

# Anatomy of Apple Russet Caused by the Fungus *Aureobasidium pullulans*

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This research is supported in part by the New York Apple Research and Development Program.

The causes of apple russet are many, but all causes lead to serious concern for growers, storers and sellers of fresh-market varieties. Varietal differences in apple susceptibility are well known. The most published studies of russet inducing factors have been done on the variety 'Golden Delicious', but studies of other varieties are not uncommon. Russet inducing agents are various and include temperature, relative humidity, light quality at the fruit surface, sprays (either active ingredients or adjuvants), and even biological agents that are present in the orchard. Indeed, in the last decade, much evidence has been gathered that implicates several fungi in the production of russet in fruit of a number of varieties in New York

State. Cornell's Department of Plant Pathology has been researching these pathogens along several avenues: surveying New York orchards for presence of these fungi; determining the cycle of inoculum production in season; correlating increase of inoculum and incidence and severity of russet in fruit of many apple varieties; surveying growers and packing houses for information on severity of russet and its economic consequences; planning control strategies that limit or eliminate russet production in the orchard; and determining how the fungus elicits russet formation on the fruit surface at the microscopic level. Some of these findings were reported in a previous issue of this publication (See Heidenreich, et al., *New York Fruit Quarterly*, Vol. 8, No. 2, Summer 2000: pp. 22-24). In this issue, we report on the interaction of the fruit surface with the russet-inducing fungus *Aureobasidium pullulans* and the production of russet as fruits develop.

There are apple varieties that russet naturally and these are even admired by some apple lovers. The skin of such varieties is rough and mottled, but the apple is not deformed. Russetting in other apple varieties is abnormal, so that normally smooth surfaces become crusty, "blistered," or "alligatored," and there may be growth deformities and surface cracks or splits. Figure 1 shows russet in mature fruits of 'McIntosh' (top) and 'Crispin' (bottom) after inoculation with *A. pullulans* spores at an early stage in fruit growth. Less severe russet is often seen (e.g., Figure 2) and may be due to strain of fungus, apple variety, weather conditions, amount of inoculum present in an orchard, and how late in fruit



Figure 1. Russet of 'McIntosh' (top) and 'Crispin' (bottom) fruit caused by inoculation with spores of the russet-inducing fungus *Aureobasidium pullulans*.

Apple russet caused by fungal pathogens has been found to be a problem in many New York orchards. The pathogens' life cycles and host-pathogen infection processes are little known. Our research shows that these pathogens feast on the fruit's epidermal cuticle (outer skin), but not on fruit cells themselves. This cuticular "hull breach" initiates the fruit's protective mechanism, namely, russet. Russeted tissue is formed as a response to penetration of the fruit cuticle and exposure of the fruit cells beneath to oxygen. In effect, it represents an attempt to wall-off invading fungi by developing a corky, suberized layer at the site of the cuticular breach.

development inoculation occurs. Older fruits appear less susceptible than those less than a month from bloom. Our inoculation of 'McIntosh' out of winter storage, for example, elicited only a slight reduction in "gloss" of the skin surface.

*A. pullulans* is a fungus that spreads out after inoculation as a colony of yeast-like cells that produce infective spores (conidia) at the ends of many of the colony's branches (Figure 3, top). The mycelium (the fuzzy, thread-like mat we associate with fungi on a leaf or fruit surface) of this fungus is relatively sparse. The mycelium will eventually produce fungal strands (hyphae) that darken and become compartmentalized as thick-walled spores (arthrospores) (Figure 3,

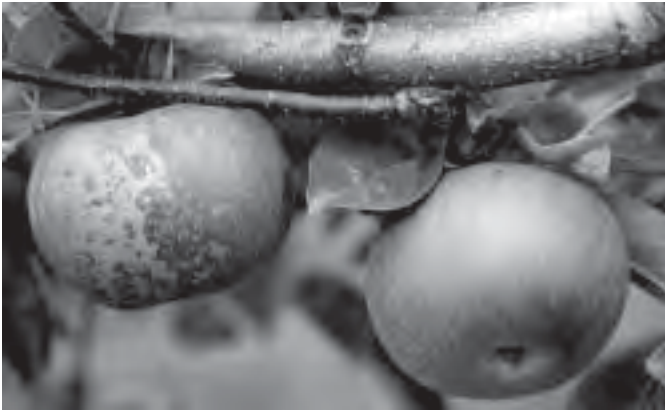


Figure 2. A less severe form of fungus-induced russet on 'McIntosh' fruit (left), while a nearby fruit is russet free.

bottom). This gives rise to the fungus' common name, "black yeast." Notice the black, crusty spots and cracks in 'Crispin' in Figure 1. This fungus is very common and found on a variety of substrates, including wood, cloth, painted surfaces, many plant species and organs, and even on insects, crustaceans, and man (F.D. Heald, 1933. Manual of Plant Diseases).

We have isolated this fungus and tested various strains of it for its ability to induce russet in apple. We then began a series of anatomical studies to see what happens at the microscopic level when a high concentration of a virulent strain is inoculated onto the developing skin of young fruits. Spore suspensions at the concentration of ten million spores per ml water were inoculated onto 'McIntosh'

fruits as a fine mist (to "run-off") in our early studies. More recently we have been devising small micro-containers that can be applied to young fruits and then filled with the spore suspension using a micropipette. This, we think, will give us a known, small-area sample of fruit skin to examine for russet formation. This is especially important when collecting skin

samples colonized by spores but before russet becomes obvious.

Early trials of spore suspensions misted onto 'McIntosh' fruits at time of fruit set showed us several things. The first is that fruits at this young stage are quite covered with long epidermal hairs (trichomes) (Figure 4, left) and that uninoculated fruit did not russet. Inoculated fruit indeed showed the presence of conidia on the fruit hairs, but also on the developing cuticle of the fruit's epidermal surface (Figure 4, right). It also appeared that these spores and their offspring were capable of digesting the protective cuticle of the fruit, leaving disruptions in that layer (Figure 4, right). Fruits at this stage are developing rapidly, mostly by increase in cell numbers. To

keep pace with the increase in internal volume, the epidermal and hypodermal layers of the fruit (the "skin") must also undergo cell division and cell enlargement, or the skin would be stretched and broken. This is a major reason why fruit inoculated at a young stage often show the most severe russet — the fruit has tried to repeatedly heal and seal a continually fractured epidermal surface as fruit size increases. Normal cell divisions in apple fruit decline to a very low frequency about 4 weeks post bloom, while cells enlarge tremendously after that time.

A cross section of the epidermal cells and overlying cuticle layer in a 'McIntosh' fruit at bloom is seen in Figure 5, top. The fruit was inoculated with *A. pullulans* a few hours before the fruit was collected and processed for this section. Spores are seen on the cuticle layer above an epidermis composed of a single cell layer. Within a week or so of inoculation we find a response of the apple skin to the presence of the fungus. Note in Figure 5, middle, that the spores are then associated with changes in fruit skin organization. The epidermal cell just below the spores has likely been induced to divide to produce an inner and an outer epidermal cell. Also, the hypodermal layers of the fruit have begun to produce a thickened layer of actively dividing files of cells, the periderm tissue. A major function of periderm in plant organs is to

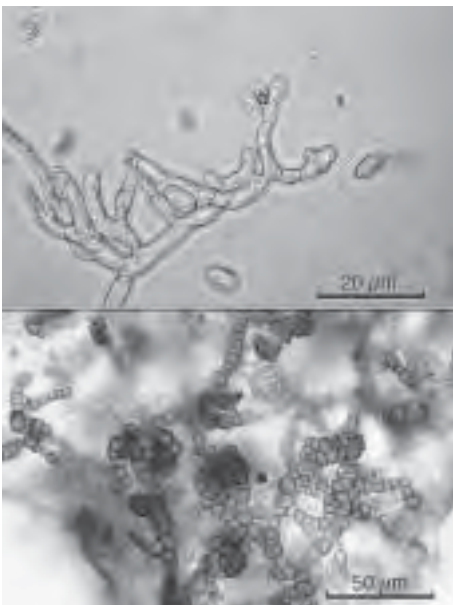


Figure 3. Cultures of the russet-inducing fungus *A. pullulans* showing production of conidiospores from the ends of the branching filaments (top) and chains of dark-staining arthrospores taken from russeted 'Cortland' apple (bottom).

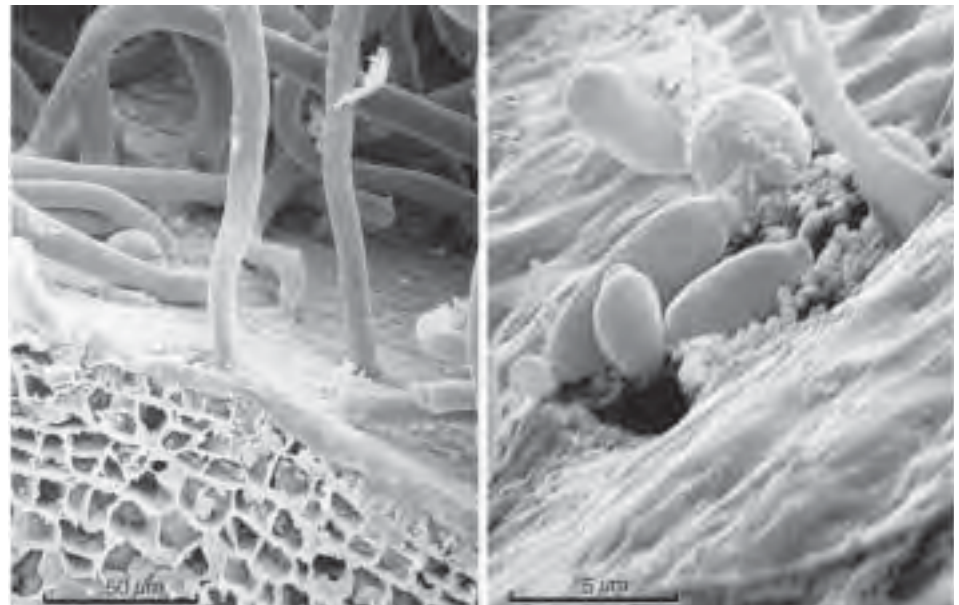


Figure 4. Scanning electron microscope images of 'McIntosh' fruit surfaces at 10 days post bloom, either uninoculated (left) or inoculated (right) with *A. pullulans* spores. Note the dense hairs on the young, intact epidermis (left). The spores are associated with degradation of the protective cuticle in inoculated fruit (right). Spores are about 1/5000 inch (5 µm) long.

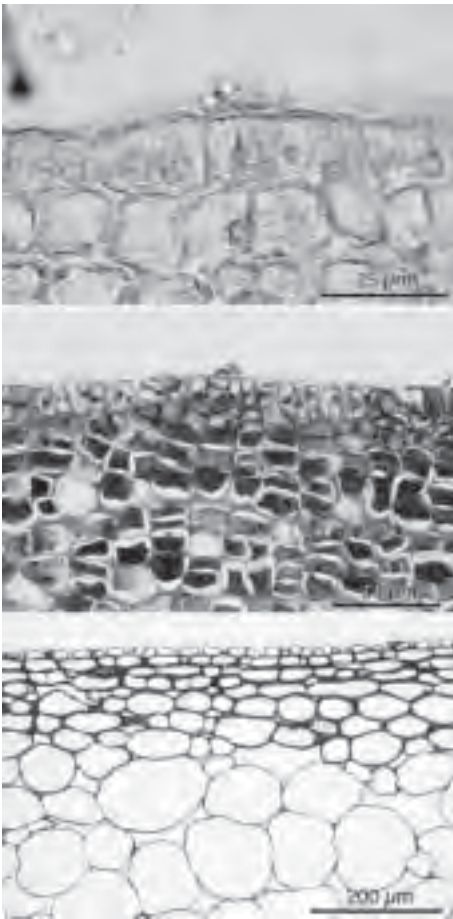


Figure 5. Cross-sections of 'McIntosh' apple skin: *A. pullulans* spores attached to the cuticle of the epidermal cell layer (top); spores of *A. pullulans* on the fruit 12 days post bloom and induction of heavy layers of periderm (russet) (middle); and normal apple skin at maturity, without periderm or russet (bottom).

seal off wounds, tears, or cracks in the organ surface. There is evidence that a cell's exposure to an oxidizing environment (air, in this case) will trigger cell division and, if necessary, periderm formation. The files of periderm cells are usually filled with sealing substances and antimicrobial materials, such as phenolics, that help prevent tissue degradation. A section through a mature fruit's skin should appear as in Figure 5, bottom. Note that there are no heavy layers of periderm, no files of cells with dark phenolic materials, even though the cells of the flesh have increased tremendously in volume.

As the fruit continues to develop, the small isolated packets of periderm coalesce into small, then increasingly larger, scabby areas (Figure 6). If such activity begins at a stage where much fruit growth has yet to occur, then huge areas of russet result, often with large scabs, cracks, or leathery patches. If only a small

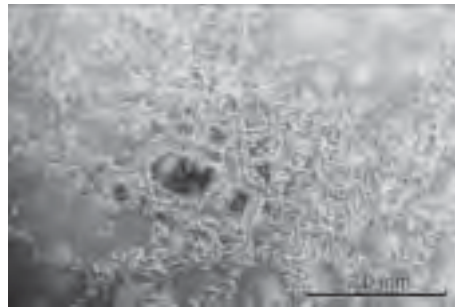


Figure 6. Magnified surface view of 'McIntosh' skin inoculated 4 weeks post bloom and examined three weeks later. Note scabby patches of periderm. The later the fruit is inoculated, the less severe the russet, both in surface area russeted and intensity of the russet.

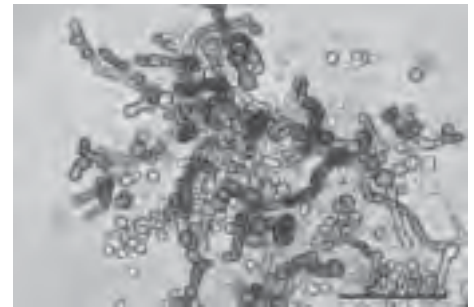


Figure 7. Colony of *A. pullulans* strain YT16 growing and multiplying on isolated cutin taken from the epidermis of a mature apple. The fungus is thus capable of digesting cutin and using it as a food for growth.

amount of cell division and fruit enlargement remains when fruits are exposed to inoculum, then russeting is more isolated and russeted surface area reduced in proportion to total fruit surface.

In collaborations with Dr. Wolfram Koeller and Diana Parker in the Department of Plant Pathology at the Geneva Experiment Station, the cutin and wax components of the apple fruit cuticle were extracted, isolated, and subjected to inoculation with *A. pullulans*. The fungus was able to digest both crude and purified components of the fruit cuticle and use it as its sole carbon source for fungal growth and differentiation (Figure 7). All *A. pullulans* isolates produce esterases in culture with apple cutin. The esterase produced by the strain used in our studies is a cutinase. This provides supporting evidence for our interpretation that it is the digestion of the waxy protective layer that sets up the russeting process. The cuticle of young fruits is both thinner and simpler than the cuticle of maturing apples. The fungal-induced "hull breach" in the young fruit surface initiates a repair mechanism that is outpaced by growth in fruit volume, while similar breaches in older fruit are repaired at a time when tensile forces in the skin are being reduced. We have not yet determined, however, if production of these cuticle-degrading enzymes by the fungus actually results in russeted fruit in the orchard, but it is highly likely considering the evidence.

In summary, russet formation in apple fruit in the presence of *A. pullulans* appears to be the result of: 1) spore deposition on the fruit cuticle; 2) spore adherence for enough time with the right conditions to begin cuticle degradation; 3) epidermal cell responses, such as phenolic deposition, cell senescence, and

death; 4) localized sub-epidermal changes due to loss of epidermal integrity; 5) a tissue recovery period involving cell divisions in the hypodermis (sub-epidermal layer); and 6) production of a corky layer (periderm) that re-isolates the flesh from the desiccating and oxidizing environment surrounding the fruit.

This year we are continuing anatomical studies on 'McIntosh' fruit that had inoculum applied to small micro-wells attached temporarily to the fruit surface at about 7–10 days post bloom. This allows a time-course study of a very small area of known exposure to a known concentration of inoculum. And, of course, further field trials are being made of spray materials that may have an ability to retard or prevent russet in a range of apple varieties.

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*We give special thanks to Mary Jean Welser, Research Support Specialist in Goffinet's anatomy laboratory for excellent histological preparations over several years of this study.*

