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EMBRYO DEVELOPMENT IN BOLLS EXHIBITING THE "HOLLOW SEED" SYNDROME IN SOUTH CAROLINA

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Abstract

Seed failures and a premature brown coloration to seeds in bolls from fields in South Carolina in 1999 gave rise to reference to "seed rot" by growers as a new malady in the area. Subsequent research has failed to identify any rot organism in the discolored, empty seed. We will now use the phrase "Hollow Seed" to refer to this malady and have attempted to define its developmental symptoms.

Seed from the cultivar "Maxxa" from fields near Florence, SC, were examined in September, 2002. The hollow seed malady was observed to be the result of abnormally slow growth of the embryo within these seed. Fertilization, which results in formation of the embryo and endosperm, appeared normal. Expansion of the endosperm and spongy parenchyma was normal, so that the seed volume at 20 days post-anthesis (DPA) appeared similar to seed with robust embryos. However, in hollow seed the embryos had not attained sufficient size at 20 DPA to fill the cavity, so that upon collapse of the endosperm, which normally takes place at about 20 DPA, an air gap was created around the embryo. With an air gap separating the embryo and the surrounding spongy parenchyma it was no longer possible for the embryo to receive nourishment and no further growth of the embryo was possible. Thus, the seed remained hollow to maturity.

No physical deformity or anatomical anomaly was observed which could explain this abnormally slow growth of the embryos in these seed.

Introduction

In 1999 a high incidence of unfluffed or "tight-loc" bolls that could not be mechanically picked occurred in the cotton crop in South Carolina and resulted in significant yield loss in some fields. Initially the syndrome of symptoms was called "seed rot" because of brown coloration of the internal tissues of the developing seeds that was suggestive of rotting organisms. Subsequently, Jones et al. (2000) concluded that the name was a misnomer since no rot organism could be identified that was consistently associated with the symptoms. Based on recent observations we have chosen to call the phenomenon "Hollow Seed" as a descriptive term that implies only that embryo development is incomplete, without a causal attribution, in a normal-sized seed at the time of boll maturation.

Bolls for this study were selected from plots of *G. hirsutum* cv. 'Maxxa" at the Pee Dee Substation, Florence, SC, during late August, 2002. Although Jones et al. (2001) observed the hollow seed phenomenon in all cultivars examined, this cultivar exhibited the highest incidence of any cultivar when grown in South Carolina. Selected bolls were brought to the laboratory and dissected and observed under 10X to 40X magnification with a dissecting microscope. The results are presented as descriptive observations because no causal basis could be associated with the symptoms.

Observations

Normal Seed Development

As a base-line with which to compare the abnormal embryo development associated with Hollow Seed, we will first describe the course of events and degree of variation in embryos found in healthy seed production.

The normal course of development of cotton embryos is described in detail in Hector (1936) and Baranov and Maltzev (1937). Early stages of the expansion of the ovule are characterized by expansion of the endosperm and by development of a layer of spongy parenchyma cells that are derived from the inner integument of the ovule. This expansion results in the ovule attaining maximum dimensions at about 20 days post-anthesis (PA). This expansion, consisting almost entirely of cell expansion and not division, is powered by a low osmotic potential (high solute concentration) in the endosperm and surrounding tissues of the ovule (Mauney et al., 1967). The embryo is not physically involved in expansion of the seed, and in fact is quite small at the time the seed reaches its maximum volume (Stewart, 1986).

At about 20 days PA the distinctive palisade layer of the seed coat becomes crystalline and further expansion of the ovule ceases. By that time the previously liquid endosperm has become cellular. At three weeks of age the embryo is about 6 to 8 mm. in length and has begun to fold and fill the embryo sac. From this age to maturity the embryo absorbs the tissue of the endosperm inside the embryo sac and the spongy layer surrounding the embryo sac. At maturity, the endosperm is represented by fragments of cells scattered between the convoluted cotylendons. The spongy layer is reduced to a layer of crushed cell fragments inside the seed coat and surrounding the embryo, however it remains attached to the seedcoat at the chalazal plug.

Throughout it's development the embryo is nourished by intimate contact with the endosperm and the spongy parenchyma of the inner integument. As the embryo matures it fills the space within the confines of the seed coat and appears to exert pressure on the spongy layer causing those cells to collapse, thus rendering their contents to be used for embryo growth.

Within any boll there is a range of embryo sizes most often related to positional effects. Bolls selected from fields in Mississippi and Arizona in September, 2002, had embryos that varied about 10% around the average length. This observation agrees with that of Baranov and Maltzev (1937) whose drawings show a similar range of embryo sizes.

Hollow Seed Development

Bolls for examination were selected from the culivar "Maxxa." Following slicing, the most easily visible characteristic of hollow seed during boll development is the premature darkening of the seed coat and the interior spongy layer. This brown coloration, which begins at about 20 days PA, gave rise to the term "seed rot." This is the term for the malady that became popular soon after it was first observed in South Carolina. In September 2002 our examination of bolls with seeds displaying the symptomatic coloration confirmed that fertilization had occurred in the affected seeds. Additionally, we concluded from our observations that no other classical rot symptoms were present, thus a rot organism was probably not the causal agent. The browning seeds and other non-browning, but hollow, seeds displayed poor endosperm development and minute to undersized embryos compare to other seeds in the same boll.

Upon dissection of the developing seeds a small percentage of them had embryos in which growth had slowed or ceased very soon after fertilization. Embryos in the hollow seeds ranged from heart stage to late spade stage (0.1 to 8 mm in length) at times when normal seeds within the same boll had filled the embryo cavity and had developed the massive cotyledons that characterize the mature cotton seed.

Observations

From these dissections the following observations could be made.

1. Fertilization of these seed appeared normal. That is, both the embryo and endosperm that result from double fertilization by the two sperm nuclei were present in the seed.
2. The seed size of hollow seed was close to that of the normal seed in each boll. The endosperm and spongy parenchyma of the integuments expanded for the typical 20 days of development, producing wet seed volume of normal dimensions. (Fig. 1)
3. Embryo development in the seed was retarded or unsupported. Thus, at the stage of endosperm disintegration and collapse, the embryos did not fill the embryo cavity. At our level of magnification we were unable to determine if the endosperm was liquid or cellularized in these seeds.
4. The failure of the embryo to fill the cavity resulted in an air gap between it and the surrounding spongy parenchyma. The presence of the gap also suggested that the endosperm had lost turgor.
5. The spongy parenchyma tended to expand to partially fill the interior of the seed, but contact between the parenchyma and the embryo was broken so that transfer of nutrients from the inner integument into the embryo did not appear possible.
6. Seed coat coloration typical of seeds in the maturation stage of normal bolls began prematurely in those seed with arrested embryo development. The spongy tissues also because to develop a brownish color. No assessment could be made of the length of time between cessation of embryo development and on-set of pigmentation in the seed coat.

Conclusions

Comparison of normal seed development with that of the failing "hollow seed" leads us to the following conclusions regarding the anatomical and physiological events leading to the seed failure:

If the cells of the endosperm collapse and disintegrate before the embryo has attained sufficient size to fill the embryo cavity and remain in intimate contact with the spongy parenchyma, then the path for nutrient flow from the funiculus to the embryo is broken and the embryo ceases development. Likewise, hormonal signals from the embryo to the plant probably cease. Eventually the parenchyma cells of the spongy layer expand to occupy a large portion of the embryo cavity, but in the absence of a hormonal signal, the plant ceases to provide nutrients and the seed becomes prematurely senescent. That is, the

hypostase is plugged, the chalazal cap begins to turn brown, the seed coat matures with its dark coloration, and the spongy layer takes on the brown coloration that is ordinarily seen as the collapsed cells surrounding the embryo.

There were very few visual clues indicating possible causes for this seed failure. In some cases there was evidence of insect punctures in the carpel wall near the affected seed. However, in most instances no such evidence could be seen. The attachment of the seed at the funiculus appeared to be normal and the development of the internal structures appeared robust until the retarded embryo growth produced the disruptions described here.

References

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Figure 1. Comparison of two seeds from the same boll of Acala Maxxa. Left, normal embryo development. Right, delayed embryo development with collapsed endosperm. Note that seed size is approximately the same. At boll opening the seed on the right will be hollow. (Photo courtesy of J.D. Mueller and M.A. Jones.)