages that they may cause;
- e) requires from the analyst a decision making capability, due to the characteristics of the test.

Mason *et al.* (1982) reported that the tz-test was not effective in detecting recently induced mechanical damage. This problem can be easily overcome with the use of lower concentrations (0.075%) of the tz-solution, as suggested in the present manual.

8

WHEN THE TEST SHOULD BE APPLIED

The seed quality control system can be improved by the use of the tz-test in all phases of seed production: harvesting, receiving, before and after seed processing and drying, during storage and before sowing. The test has been applied with success even before harvest: mature plants are sampled from the seed production field one to two days before harvest; pods are hand threshed, and seeds are then taken for analysis. The tz-test will provide the results of viability, vigor, weathering and stink bug damages. Depending on these results, the seed producer will decide whether seeds from this field show adequate quality to be harvested as seed or grain. The adoption of this procedure may result in significant savings for the seed producer, avoiding unnecessary expenses related to transportation, drying, processing, bagging and storage of seed lots of low quality.

FINAL OBSERVATION

The information contained in the present manual is illustrated in the video "DIACOM: complete diagnosis of soybean seed quality - Part I: Methodology of the tetrazolium test" (DIACOM, 1994 - in Portuguese), which is available from the Department of Publications of Embrapa Soja.

9

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APPENDIX



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