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# Survey of Moldy Core Incidence in Germplasm from the Three U.S. Apple Breeding Programs

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## Abstract

Moldy core, mainly caused by *Alternaria* spp., in apples has been studied in a limited number of cultivars, and susceptibility is attributed to open sinuses and calyxes in the fruit. In three US apple breeding programs, a diverse germplasm collection was characterized for core opening, calyx opening, and moldy core incidence at several time points during storage. Ten cultivars showed signs of moldy core, all had open cores while only three had open calyxes. Fruit with either an open core or an open calyx increased the likelihood of moldy core incidence. Two susceptible cultivars, 'Gingergold' and 'Pinova', also had progeny with high incidence of moldy core. A separate project screened 707 seedlings with diverse parentage in the Washington State University breeding program for core opening, calyx opening, sinus opening and moldy core incidence. Only four of the seedlings had open sinuses, and all failed to develop moldy core. The cultivar survey information presented here may be useful to other breeders, horticulturists, and pathologists interested in determining the heritability for moldy core susceptibility.


Moldy core (mouldy core, dry core rot) is caused primarily by *Alternaria* spp., but has also been reported as a complex consisting of *Stemphylium* spp., *Cladosporium* spp., *Ulocladim* spp., *Epicoccum* spp., *Coniothyrium* spp. *and Pleospora herbarum* (Pers.) Rabenh., with *Alternaria alternata* (Fr.) Keissl. as the dominant species (Combrink et al., 1985a; Hickey 1990; Niem et al., 2007). Only a few commercial apple (*Malus* × *domestica* Borkh.) cultivars including 'Fuji', 'Red Delicious' and sports of 'Red Delicious' such as 'Starking' are reported to be affected; their open sinus is thought to be responsible as a port on entry for inoculum (Miller, 1959; Combrink et al., 1985a). Combrink et al. (1985b) found 38-66% of 'Red Delicious' fruit cut at harvest had moldy core. It has recently been reported that *A. alternata* and *A. tenuissima* (Kunze) Wiltshire can also cause external lesions on stored 'Nittany' apple fruit that manifests during storage (Jurick et al., 2014; Kou et al., 2013). Research on *Alternaria* spp., *Stemphylium* spp. and *Cladosporium* spp. has shown that their spores are ubiquitous in temperate zones, with concentrations affected by diurnal fluctuations, the time of year and nearby sporulating plants (Sreeramulu, 1959; Bergamini et al., 2004; Rodriguez-Rajo et al., 2005).

The disease cycle starts soon after blossoms open. Spores germinate and mycelia grow down the open style/sinus to the developing core where colonization of the locular region occurs. Sensitivity to colonization drops significantly after petal fall (Reuveni et al., 2002). During fruit development, as cells divide and enlarge and fruit tissue swells, the style/sinus opening closes. An open sinus could be a characteristic of the cultivar, as in the case of 'Red Delicious' (Combrink et al., 1985a), or could be caused by rapid, irregular growth or when early season dry weather is followed by heavy rain (Hickey, 1990). Warmer weather during early fruit development has also been indicated in increased susceptibility, as well as reducing the length of the fruit (Sugar, 2002). Mycelial growth is generally restricted to the locules and carpellate region, but can develop into dry rot if it

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colonizes the surrounding mesoderm (Hickey, 1990). Wet core rot is distinct from moldy core/dry core rot in that it is a postharvest disease caused mainly by *Penicillium expansum* Link, other *Penicillium* spp. and *Mucor piriformis* Scop. (Warner, 2006; van der Walt et al., 2010; Peter et al., 2012; Li et al., 2014).

While environmental conditions and inoculum density likely contribute to moldy core incidence, host physiology is the main driver of susceptibility during bloom (Combrink et al., 1984; Shtienberg, 2012). Larger calyx openings, smaller length to diameter ratio and larger fruit have all been positively correlated to moldy core susceptibility in 'Gala' and 'Fuji' (Silveira et al., 2013). In 'Red Delicious' and 'Golden Delicious', sinus opening is inversely related to the length-diameter ratio of fruit (Spotts, 1990). Open calyx and open core cultivars are considered more susceptible to moldy core than cultivars with a closed calyx, though research has focused mainly on 'Red Delicious' (Combrink et al., 1985a; 1985b; Hickey, 1990). The heritability of this trait has been difficult to discern as studies have focused on only a few commercial cultivars. While symptoms typically do not affect the edible portions, it is off-putting to consumers that slice their apples to find mold in the center of the fruit. There are currently no postharvest fungicides labelled for use on pome fruit to control moldy core, and this coupled with the fact that *Alternaria* spp. also produce mycotoxins such as alternariol and alternariol monomthyl ether, make this an important disease to combat (Biggs, 1994; Scott, 2012; Warner, 2006). At least one cultivar has been released and largely rejected by industry due to its susceptibility to moldy core (Janick et al., 2000). Most apple breeding programs should or currently select against moldy core susceptible cultivars.

In this survey, data were collected over three years in pursuit of an overarching trend for moldy core incidence in two large sets of genetically diverse individuals from the USDA-Specialty Crops Research Initiative project, RosBREED (Iezzoni et al., 2010) and the Washington State University apple breeding program (WABP)(Evans, 2013).

## Materials and Methods

*Plant Materials.* The RosBREED apple Crop Reference Set included 154 cultivars and elite selections as well as 313 seedlings. Subsets of these individuals were grown at the Cornell University (CU) New York State Agricultural Experiment Station in Geneva, NY; at the University of Minnesota (UMN) Horticultural Research Center near Chaska, MN; and at the Washington State University (WSU) Tree Fruit Research & Extension Center Sunrise Orchard in Rock Island, WA. The RosBREED material grown at CU were on their own roots and planted in single rows at approximately 1 m within-row and 5.8 m between-row spacing (3 x 19 feet). Seedlings grown at UMN were grafted on B9 rootstocks, were in double rows at 0.6 m x 0.6 m within-row (2 x 2 feet) and 3.7 m (12 feet) between-row spacing. The cultivars and elite selections at UMN were a mix of older accessions and test plantings grafted on M26, with most planted at approximately 3 m within-row and 4.8 m between-row spacing (10 x 16 feet). The RosBREED material grown at WSU were grafted on M9-337 and planted at 1.8 m within-row and 3.7 m between-row spacing (6 x 12 feet). There was little overlap of cultivars in the population structure between sites (Peace et al., 2014); however, in 2010 'Silken' was evaluated at WSU and UMN, and seven cultivars in 2011 were available for evaluation at more than one location (CU/UMN: 'Autumn Crisp'; CU/WSU: 'Empire', 'Fuji'; UMN/WSU: 'Arlet', 'Ginger Gold', 'Zestar'; CU/UMN/WSU: 'Honeycrisp'). The WABP seedling set comprised of 29 families derived from crosses using the following parents: 'Ambrosia', 'Aurora Golden Gala', 'Braeburn', 'Chinook', 'CrimsonCrisp', 'Cripps Pink', 'Delblush', 'GoldRush', 'Honeycrisp', 'Huaguan', 'Pinova', 'Sciros', 'Splendour' and several WSU breeding selections. Seedlings were vegetatively propagated on to Malling 9 rootstocks and planted approximately 0.3 m (12 inches) apart on a three-wire trellis in groups by family. The 707 seedlings were randomly selected from progeny of the 29 families. At the time of fruit evaluation in 2013, seedlings were in their third or fourth leaf, depending on the family.

*Fruit Evaluation.* Fruit maturity of the RosBREED individuals was monitored weekly and a 25-fruit sample was harvested when the starch index was between three and four (Blanpied and Silsby, 1992). Five fruits were evaluated at three time points: harvest, 10 weeks 2°C regular atmosphere (RA) storage with one week shelf-life at room temperature (approx. 20°C), and 20 weeks in RA storage with one week shelf-life at room temperature. Calyx openings were evaluated in five fruit samples at harvest only and scored as "closed" when the calyxes of all five fruits were closed, "mixed" when the calyxes were a mix of open and closed, and "open" when the calyxes of all five fruits were open. Core opening was also evaluated at harvest only as open or closed. A core was considered closed when there was no break in the endocarp, thus allowing no transfer from one locule to another. Moldy core was evaluated at all three time points. Moldy core was considered present if mycelium could be seen in the core region of any of the five fruits without the aid of a dissecting microscope. Moldy core was scored as absent if there was no visible infection. Fruit evaluation was based on the RosBREED protocol for apple (Evans et al., 2012). Photographs of the scoring guides for calyx opening, core opening and moldy core can be found at http://www.rosbreed.org/sites/default/files/files/RosBREED_2010Phenotyping_protocol_Malus.pdf

Twenty fruit were harvested from the WABP seedlings in 2013 at a starch index between three and five. Fruit evaluations occurred at four time points: harvest, 8 weeks 2°C RA storage with one week at room temperature shelf-life, 16 weeks 2°C RA storage with one week at room temperature shelf-life, and 24 weeks in 2°C RA storage. At harvest, all 20 fruit were evaluated visually for calyx opening. At 8 weeks, subsets of five fruits were evaluated using the RosBREED protocol for moldy core. At 16 weeks, subsets of five fruits were examined for moldy core, core opening and sinus opening. To evaluate sinus opening, each fruit was cut transversely approximately 2 cm from the calyx end and scored either as open or closed. At 24 weeks, subsets of five fruits were scored only for moldy core.

As some fruit succumbed to storage rots over the course of the experiment, not all seedlings could be scored for the traits of sinus opening, core opening and moldy core incidence.

*Statistical Analysis.* Due to the low incidence of moldy core in both sets of data, traditional statistical methods (chi-square, zero-inflated Poisson regression, data transformation, etc.) were not robust. Hence, the results presented here represent observations drawn from the data without the aid of statistical analysis. RosBREED data is presented as a combination of data taken in 2010 and 2011. The WABP seedling data is from 2013.

## Results

The number of individuals within the RosBREED germplasm with open cores was similar to the number with closed cores (Table 1). Closed calyxes, however, were more numerous than either open calyxes or mixed calyxes. Incidence of moldy core was highest in those apples with open cores or open calyxes both at harvest and after 10 weeks of storage. At harvest, open core/open calyx individuals had the highest incidence of moldy core but after ten weeks in storage, the open core/open calyx individuals showed a similar level of incidence to the open core/closed calyx and closed core/open calyx individuals (Table 1). When the data is separated by sites, UMN and CU had similar trends, with open core/open calyx individuals having the highest incidence while open core/closed calyx individuals had the highest incidence of moldy core in WSU (data not shown). Differences between

**Table 1:** Core opening, calyx opening and incidence of moldy core at both harvest and after 10 weeks in 2°C RA storage of RosBREED germplasm evaluated in 2010 and 2011. Incidence of moldy core is presented both as a number of individuals in each core opening/calyx opening class and as a percentage.

| Core at harvest | Calyx at harvest | No. apples | Incidence at harvest | | Incidence after 10 weeks storage | |
|---|---|---|---|---|---|---|
| | | | Number | % | Number | % |
| Open | Closed | 296 | 32 | 10.8 | 34 | 11.5 |
| | Mixed | 161 | 16 | 9.9 | 12 | 7.5 |
| | Open | 126 | 21 | 16.7 | 14 | 11.1 |
| Closed | Closed | 279 | 8 | 2.9 | 5 | 1.8 |
| | Mixed | 136 | 5 | 3.7 | 7 | 5.1 |
| | Open | 119 | 10 | 8.4 | 14 | 11.8 |

sites are likely due to the different germplasm evaluated at each site, availability of fungal inoculum, and the different weather conditions, particularly differences in humidity and precipitation.

Of the approximately 70 cultivars evaluated, ten showed symptoms of moldy core: 'Fortune', 'Ginger Gold', 'Honeygold', 'Hudson's Golden Gem', NJ90, 'Pinova', 'Sawa', 'Sonya', 'SunCrisp' and 'Zestar' (Table 2). All but 'Ginger Gold' had open cores; 'Ginger Gold' had an open core at UMN and a closed core at WSU. The calyxes of 'Fortune', 'Ginger Gold', 'Honeygold', NJ90, 'Pinova', 'Sawa', and 'Zestar' were closed while 'Hudson's Golden Gem', and 'SunCrisp' had open calyxes. 'Fuji' and 'Red Delicious', two cultivars known for their susceptibility, did not develop moldy core in the two years of evaluation. 'Red Delicious' was evaluated only at WSU and 'Fuji' was evaluated at WSU and CU (Table 2). This could be a result of a lack of viable pathogen and/or unfavourable weather conditions.

In the 707 WABP seedlings, there were more open core individuals (88%) than closed core individuals (12%). There were also more open calyx individuals (59%) than either mixed (22%) or closed (19%) calyx individuals. There was extremely low incidence of moldy core in 2013, with only 49 of the total 707 individuals (6.9%) showing moldy core. Only four selections had open sinuses out of those evaluated, all of which had open cores and open calyxes. None of those selections had moldy core at any of the evaluation points which could again be attributed to inoculum load or environmental conditions.

Few of the cultivars shown to be susceptible to moldy core had been used as parents in the families screened, however there were three families derived from 'Pinova'. In the two New York families with 'Pinova' as the female parent (NY-A, NY-F), several of the progeny showed susceptibility to moldy core with the highest incidence being 17 of the 24 NY-F seedlings in 2010 (Table 3). Three of the 22 WABP 'Cripps Pink' × 'Pinova' seedlings had moldy core. Although interesting to breeders, no conclusions can be drawn regarding heritability due to the small sample size in each case.

A second susceptible parent, 'Ginger Gold', was used at both CU and UMN with different results. In NY-D with 'Ginger Gold' as the pollen parent, ten progeny in 2010 and eight progeny in 2011 showed susceptibility to moldy core. In two UMN families with 'Ginger Gold' as the female parent (MN-G, MN-H), no susceptibility was seen in 2010 and only one progeny from each family showed susceptibility in 2011. This seeming discrepancy could be the result of the effect of the other parent - 'Braeburn' at CU and two seedlings at UMN - or the effect of the environment, or inoculum load. A study using controlled inoculation would be required to better predict the cause of the discrepancies or the heritability of moldy core susceptibility in these families.

The RosBREED germplasm was structured to include a large number of individuals but only a small number of fruit sampled for each individual, primarily for discovery of quantitative trait loci associated fruit quality traits (Peace et al., 2014). With the low incidence of moldy core in this fruit, statistically sound conclusions were not possible. However, as general observations, the data have value to breeders and pathologists to confirm and dispel some previous conclu-

**Table 2:** Moldy core susceptibility of cultivars tested at Cornell University[1], University of Minnesota[2], and Washington State University[3]. Trademarked names are in parenthesis.

| Parents with Moldy Core | |
|---|---|
| Fortune [2] | Nevson (Sonya™) [3] |
| Ginger Gold [2,3] | NJ55 (SunCrisp™) [1] |
| Honeygold [2] | NJ90 [3] |
| Hudson's Golden Gem [1] | Pinova [3] |
| Minewashta (Zestar™) [2,3] | Sawa [2] |
| **Parents without Moldy Core** | |
| 8S6923 (Aurora Golden Gala™) [3] | Granny Smith [3] |
| Akane [2] | Haralson [2] |
| Ambrosia [3] | Honeycrisp [1,2,3] |
| Arlet [23] | Keepsake [2] |
| Autumn Crisp [1,2] | Kerr [2] |
| Beacon [2] | Macoun [2] |
| Braeburn [3] | Malinda [2] |
| Cameo [3] | Mantet [2] |
| Co-Op 15 [3] | McIntosh [2] |
| Co-Op 29 (Sundance™) [3] | MN 447 (Frostbite™) [2] |
| Co-Op 33 (Pixie Crunch™) [2] | NY1 (SnapDragon™) [1] |
| Co-Op 39 (CrimsonCrisp™) [3] | NY2 (RubyFrost™) [1] |
| Cortland [2] | Oriole [2] |
| Cripps Pink (Pink Lady™) [3] | Pitmaston Pineapple [2] |
| Cripps Red (Sundowner™) [3] | Red Delicious [3] |
| Delblush (Tentation™) [3] | Regent [2] |
| Delorgue [3] | Scifresh (Jazz™) [3] |
| Discovery [2] | Scired (Pacific Queen™) [3] |
| Dolgo [2] | Sciros (Pacific Rose™) [3] |
| Duchess of Oldenburg [2] | SPA 440 (Nicola™)[3] |
| Early Cortland [2] | Splendour [3] |
| Empire [1,3] | StateFair [2] |
| Fiesta [3] | Sunrise [2] |
| Fireside [2] | Sweet Sixteen [2] |
| Fuji [1,3] | Tsugaru [2] |
| Gala [3] | WA 38 (Cosmic Crisp™) [3] |
| Golden Delicious [3] | Wealthy [2] |
| GoldRush [3] | Wildung (SnowSweet™) [2] |

sions regarding fruit morphology and disease incidence. Open core/open calyx individuals from diverse backgrounds are more susceptible to moldy core than other core/calyx combinations; however, an open core is more likely to lead to moldy core incidence than an open calyx. Larger diameter fruit, which are often the individuals targeted in breeding programs, are more likely to have open cores than smaller diameter fruit. Open sinuses in 'Red Delicious' and its sports have been attributed for the relatively high incidence of moldy core in this cultivar, however open sinuses are relatively rare, certainly in the germplasm within this study. A more definitive way of determining whether the calyx is open or closed, such as that used by Spotts et al. (1999) where air pressure is applied to the flesh side of a transversely cut apple submerged in water while the calyx end is monitored for air bubbles, could be used in future studies. In order to further explore this concept, it would be interesting to examine the sinuses of the cultivars found to be susceptible at different developmental stages of the fruit to determine whether the sinus is particularly slow to close in these cultivars compared to those that show no symptoms.

**Table 3:** Number of seedling individuals evaluated and incidence of moldy core in families as part of the RosBREED project in 2010 and 2011.

| Family Code | Female parent | Pollen parent | No. seedlings 2010 | Moldy core incidence 2010 | No. seedlings 2011 | Moldy core incidence 2011 |
|---|---|---|---|---|---|---|
| MN-A | Honeycrisp | Jonafree | 3 | 1 | 10 | 1 |
| MN-B | Honeycrisp | Monark | 10 | 1 | 23 | 4 |
| MN-C | Honeycrisp | Pitmaston Pineapple | 2 | 0 | 17 | 1 |
| MN-D | Honeycrisp | Regent | 3 | 0 | 6 | 0 |
| MN-E | Zestar | BC-8S-27-43 | 4 | 0 | 9 | 2 |
| MN-F | Sunrise | GMAL4329 | 3 | 0 | 19 | 0 |
| MN-G | Ginger Gold | GMAL4328 | 3 | 0 | 15 | 1 |
| MN-H | Ginger Gold | GMAL4332 | 1 | 0 | 22 | 1 |
| MN-I | SnowSweet | Pixie Crunch | 8 | 1 | 11 | 2 |
| MN-J | Dayton | Zestar | 4 | 0 | 25 | 3 |
| MN-K | Honeycrisp | GMAL4327 | 9 | 0 | 19 | 0 |
| MN-L | Sweet16 | BC-8S-27-43 | 3 | 0 | 4 | 1 |
| MN-M | Honeycrisp | Akane | 1 | 0 | 22 | 4 |
| MN-N | Honeycrisp | Silken | 6 | 1 | 15 | 2 |
| WA-A | Delicious | Honeycrisp | 7 | 0 | 9 | 0 |
| WA-B | Honeycrisp | Splendour | 11 | 2 | 11 | 2 |
| WA-C | Cripps Pink | Honeycrisp | 8 | 0 | 8 | 1 |
| WA-D | W7 | W5 | 19 | 1 | 23 | 5 |
| WA-E | Aurora Golden Gala | Arlet | 13 | 0 | 13 | 0 |
| WA-F | Aurora Golden Gala | Enterprise | 13 | 2 | 13 | 2 |
| WA-G | Aurora Golden Gala | Honeycrisp | 15 | 2 | 25 | 2 |
| WA-H | Aurora Golden Gala | Granny Smith | 11 | 0 | 0 | 0 |
| WA-I | Enterprise | Arlet | 9 | 0 | 9 | 2 |
| WA-J | Honeycrisp | Arlet | 16 | 2 | 16 | 1 |
| WA-K | Cripps Pink | W7 | 23 | 5 | 23 | 4 |
| WA-L | Cripps Pink | Aurora Golden Gala | 12 | 0 | 12 | 0 |
| NY-A | Pinova | Cameo | 13 | 3 | 14 | 4 |
| NY-B | Sansa | Granny Smith | 18 | 6 | 15 | 2 |
| NY-C | Hudson | NY-S1 | | | 29 | 3 |
| NY-D | Braeburn | Ginger Gold | 25 | 10 | 35 | 8 |
| NY-E | Autumn Crisp | Fuji | 9 | 5 | 10 | 0 |
| NY-F | Pinova | NY-S2 | 24 | 17 | 18 | 4 |
| NY-G | NY-S3 | Sonya | 3 | 1 | 4 | 0 |
| NY-H | Sonya | NY-S4 | 4 | 1 | 3 | 0 |
| NY-I | Fuji | NY-S5 | 9 | 5 | 10 | 1 |
| NY-J | Honeycrisp | NY-S6 | 1 | 0 | 1 | 0 |

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by Washington State University.

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